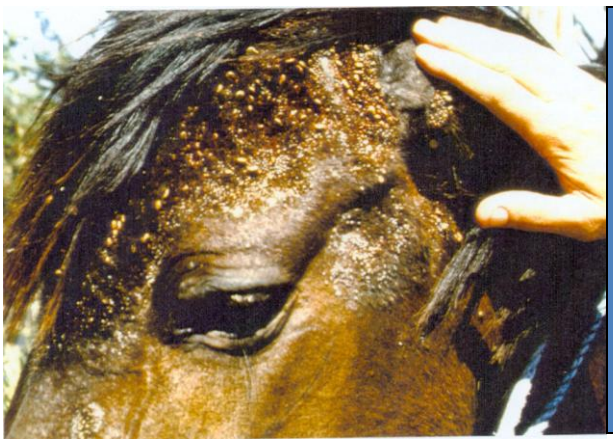
EFTBA Veterinary Newsletter 13



Equine Piroplasmosis (EP)

Epidemiology, symptoms, diagnosis, pathological findings and therapeutic options

September 2014

. Efficient protection wants detailed and comprehensive information
. Diagnosis is demanding and diverse techniques must be differentiated
. In endemic and non-endemic regions, therapeutic options differ
.Therapy may be toxic and must be well reflected
. It is demanding to deal with EP and all knowledge is worth having

"Many thanks to Mrs. Eva-Maria Bucher-Haefner, Moyglare Stud Farm, for her valued sponsorship of this newsletter."

Welcome to EFTBA's veterinary newsletter

Further to the newsletter 12, much more information on the complex nature of Equine Piroplasmosis (EP) becomes available with this issue. It is a quite voluminous edition, but this fact is just the expression of the complex nature of EP. Of course, we hope that you will not encounter problems with this disease and that all these informations may only serve for reference. However, we know that prevention is better than cure generally, and with such a complicated subject as EP, efficient prophylaxis is most welcome, especially.
This newsletter is meant to be a benefit to all breeders and we thank you for your devotion to the cause.
Wishing all the readers an enjoyable discovery

Loïc Malivet and Hubert Honoré

Loïc Malivet and Hubert Honoré

Chairman EFTBA, president and vice-president Syndicat des Eleveurs, respectively

Editorial

Issue 12 of our newsletter informed that Equine Piroplasmosis (EP) has to be looked at as a demanding subject for all of us, breeders and veterinarians alike. But so far, we only looked at historical aspects, the very special and complex nature of the disease, its etiology and pathogenesis, the life cycles of the parasites and economic aspects. But for the sake of our industry, economic reflections also ask for informations on epidemiology, clinical signs, diagnosis, pathological findings and therapeutic options - the contents of this number now.

Means of prevention will finally be the subject of a further newsletter.

Dr Hanspeter Meier

EFTBA veterinary advisor & Newsletter editor

Profound Beauty (Danehill) owned and bred by Moyglare Stud.

Introduction

The breeding of Thoroughbreds is a global enterprise, wherefore the mechanisms of transmission and occurrence of infectious diseases ask for our attention. Such investigations belong to the field of epidemiology which studies the patterns, causes and effects of health and disease conditions and tries to identify risk factors for allowing evidence-based practice and prevention. For achieving all these goals, both clinical and pathological findings and the methods for diagnosis and treatment must be considered carefully.
In regard to the most interesting nature of EP, we cannot help going into pretty detailed technical aspects of the diagnostic methods. This isn't really entertaining, but may at least be helpful for quick reference, hopefully.

Epidemiology of EP

In regard to our endeavours for maintaining free trade and transport, epidemiological facts of any infectious or contagious disease are of prime importance for us. In the case of EP and according to informations of the OIE and Rothschild & Knowles (2007), this is a difficult task. Only few countries in the world are free from native infections with EP, and it is estimated that just about 10% of horses globally inhabit regions that are free of it.

The countries **currently recognized as nonendemic** by the World Organization for Animal Health (OIE) include **Australia, Canada, United States, England, Ireland, and Japan**. Further information on this subject of national information is available from the OIE website, http://www.oie.int.

Moreover, one has to keep in mind that many areas currently free of EP climatically are suitable for appropriate tick vectors or already even possess competent tick vectors. Thus, there is the continual possibility of introducing *T. equi* and *B. caballi* into hitherto free areas either by infected ticks or horses (Rothschild and Knowles, 2007).

As the prevalence of EP is consistent with the distribution of competent tick vectors, piroplasmosis is endemic in tropical and subtropical climates. In most regions, however, **infections with *T. equi* are more common than infections with *B. caballi***.

Outbreaks of overt clinical disease are uncommon in endemic areas, with the exception of India, despite the endemic status of implicated parasites.
Contrary to this, acute clinical disease is most often observed when naïve horses are moved into endemic areas (Rothschild and Knowles, 2007).

