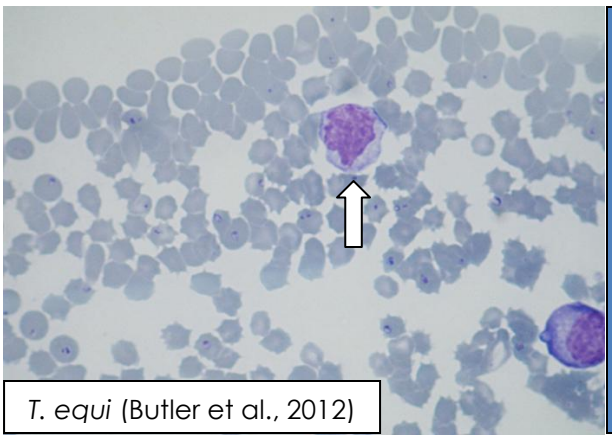
EFTBA Veterinary Newsletter 12



T. equi (Butler et al., 2012)

Equine Piroplasmosis

Babesia caballi* and *Theileria equi

June 2014

. Free trade and movement of our horses are essential
. prevention of infectious diseases is equally important
. One has to find the right balance for all these requirements
. new regulations of the OIE for ex- and import of horses in regard to Equine Piro-Plasmosis ask for good informations for this economically important disease

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

Welcome to EFTBA's veterinary newsletter

I am very honoured to introduce the Veterinary Newsletter of the EFTBA as I value this as one of the major assets of our Federation.
Dr. Hanspeter Meier of the Veterinary Committee brilliantly writes on topics that concern us all and since the EFTBA was created, there are still many common interests that oblige getting together at the European level to defend our Industry.
Today we all recognize that health, sanitary and veterinary issues have been one of the foundation pillars of our work.
A Veterinary Committee cannot function without good communication, so thank you Hanspeter for continuing to contribute with such a professional and user-friendly Newsletter.

Loïc Malivet

Loïc Malivet

Chairman, EFTBA

Editorial

Free trade and movement are most important prerequisites for the well-being and development of our industry, on one side. On the other side, the health of our horses is equally important, and here, prevention is one of the best means, e.g. restrictions for import. For the protection of both interests, one always has to find an optimal solution and to weigh carefully the pros and cons of all subjects. At our Annual General Meeting in Paris on the 10th of May (item 8, veterinary matters), we were informed that the OIE (Office International des Epizooties) adopted a new version of the chapter on Equine Piroplasmosis. According to this, export certification by vets require a statement of no outbreak within 60 days. This requirement may affect the issues as above, especially as the definition of an outbreak isn't clear yet and there also are different regulations in regard to the notifiability of this disease in different countries. It therefore seems to be worthwile to get better acquainted with this parasitic infection and its means of transmission.

Dr Hanspeter Meier

EFTBA veterinary advisor & Newsletter editor

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

Introduction and Nomenclature

If ever one wants to discuss matters, names and terms must be defined and understood properly. Normally, this is self-evident and clear, but while studying Equine Piroplasmosis (EP), at the beginning, even the usual nomenclature may be perceived confusing – but actually quite fitting for this fairly complicated parasitic disease.

In these days, one normally speaks of **Piroplasmosis**, an **accepted term** which also can be considered as official as the OIE uses it. However, the **parasites** responsible for the infection are called ***Babesia caballi*** and ***Theileria equi***, and until recently, the latter was also called *Babesia equi*. The change of name was necessary, as phylogenetic investigations showed that this parasite is closer related to the *Theilerids*. These studies even suggest that *T. equi* belongs to a distinct paraphyletic group, different from both the *Babesia* and the *Theileria* genus. Currently, both nomenclatures may be found interchangeably, because **both pathogens are piroplasms** and the term equine piroplasmosis (EP) therefore conforms with either classification (Rothschild and Knowles, 2007)

The name *Babesia* is honouring the Rumanian pathologist Victor Babes and the definition *Theileria* the Swiss/South-African veterinarian Arnold Theiler who both did pioneer work on these parasites.

The name of the disease therefore has no obvious relation with the name of the parasites themselves, but with the appearance of the intraerythrocytic replicative forms, which look pearshaped - in Latin "piriform" (or also "pyriform"). The term "piroplasm" therefore is fitting well enough.

Babes discovered piroplasms in red blood cells of bovines and Theiler studied the parasites in South African horses (Babes, 1888; Theiler 1902 & 1903; Naucke, 2008).

This introduction already sounds quite technical, but that lies very much in the nature of EP. Moreover, in the more temperate climates of Europe, EP is not well-known – yet. Therefore, this and the next newsletter may be felt somewhat detailed. My apologies for this, but all the extensive information may at least get us well prepared for probable further developments.