Western Europe

T. equi and ***B. caballi*** are **widely present in Portugal, Spain, France, and Italy**. Austria, Belgium, the Czech Republic, Germany, Netherlands, Switzerland are considered infection free, although autochthonous infections occasionally occur. Publications from Switzerland, Germany and the Netherlands inform about such autochthonous, very similar and exemplary clinical cases (in chronological order):

Gottstein et al. (1995): ***Babesia equi*-Infektion bei einem Pferd ohne Auslandaufenthalt in der Schweiz** (Possible autochthonous infection with *T. equi* in a horse in Switzerland)
The first case of a possibly autochthonous *T. equi*-infection in Switzerland was already described in 1995. It was a seven year old homebred gelding in the canton of Berne which was presented because of poor performance, apathy and intermittent fever (41° C). The attacks of fever were accompanied by inappetence and slight shivering all over the body. Laboratory examinations revealed anemia, a low white blood cell count and positive *T. equi* IFAT- and CFT-titers. This horse was never abroad and competed only in the cantons of Berne and Vaud. The examination for ticks was positive, both *Rhipicephalus sanguineus* and *Ixodes ricinus* were found; an acute infection was assumed.

Scheidemann et al. (2003): **Equine Piroplasmosis – a case of an acute infection with *Theileria equi* (syn. *Babesia equi*) in Germany**.
An acute case of EP in a horse from Germany (Westphalia) caused by infection with *T. equi* also showed typical clinical symptoms (fever (41° C), inappetence, anaemia, haemolysis, bilirubinaemia and haemoglobinuria) and an edema of the head and colic symptoms as well. In Giemsa-stained blood smears typical Maltese-cross intraerythrocytic merozoites of *T. equi* were seen and EP was serologically confirmed by complement fixation (CF) and infection with *T. equi* was diagnosed also by Indirect Fluorescent Antibody Test (IFAT). Finally the horse was successfully treated with Imidocarb (Imizol®) and with 2 injections no side effects were observed. From 21 days after the second treatment no parasites were seen in the blood smears. This

horse was reared in Westphalia and had only spent a short time in the Netherlands; however, the clinical picture was typical for an acute infection.

Butler et al. (2012): **Prevalence of the causative agents of equine piroplasmosis in the South West of The Netherlands and the identification of two autochthonous clinical *Theileria equi* infections**. EP has not been considered indigenous in the Netherlands so far, but following detection of an apparently indigenous subclinical *Babesia caballi* infection in a horse on Schouwen-Duiveland (an island in the Zeeland Province), a survey was undertaken between May and September 2010 to assess the prevalence of the causative agents of EP in the South West of The Netherlands. Blood samples from 300 randomly selected horses were tested for specific antibodies against *T. equi* and *B. caballi* using an indirect fluorescence antibody test (IFAT), and for parasite DNA using a specific polymerase chain reaction.
Twelve of the horses (4%) were seropositive for EP. Of these, nine were positive (titre ≥1:160) for *B. caballi* alone and three were also positive for *T. equi*. PCR detected *T. equi* DNA in five horses (1.6%), two of which were seronegative. Four (1.3%) of the positive horses (three positive for *T. equi* and one for both *B. caballi* and *T. equi*) were considered truly indigenous. During the study, two indigenous ponies from a farm situated outside the sampling area were diagnosed with acute clinical piroplasmosis (severe anaemia and pyrexia). Blood smears showed *T. equi*–like inclusions in red blood cells, and *T. equi* infection was confirmed in both ponies by PCR. The initial subclinical *B. caballi* infection, the survey results and the two acute clinical EP cases confirmed the autochthonous transmission of *B. caballi* and *T. equi* infections in The Netherlands.

Eastern Europe

Piroplasmosis is also endemic **in the Balkan Peninsula, Hungary, Romania, several countries of the Commonwealth of Independent States (CIS, the southern parts of Russia)**, and the states of the **Caucasus region**. In **central Russia**, *B. caballi* infections have a common distribution with the tick vector *Dermacentor reticulatus*. Most infections of horses in nonendemic European countries have been traced back to Spain, France, Italy, or the CIS. Poland is considered infection free (Rothschild and Knowles, 2007).

Potential vectors of the genus *Dermacentor* are present in most Northern European regions. *Hyalomma* and *Rhipicephalus* ticks are predominate in southern Europe, the southern regions of Russia, and the CIS (Rothschild and Knowles, 2007).

Latin America

T. equi and *B. caballi* are endemic in almost all of Latin America (incl. the Caribbean), except for the southern parts of Chile and Argentina. Almost all horses in Brazil, Colombia, Puerto Rico, and Mexico are seropositive for *T. equi* and *B. caballi*. Despite the widespread distribution of infection and the intense tick parasitism of the horse population in many parts of Latin America (see Fig. 1), data are limited regarding which ticks are responsible for transmission of EP in these countries.