Historical Perspective of EP

In 1893, the Americans Smith and Killborn published the discovery of *Babesia bigemina* as the causative agent of the texas fever, a severe bovine epidemic. Two years later, Piana and Galli-Valiero found a very similar pathogen in the red blood cells of dogs and called it *Babesia canis* (Naucke, 2008).

But before 1901, Piroplasmosis was not recognized as a distinct disease of horses and was often confused with other problems (Traub-Dargatz et al. 2010). EP has been called "anthrax fever, bilious fever or bilious form of horse sickness, biliary fever, and equine malaria" (Roberts et al., 1962). EP was referred to as equine malaria because the clinical signs of the hemoparasitic infection (observed in equids in South Africa), were similar to malaria infection in humans (Theiler, 1903). Based upon morphology, the hemoparasite was classified with other pear-shaped protozoa known as piroplasms, and was named *Piroplasma equi* in 1901. Just about a decade later, Nuttall and Strickland (1912) from Cambridge discovered that EP could be caused by two different agents, *B. caballi* and *B. equi*. They wrote: *"Piroplasmosis," or "biliary fever" in horses, has hitherto been regarded as a disease due to a distinctive parasite, Piroplasma equi Laveran. We propose to show that there are two specifically distinct parasites concerned in the production of biliary fever, and that consequently two distinct diseases have hitherto been confused under this name and under the name of piroplasmosis* (Nuttall and Strickland, 1912).

In the USA, the first reported infections of *B. caballi* and *B. equi* in equids occurred in 1961 (Florida) and 1964, respectively (Strickland and Gerrish, 1964). The mode and time of introduction of *B. caballi* into the United States was reported as unknown by one author and speculated by another to be the result of the importation in 1959 of 50 Cuban Walking Horses into Davie, Florida in 1959 (Sippel et al., 1962; Knowles et al., 1966; Stiller and Coan, 1995). However, Knowles et al. (1966) assumed that EP might have gone undiagnosed for years because it was confused with EIA. Initially, the only means for diagnosis were clinical signs consistent with the disease and the detection of the parasite in erythrocytes, using specific staining techniques and confirmation through animal inoculation studies (Roberts et al., 1962; Traub-Dargatz et al., 2010).

The disease and the responsible parasites

Equine piroplasmosis (EP) is a **tick-borne protozoal disease of horses, mules, donkeys and zebras** and is characterized by **acute hemolytic anemia**.

In the past, EP was synonymous with "equine malaria, equine biliary fever, equine babesi**a**sis or babe-

si**o**sis, Oyns, horse tick fever and equine theileriosis" (Rothschild and Knowles, 2007).

The etiologic agents are the two hemoprotozoan parasites *Babesia caballi* and *Theileria equi* that are transmitted primarily by *ixodid* ticks. Equine piroplasmosis is found globally where tick vectors are present and is endemic in tropical, subtropical and some temperate regions. **Horses infected with *T. equi* remain seropositive for life; horses infected with *B. caballi* are seropositive for several years to life** (Rothschild and Knowles, 2007).

The genera *Babesia* and *Theileria* belong to the family *Piroplasmidae*, and the piroplasms are members of the phylum Apicomplexa, which also includes other pathogens as the genera *Plasmodium*, *Cryptosporidium*, and *Toxoplasma*.

Phylum: ***Alveolata***
(single-celled eukaryotes)

Underphylum: ***Apikomplexa***

Class: ***Sporozoa***

Underclass: ***Piroplasmea***

Order: ***Piroplasmida***

Family: ***Babesiidae*** Family ***Theileriidae***

Genus: ***Babesia*** Genus: ***Theileria***

B. caballi is regarded as a true *Babesia* because it exclusively replicates within erythrocytes in the vertebrate host (Fig. 1 & 3).

Currently, there is still uncertainty as to the appropriate taxonomic classification for *T. equi*. Although considered a "small *Babesia*," *T. equi* has several characteristics that distinguish it from other species within the genus, including

- apparent initial development in lymphocytes before the erythrocytic stage (as occurs with *Theileria* spp.)
- division into four merozoites within the erythrocytes (forming the "Maltese cross," Fig. 2)
- only transtadial transmission by ticks (not transovarial, Fig. 3), and
- resistance to babesicidal drugs

(Rothschild and Knowles 2007).

Etiology

According to Rothschild and Knowles (2007), *Babesia caballi* and *Theileria equi* are usually present in the same geographic regions and frequently co-infect horses. They also share many of the same tick-vectors and are transmitted by more than 15 species of the tick genera *Dermacentor*, *Hyalomma* and *Rhipicephalus*, occasionally also by *Boophilus microplus*.