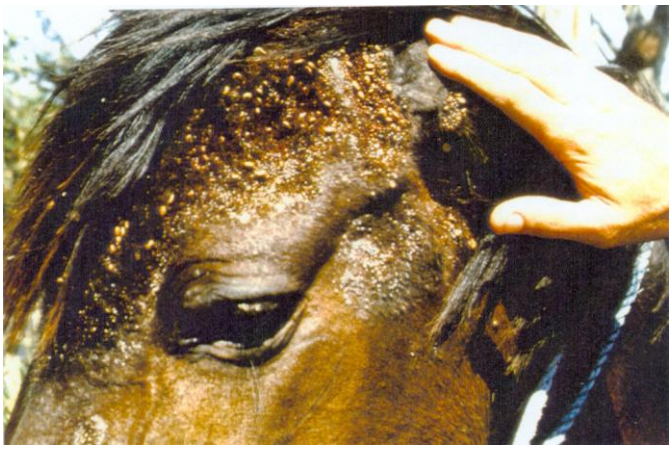


Fig. 1 Severe infestation with ticks in Brazil *(Anocenter nitens)*
Photo: courtesy of Prof.K.T.Friedhoff, Hannover

B. caballi is transmitted by *Dermacentor* (*Anocentor*) *nitens*, the "tropical horse tick." *Boophilus microplus* is believed to be one of the ticks involved in the transmission of *T. equi*. In a study in Brazil using nested polymerase chain reaction (PCR), both *T. equi* and *B. caballi* were identified in *B. microplus* ticks. The significance of this finding is uncertain because these ticks are not generally considered to be a vector for *B. caballi*. *Boophilus microplus* primarily feeds on cattle, although it will occasionally feed on horses, especially if horses are kept in pasture with cattle. As a result, the seroprevalence of *T. equi* is higher in horses in close contact with cattle. The most common species of tick found on horses in Brazil are *Dermacentor* (*Anocentor*) *nitens* (see Fig. 1) and *Amblyomma cajennense*. Intense parasitism by these ticks is observed in horses at an

early age in many regions of the country. In 1995, Barbosa et al. found that 100% of foals in south-eastern Brazil seroconvert to *T. equi* by 127 days of age and to *B. caballi* by 150 days (Rothschild and Knowles, 2007).

Middle East and India

High infection rates have also been reported in the Middle East, including Kuwait, Oman, and India, where *Theileria equi* is reported to have higher seroprevalence. Ticks involved are of the *Hyalomma* and *Rhipicephalus* species. Jordan recently has reported fatalities in strenuously exercised horses with high-level *T. equi* parasitemia on blood smears and consistent necropsy findings (Rothschild and Knowles, 2007).

USA

B. caballi and *T. equi* are said to have been introduced into the United States (U.S.) in 1959 when Cuban horses were imported. Limited *B. caballi* infections became established in **Florida, Southern Georgia, Texas**, and some adjacent states in which the tick vector *Dermacentor (Anocentor) nitens* was present. Since then, a few new cases and epizootics have occurred in Florida, but with minor exceptions, further spread was prevented by intensive control measures. Despite sporadic occurrence of EP in the U.S., it is considered free of EP (Rothschild and Knowles, 2007).

Australia

Although *T. equi* was introduced into Australia in the 1950s and 1960s with Quarter Horses imported from Texas and in 1976 with the importation of Andalusian horses from Spain, the organism did not become established in Australia. As in other non-endemic countries, transmission occurred by contaminated needles and instruments.
Boophilus microplus is present in parts of Australia, and other tick vectors capable of transmitting EP could potentially be introduced by horses or other hosts (Rothschild and Knowles, 2007).

Asia

T. equi and *B. caballi* are **widespread** in the horse populations of **Mongolia, China**, and **many parts of Southeast Asia and Asia**. In both China and Mongolia, *T. equi* is more prevalent and is primarily transmitted by *Dermacentor nuttallii*. High infection rates are similarly reported in **Korea**. Historically considered free of infection, recent reports demonstrate both *T. equi*–seropositive and *B. caballi*–seropositive horses in **Japan**.
The "brown dog tick," *Rhipicephalus sanguineus*, listed as a potential *T. equi* vector, is also present in Japan. (This tick has been shown to transmit *B. caballi*, although it is not believed to be involved in the natural transmission of the disease because of its habit of feeding almost exclusively on dogs.) (Rothschild and Knowles, 2007).

Africa

Morocco, Republic of South Africa, Madagascar, and **nearly all other parts of the African continent** are considered endemic for *T. equi* and *B. caballi*. Virtually all horses and zebras are infected, except in a few regions. The seroprevalence in these countries is generally greater than 50% and 60% for *T. equi* and *B. caballi*, respectively. Ticks involved in transmission include *Rhipicephalus evertsi*, which can transmit both *T. equi and B. caballi,* and *Hyalomma truncatum*, which transmits *B. caballi*.
An association between gender and the occurrence of antibodies against *B. caballi* has been observed in South Africa, with colts more likely to have antibodies than fillies. This may be linked to tick gender predilection, because male horses in some studies have a higher burden of certain tick species than females (Rothschild and Knowles, 2007).

Clinical and pathological findings

For the treatment and the prevention of further transmission, early and correct diagnosis is essential. This sounds self-evident or a truism, but is of special importance in regions, where piroplasmosis emerges – as **naive populations are especially vulnerable**. However, **in non-endemic-regions, disease awareness and experience may lack**. Therefore we better go into some details here.
Principally, clinical infections may be peracute, acute, subacute, or chronic and horses infected with *B. caballi* and/or *T. equi* may present with similar clinical signs, although the signs associated with *B. caballi* infection tend to be milder or inapparent (Rothschild and Knowles, 2007).

Inapparent Carrier
The vast majority of *piroplasm*-seropositive horses are inapparent carriers, with low levels of parasit-

emia (not detectable with blood smears) and no obvious clinical signs. However, athletic or heavy working horses may have compromised athletic performance, compared with uninfected horses. These horses are at risk of developing overt infection with clinical illness, are reservoirs for the parasites and can potentially disseminate the organism in areas where vector ticks are present.

Inapparent **carrier mares** may transmit *T. equi* by intrauterine infection throughout their breeding life, leading to **abortion, stillbirth, or neonatal piroplasmosis**. A survey of TB mares in South Africa reported that 11% of abortions in those herds were caused by *T. equi* infection. Abortion usually occurs in the last trimester of gestation, and mares are usually clinical normal. However, *T. equi* has been identified in healthy fetuses at as early as 120 days of gestation, indicating that infection may actually occur early in gestation (Rothschild and Knowles, 2007).

Peracute EP

Peracute piroplasmosis occurs primarily in **neonatal foals** born infected in utero, in **adult horses** suddenly introduced into areas with **large numbers of infected ticks**, and in adult horses infected **after strenuous exercise**.

Neonatal piroplasmosis is characterized by weakness at birth or the rapid onset of listlessness, anemia, severe icterus, and malaise soon after birth. Affected foals become progressively lethargic and are ultimately unable to stand and suckle. Fever is usually present, and petechiae may be observed on the mucous membranes. Hemoglobinuria may also be evident. Some foals are apparently normal at birth but develop clinical signs of EP 2 or 3 days later. Therefore, neonatal piroplasmosis **may appear similar to equine neonatal isoerythrolysis** (as we have seen in the history of the Poitou-mule), but differentiation is important so that adequate therapy can be implemented as affected foals have a poor prognosis.

Peracute infection with *B. caballi* results in organ damage and dysfunction caused by obstruction of capillaries or other small vessels with parasitized erythrocytes. Clinical signs in affected foals and adult horses therefore vary with the organ affected. Central nervous system involvement has been reported, with encephalitis, ataxia, and other signs, depending on the exact location of the lesions.

During peracute *T. equi* infection, parasites replicate in erythrocytes, causing cell lysis and death from anemia. Sudden death caused by piroplasmosis in adult horses is rare but can occasionally occur when naive horses are introduced into *T. equi*–endemic areas. *T. equi* has been implicated as the cause of death in two horses with high levels of parasitemia after strenuous exercise (Rothschild and Knowles, 2007).

Acute Equine Piroplasmosis

Acute cases of EP are characterized by **pyrexia** (typically exceeding 40° C [104° F]), moderate anorexia and malaise, frequent recumbency, dehydration, congested mucous membranes, tachypnea and -cardia, sweating, limb edema, supraorbital edema and tearing, anemia and in severe cases icterus, hemoglobinuria/bilirubinuria (rare in *B. caballi* infections), and death. Pneumonia may result as a complication of pulmonary edema and inflammation. ***T. equi* infection results in intermittent pyrexia**; if untreated, mortality can be moderately high. Digestive tract involvement may occur terminally, with signs of colic, constipation, diarrhea, and catarrhal enteritis. In severe cases, inflammation of mucous membranes and blood vessels occurs in various organs, and a variety of atypical presentations may result, including renal and liver failure (Rothschild and Knowles, 2007).

Subacute Equine Piroplasmosis

Horses with subacute EP exhibit varying degrees of **anorexia, malaise, weight loss, intermittent pyrexia, anemia, limb edema, poor performance, tachycardia and -pnea**. Mucous membranes vary from pale pink or pale yellow to bright yellow, with occasional petechiae and ecchymoses. Intermittent colic signs may occur. Constipation may be followed by diarrhea. Urine may be dark yellow to brown or reddish, depending on degree of hemoglobinuria. Horses with subacute EP typically have splenomegaly. If untreated, these horses may become severely anemic with marked general weakness (Rothschild and Knowles, 2007).

Chronic Equine Piroplasmosis

Horses with chronic EP typically present with a history of **nonspecific clinical signs**, including mild inappetence, poor performance, weight loss, poor body condition, and malaise. Anemia may be minimal. **Clinical signs of chronic EP may be similar to signs observed in horses with equine infectious anemia (EIA)** or other chronic inflammatory conditions.