Ticks serve as a reservoir for *B. caballi* because the organism persists in ticks throughout several generations, with transtadial and transovarial transmission (although not in all tick species).

In contrast, horses are the only known reservoir for *T. equi*, and only transtadial (not transovarial) transmission occurs within the tick vector. Because of their longevity and mobility, male ticks can transmit *T equi* to multiple horses. Importantly, *Boophilus microplus* may acquire and transmit *T. equi* even when feeding on inapparent carrier horses with a low level of parasitemia. Under experimental conditions, adult stages of *Hyalomma anatolicum* and *Rhipicephalus turanicus* transmit *T. equi* to horses after acquisition of the organism in nymph stages from infected horses.

Parasitemia (percentage of erythrocytes infected) typically does not exceed 1% in *B. caballi* infections and may be as low as 0.1% even in clinical cases. Maximum reported parasitemia is 10%.

T. equi parasitemia usually ranges from 1% to 7%, with a maximum of 95%. **Once infected with *T. equi*, horses remain carriers for life,** regardless of whether clinical signs resolve naturally or with drug treatment.

Infection with *B. caballi* has been said to be self-limiting, lasting up to 4 years after infection. However, many horses that recover from *B. caballi* infection later relapse, suggesting temporary non-detection of organisms with possible lifelong infection.

Carrier horses represent a potential reservoir for maintenance and dissemination of parasites to ticks and horses. Because new tick species capable of transmitting EP are being recognized and reliable control methods do not currently exist, **it is important to prevent the introduction of both infected horses and ticks into equine piroplasm-free areas**.

***T. equi* can also be transmitted iatrogenically** by contaminated needles (Hermann et al., 1987) or surgical instruments, administration of contaminated blood transfusions, or failure to properly clean and sterilize equipment that contacts equine blood (e.g., stomach tubes, dental instruments).

Chronically infected pregnant mares are at risk of transplacental transmission, resulting in abortion, stillbirth, or birth of a sick foal that typically succumbs to the disease. *T. equi* is most frequently involved in these cases. Transmission of parasites in semen has not been documented; however, this may be possible if blood contamination of semen occurs (Rothschild and Knowles 2007).

Pathogenesis

In piroplasmosis-endemic areas, most horses become infected within the first year of life. **Mortality** in native horses may range from 5% to 10%, depending on the strain of protozoa, the general health of the animals, and the availability of treatment. Mortality rates exceeding 50% may occur when previously unexposed mature horses are introduced into endemic areas. Importation of infected animals into southern France resulted in 69% mortality among untreated horses.

The incubation period varies from 12 to 19 days for *T. equi* and 10 to 30 days for *B. caballi* and coincides with the peak in fever and erythrolysis.

Alterations in erythrocyte membrane protein, lipid content and further reactions during severe parasitemia suggest that accumulation of oxidative ions alter the erythrocytes' biochemical composition, leading to hemolysis. These alterations may result in increased erythrocyte rigidity and decreased deformability, contributing to microvascular stasis. The packed cell volume (PCV) typically decreases to 20% but may fall to 10% or lower. Extreme anemia is more common in *T. equi* infections.

Hemoglobinuria of varying severity, secondary to severe hemolysis, is observed in *T. equi* infection. Icterus, here also caused by hemolysis, is the result of an increase in the unconjugated bilirubin, which is deposited in mucosal surfaces and elsewhere, imparting a yellow color to those tissues. In the case of *B. caballi*, clumping of parasitized erythrocytes can lead to microvascular occlusion. Simultaneous thrombocytopenia and the systemic inflammatory response result in endothelial damage, increased vascular permeability, and in severe cases, disseminated intravascular coagulation. Severely affected horses experience edema, hemorrhage, ischemia, and anoxia, culminating in organ dysfunction (Rothschild and Knowles, 2007).

The pregnant mare and the foal

For us breeders, special interest finds the fact that passage of infected erythrocytes across the placental barrier has been implicated as the probable mode of in utero transmission of disease and may result from damage to placental blood vessels, leading to mixing of maternal and fetal blood near or at the time of foaling. Not all foals from an infected mare are born infected, and the factors that determine whether prenatal infection occurs have not been identified yet.

Protective immunity is present as long as a horse harbors the organism, which in most cases is for life. But there is no cross-immunity between *T. equi* and *B. caballi*. The resistance to clinical disease acquired by horses after infection is a result of continued stimulation of immunity by the persisting parasite, but the exact mechanisms involved are unknown. Innate immunity likely plays a central role in control of parasites.