Clinicopathologic abnormalities in horses with *T. equi* or *B. caballi* infection may include reduced red blood cell count, platelet count, and hemoglobin concentration. Acute infections are characterized by neutro- and lymphopenia. Decreased

plasma fibrinogen, serum iron, and phosphorus concentrations, increased serum bilirubin concentration; and prolonged clotting times may also occur. Varying degrees of hemoglobinuria are observed in *T. equi*–infected horses. After about 10 days of infection, lymphocytosis develops, and PCV often decreases to approximately 20% but may fall to 10% or lower. Extreme anemia is more common with *T. equi* infection. In chronic cases, serum bilirubin concentration is not usually increased, and only mild anemia may be observed (Rothschild and Knowles, 2007).

Pregnant Carrier Mares (*T. equi* or *B. caballi*)
Effective measures to prevent abortion or stillbirth from EP have not been described. It is unknown at what stage *T. equi* infects the fetus, and therefore the optimal time for treatment has not been determined. Abortions usually occur in the last trimester of gestation, but *T. equi* has been identified in healthy fetuses as early as 120 days of gestation. Imidocarb is detectable in the fetal circulation at a similar concentration as found in the dam's blood, but correlation between treatment of mares with the birth of sick or healthy foals has not yet been described. Imidocarb is eliminated in the milk for approximately 2 hours after administration in the dam. It is uncertain if this can cause toxicity effects in nursing foals (Rothschild and Knowles, 2007).

Neonatal Piroplasmosis
Even with appropriate intensive therapy, prognosis for most neonatal foals with EP is poor. Infected foals frequently become severely anemic. Transfusion with a crossmatched non-infected blood donor should be performed. The PCV should be closely monitored for 2 weeks or longer for recrudescence of severe anemia. Other supportive therapy may include judicious fluid therapy, adequate nutrition (bottle, nasogastric tube, or parenteral), and broad-spectrum antibiotic therapy if concomitant sepsis is a concern.
Except one brief report, no data exist regarding the safety or efficacy of babesicidal drugs (e.g. imidocarb) in equine neonates (Rothschild and Knowles, 2007).

Diagnosis

Clinical diagnosis of EP can be made based on clinical signs and examination of blood smears (Fig. 2 & 3). **Clinical signs may be nonspecific, and the disease may be confused with a variety of other conditions**. Important differential diagnoses for EP include equine infectious anemia, African horse sickness, purpura hemorrhagica, and toxicities, among others.
Although it may be difficult to differentiate clinically between *T. equi* and *B. caballi*, differenttiation may be important for successful treatment. Serologic and PCR testing may be necessary in subclinical cases and for regulatory purposes.
In a *Babesia*-regulated country, the proper state and federal authorities should be contacted before collection or submission of samples from suspect animals. Careful and secure handling of samples is necessary, and samples should only be submitted to authorized laboratories (Rothschild and Knowles, 2007).

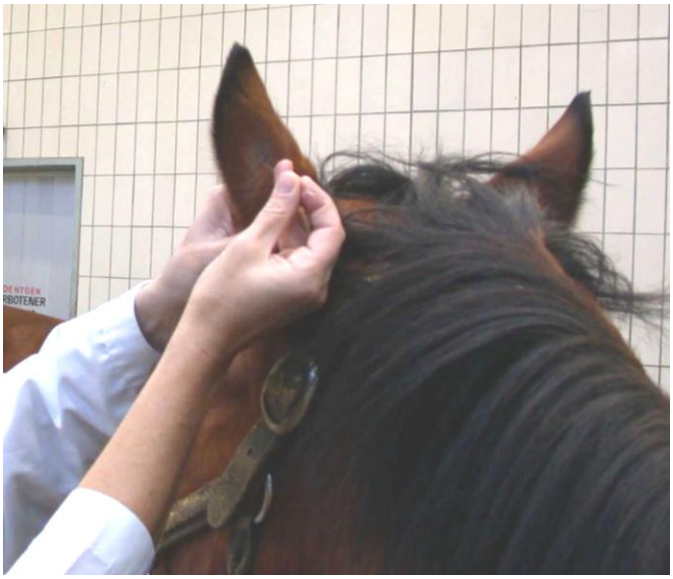


Fig. 2 Collection of a capillary blood sample for blood smears

Further Information on diagnostic procedures can be found on the website of the World Organization for Animal Health (OIE) at http://www.oie.int.

Microscopy

If present, parasites can be observed in blood smears. In the acute phase of EP, diagnosis by microscopic examination of blood smears is possible. In inapparent carrier horses, however, the low number of piroplasms in circulation decreases the sensitivity of microscopy, and serologic diagnosis is more reliable. Because parasitemia with *B. caballi* is very low, observation of parasites in thin blood smears is often difficult (Rothschild and Knowles, 2007).
As already mentionend in newsletter 12, the merozoites of *B. caballi* within erythrocytes are pyriform in shape and often form pairs joined at their posterior ends. Trophozoites may also be observed in erythrocytes and are polymorphic in shape, varying from round to oval or elliptic.
T. equi merozoites in erythrocytes typically appear as four pyriform parasites and arranged in a "Maltese cross" formation (see Fig. 3). The trophozoites can appear as oval, round, elliptic, or spindle in shape and within erythrocytes (Rothschild and Knowles, 2007).

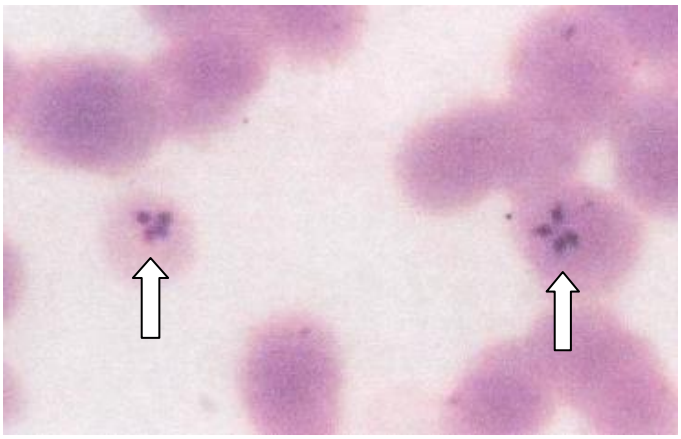


Fig. 3 blood smear with intra-erythrocytic stages (merozoites) of *T. equi* showing Maltese-cross stages (arrows) (Scheidemann et al., 2003)

Serological diagnosis

Complement Fixation Test (CFT)
The complement fixation test (CFT) **was the previously official standard** test for equine piroplasmosis and has been used worldwide to test horses entering EP-free countries. **Recently it has been determined that the regulatory assay for entrance of horses into the United States is the cELISA.**
The underlying principle of the CFT is the fixation of complement during the reaction between specific antigen and antibody. Horse sera that react positively at a dilution of 1:5 are considered positive. The CFT detects antibody titers from day 8 after infection, with titers declining at 2 to 3 months after infection. **CFT reactions may become transiently negative** within 3 to 15 months of treatment of *B. caballi*–infected horses and within 24 months for *T. equi*–infected horses. For this reason, retesting of treated horses must be done 4 to 6 weeks after treatment.
Many disadvantages are associated with the use of CFT, including the need for production of large quantities of antigens, the occurrence of false-negative results, and crossreactivity between *B. caballi* and *T. equi* sera. CFT is a very specific test; however, it has low sensitivity in chronic cases because of the presence of IgG(T) antibodies (non-complement fixing) (Rothschild and Knowles, 2007).

Indirect Immunofluorescent Antibody Test (IFAT)
The indirect immunofluorescent antibody test (IFAT) is **more sensitive than the CFT** and has been used as **a supplementary test when CFT results are inconclusive**. In this assay, parasite antigens are bound to glass slides and allowed to react with test sera. Bound antibodies are visible under ultraviolet light after binding of a fluorescein-labeled anti-equine serum. Sera are considered positive if they show strong fluorescence of the parasites at a dilution of 1:80 and higher. The earliest antibody responses in horses experimentally infected with *B. caballi* and *T. equi* in one study were at 3 to 20 days after infection, with titers still detectable during the latent period of infection. IFAT titers are detected more consistently than CFT titers, and sera remain positive by IFAT longer than by CFT. To increase specificity with IFAT, serum must be diluted, which concurrently results in loss of sensitivity.
The IFAT is time-consuming, requires large amounts of antigen, and because of subjectivity in interpreting fluorescence, is difficult to standardize (Rothschild and Knowles, 2007).

Enzyme-Linked Immunosorbent Assay (ELISA)
The enzyme-linked immunosorbent assay is used to detect dominant antibodies to both *T. equi* and *B. caballi*, although cross-reactivity may occur. In 1991, Knowles et al. using *T. equi* EMA-1 and specific monoclonal antibodies, developed a **competitive inhibition ELISA (cELISA)** for *T. equi* infection. EMA-1 is a specific *T. equi* surface erythrocyte-stage protein that possesses an epitope shown to be both immunodominant and conserved worldwide. This cELISA was later improved by the use of a recombinant protein instead of culture-derived whole

parasites. This test overcomes the problem of antigen purity because specificity depends only on the monoclonal antibody used. **ELISA has improved performance compared with CFT and IFAT**, and has detected latent infections of experimentally infected horses not detected by CFT. The use of a recombinant protein facilitates standardization of the assay and overcomes the need for in vitro cultivation of the parasite or the artificial infection of horses for antigen production, making **cELISA an ideal test for screening of *Babesia* infection**.
In 1999 a cELISA using recombinant *B. caballi* rhoptry-associated protein-1 was developed by the same group. In a field survey, this test identified 25% more sera as positive for *B. caballi* than the CFT. **In 2004, OIE** (**http://www.oie.int**) **approved the cELISA for both *B. equi* and *B. caballi* as the prescribed test for international horse trading** (Rothschild and Knowles, 2007).

Polymerase Chain Reaction
Detection of parasite deoxyribonucleic acid (DNA) using polymerase chain reaction (PCR) is more sensitive than microscopic detection of parasites in blood smears and is **ideal for the detection of carrier infections**. The PCR systems may be useful tools in the expeditious detection and identification of *T. equi* and *B. caballi* in blood, as supplements to microscopy and serology for augmenting diagnostic results; at this time, however, **these systems are only used for research purposes**. Surveys of horses in endemic areas are necessary for assessment of the diagnostic sensitivity and specificity of these assays.
Primary PCR assays have been developed to detect both *T. equi* and *B. caballi* DNA in horses. In one survey, PCR was able to detect calculated parasitemias as low as 0.0083% for *T. equi* and 0.017% for *B. caballi*. However, most horses with positive PCR results also had positive microscopic examinations of blood smears.
A nested PCR for *T. equi* based on the sequence of the EMA-1 gene has increased sensitivity and may be more reliable for the diagnosis of subclinical infection, detecting an equivalent calculated parasitemia of 0.000006%. In a field study using the same nested PCR for *T. equi*, the test was able to detect 3.6 times more infections than microscopic analysis and 2.2 times more than with primary PCR. Many subclinical infections in apparently healthy horses that could not be detected with primary PCR were detected by nested PCR. The same test has been successfully used to detect *B. caballi*–infected and *T. equi*–infected ticks in Mongolia and to detect infected ticks and horses in Brazil (Rüegg et al. 2007, Heim 2008).
PCR testing is fairly straightforward and becoming more affordable and may become commercially available (Rothschild and Knowles, 2007).

For a summary of the diagnostic techniques of the OIE, please s. page 9.

Therapy

The **efficacy of drugs** for treatment of EP **is highly variable**, and close monitoring of horses undergoing treatment is necessary to ensure success. Treatment strategies may aim at resolving the clinical signs during acute infection or at completely clearing the horse from the carrier state (sterilization). The latter is more difficult and possibly unattainable. Although it has been stated that horses with B. caballi infection may self-clear the infection, it is important to remember that most reports of successful sterilization for both *B. caballi* and *T. equi* were before the development and availability of more sensitive tests such as nested PCR. **Attempts to eliminate the carrier state of EP is not recommended in endemic areas**, but may be desirable for movement of horses to areas that are considered EP free (Rothschild and Knowles, 2007).

***Babesia caballi* infection in adult horses**
For the treatment of adult horses with acute clinical signs of *B. caballi* infection, intramuscular (IM) administration of *imidocarb dipropionate* (Imizol) for two treatments with a 24-hour interval is considered most effective. Although relatively safe, **imidocarb can cause toxicity with fatal outcomes in some horses**. Mild signs of toxicity include salivation, gastrointestinal hypermotility, and colic.
The same imidocarb treatment regimen is recommended for sterilization, although this may actually result in temporary disappearance of the organisms, with later recrudescence. The time required for horses to become seronegative by CFT after imidocarb treatment varies substantially, with one study reporting an average of 39 days (maximum 116 days) to "clearance".

Summary of diagnostic techniques (OIE)

Test methods available for the diagnosis of EP and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection - surveillance	Immune status in individual animals or populations post-vaccination
Agent identification[1]						
Microscopic examination	–	+	–	++	+	n/a
PCR	+++	+++	+++	+++	+++	n/a
Detection of immune response[2]						
IFAT	++	++	++	+++	++	n/a
C-ELISA	+++	+++	+++	+++	+++	n/a
CFT	+	+	+	+	+	n/a

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = not applicable.
Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.
PCR = polymerase chain reaction; IFAT = indirect fluorescent antibody test;
C-ELISA = competitive enzyme-linked immunosorbent assay; CFT = complement fixation test.

Diminazene (Berenil) is effective for treatment of acute disease when administered intramuscularly for two treatments 24 hours apart. This therapy may result in elimination of the organism. Respiratory distress and depression are primary signs of intoxication.

Amicarbalide (Diampron) administered at 9 to 10 mg/kg IM single dose is often sufficient for treatment of horses with acute signs of EP. When administered for 2 consecutive days, amicarbalide therapy has been claimed to result in sterilization. A dosage for of 2.2 mg /kg for 2 consecutive days may be used to treat clinical signs of B. caballi infection but does not result in clearance. A delayed anaphylactic-type reaction, with respiratory and gastrointestinal disturbances, periorbital and muzzle edema, and subcutaneous edema over the back and flank, has been reported in a few horses.

Acridine dyes such as *euflavine* (Gonacrine) and tetracyclines are reported to alleviate clinical signs but not clear infection. It has been reported that it may take approximately 3 to 15 months for a horse to become seronegative by CFT after spontaneous or therapeutic clearance of *B. caballi* (Rothschild and Knowles, 2007).

***Theileria equi* infection in adult horses**

***T. equi* is resistant to most therapeutic agents**, and sterilization or even temporary clearance may not be accomplished even with persistent and repeated efforts. Most horses that survive clinical infection remain lifelong carriers of the parasite. In some cases, treated horses become seronegative by CFT for a few weeks or months but remain positive by IFAT. Additional supportive therapy is often necessary if severe hemolysis and enterocolitis are present.

Imidocarb dipropionate (Imizol) administered four times at 72-hour intervals is usually an effective treatment for clinical signs of *T. equi* infection. The recommended dose is near the 50% lethal dose (LD50) for imidocarb and may cause moderate to severe signs of intoxication and even death. Clinical signs of toxicity include salivation, restlessness, slight to moderate colic, and gastrointestinal hypermotility.

Administration of half this dose initially, followed by the remainder of the dose, may decrease toxicity. The treatment should not be repeated before 30 days after the first treatment. Liver function should be monitored in treated horses. Necropsy findings in

horses with imidocarb toxicity include acute periportal hepatic necrosis and renal cortical tubular necrosis.
Administration of imidocarb at 6 to 8 mg/kg for 4 doses with a 72-hour interval between doses may induce clearance but has a very high risk of toxicity.
Some *T. equi* strains, such as those of European origin, may have differing susceptibility to treatments with imidocarb.
Antitheilerial drugs have been used with variable success in the treatment of clinical signs of *T. equi* infection but cannot completely eliminate the parasite (parvaquone).
Buparvaquone (Butalex), 4 to 6 mg/kg given as a slow IV injection once a day for 3 consecutive days, successfully resolved clinical signs of *T. equi* infection in two experimentally infected spleen-ectomized horses; however, these horses remained inapparent carriers. No toxicity was observed if treatment began before horses were severely ill and while PCV remained above 25%. IM administration of this drug resulted in severe local reaction and lameness that lasted 2 to 3 days. A combination of buparvaquone and imidocarb therapy may eliminate *T. equi* infection. Experimental trials using this combination are necessary.
Other antiprotozoal drugs have had variable results against *Babesia* in vitro. Imidocarb is recommended for treatment of mixed infections. It is believed to take approximately 24 months for a horse to become seronegative by CFT if complete elimination of *T. equi* occurs (Rothschild and Knowles, 2007).

Conclusions

This was tough reading so far, I guess. At least I feel so myself, but just this simple fact already explains our difficulties with Equine Piroplasmosis, especially the different regulations regarding reportability and notifiability. In different countries, circumstances are not the same and recommendations for dealing with this disease must consider these differences. We therefore had to go into many details with e.g. the different stages of the disease and also the questions whether and how to treat the patients or not. In some cases, therapy may not be adequate, in others we may encounter side effects as severe as EP itself.
For a better understanding of all the problems and differing attitudes in struggling against EP, one must have a detailed knowledge of all aspects and be conscious of the fact that any action must be reflected carefully.
Finally, we therefore certainly agree to the saying that all knowledge is worth having.

References

Butler C.M., Sloet van Oldruitenborgh-Oosterbaan M.M., Stout T.A.E., van der Kolk J.H., van den Wollenberg L., Nielene M., Jongejan F., Werners A.H. Houwers D.J. (2012): Prevalence of the causative agents of equine piroplasmosis in the South West of The Netherlands and the identification of two autochthonous clinical *Theileria equi* infections.

Gottstein B., Pauli H., Böse R., Hentrich B. und Tschudi P. (1995): *Babesia equi*-Infektion bei einem Pferd ohne Auslandaufenthalt; Swiss Vet. 12, 14-19

Heim A. (2008): Untersuchungen zur Epidemiologie der Equinen Babesiose in Brasilien; vet. med. Diss. Ludwig-Maximilians-Universität München

Rothschild C.M. and Knowles D.P. (2007): Equine piroplasmosis, in Sellon D.C. and Long M.T. Equine Infectious Diseases, Chapter 60, Saunders Elsevier St. Louis Missouri, 465-473.

Rüegg S.R., Torgerson P., Deplazes P. and Mathis A. (2007): Age-dependent dynamics of *Thei-leria equi* and *Babesia caballi* infections in southwest Mongolia based on IFAT and/or PCR prevalence data from domestic horses and ticks. Parasitology, 134, 939-947

Scheidemann W., Liebisch G., Liebisch A., Budde K. (2003): Equine Piroplasmose – Fallbericht einer akuten Infektion mit *Theilerai equi* (syn. *Babesia equi*) in Deutschland. Pferdeheilkunde 19 (1) (Januar/Februar), 16-20

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