The spleen is important in eliminating piroplasms. Horses with intact spleens often control infection and survive, whereas splenectomized horses develop severe parasitemia and succumb to the infection. Despite its important role, the spleen and innate immunity are insufficient for protection against EP in the absence of adaptive immunity.

High antibody titers correlate with parasite control in horses. Some Immunoglobulins (IgG) correlate with control of *T. equi* during the acute stage of infection, and IgG(T) increases during chronic infection. Specific antibodies are first detected 7 to 11 days after experimental infection in ponies and reach a peak 30 to 45 days after infection. Passively transferred antibodies to *T. equi* and *B. caballi* may persist in the foal for 4 to 5 months. However, **foals born from *T. equi*–seropositive mares may be born infected without clinical disease and remain seropositive for life.** Foals from endemic areas are particularly resistant to clinical disease.

In regard to sick foals, interesting Informations can also be found in the history of breeding Poitou-mules in France. Almost a hundred years ago, Donatien et al. (1924) and Sausseau (1925) mentioned the "jaunisse, ictère, pissement de sang" or "hématurie des muletons", where the mule production payed a heavy tribute for unthinkable times. Veterinarians estimated the loss of approximately every tenth foal and a connection with EP was suspected (among many other causes). However, the findings as above (resistance of foals) do not support this hypothesis and another, a traditional assumption, referred to the intake of colostrum. Therefore, the mares were milked and instead of getting the first milk, the foals received two or three spoons of olive oil and some wine as well. Retrospectively, it is to assume that in one of ten cases this method worked well, as the reason for this hematurie most probably was neonatal isoerythrolysis (or icterus neonatorum), the incompatibility of the paternal blood groups of mare and donkey.

Despite the vigorous humoral immune response, in vivo and in vitro studies with serum of recovered animals failed to prove a full protective effect of antibodies, suggesting that humoral antibodies are insufficient for protection against EP. Considering the importance of cell-mediated immune responses to other protozoan parasites, such as *Theileria parva* and *Babesia bovis*, it is expected that cell-mediated immunity plays a key role in immunity against *T. equi* and *B. caballi* (Rothschild and Knowles, 2007).

Life Cycle of *Babesia caballi*

The life cycle of ***B. caballi*** is typical of most *Babesia* species in that **only erythrocytes are targeted** in the mammalian host. Infection is initiated by feeding of infected ticks on a naive equine host. Sporozoites immediately invade erythrocytes. Within the erythrocyte, the parasite develops from a small anaplasmoid body (trophozoites), consisting predominantly of nuclear material, into a larger ameboid sphere that divides into two large piriform bodies (merozoites) (Fig. 1).
When an uninfected tick subsequently feeds on the infected horse and ingests parasitized erythrocytes, most parasites are destroyed within the tick's midgut. However, some merozoites survive and form small, round bodies floating free within the gut contents of the tick. These spherical bodies give rise to large, clavate (club-shaped) bodies that penetrate the epithelial cells of the midgut and undergo multiple fissions. This is followed by infection of a variety of tick tissues, where a secondary cycle of multiple fissions occurs. Infected tissues in the female tick include all tissues and eggs except for the salivary glands. Small piriform bodies produced in the salivary glands of the larvae, nymphs, and adults of the next tick generation are infective to the horse if these tick forms are feeding.
The large, paired merozoites joined at the posterior ends in the equine erythrocytes are a diagnostic feature of *B. caballi* infection (see Fig. 1) (Rothschild and Knowles, 2007).

Life Cycle of *Theileria equi*

The details of the life cycle of ***T. equi* may vary depending on the tick species involved**. Infected ticks feed on horses and inject *T. equi* sporozoites into the new mammalian host with their saliva. It has been reported that **sporozoites initially are able to penetrate lymphocytes** and form large micro- and macroschizonts (schizogony). These parasite-forms ultimately give rise to approximately 200 merozoites per infected cell. Merozoites invade erythrocytes and reproduce by binary fission (merogony), forming piriform stages. In erythrocytes, asexual division gives rise to four pear-shaped stages and appearing as a "Maltese cross" form (Fig. 2). After rupture of infected erythrocytes, merozoites enter new erythrocytes and continue to replicate. Some merozoites eventually become spherical in shape, forming rings, believed to be gamonts. When a tick ingests gamonts, these grow in the tick's mid-gut, start nuclear reproduction and initiate formation of "ray bodies" (protrusions). By 4 to 6 days after ingestion, ray bodies divide and form micro- and macrogamonts, which will fuse (sexual replication), forming zygotes. Inside the zygote a kinete is formed, which will later penetrate through the midgut of the tick into the hemolymph. Kinetes are observed 5 to 7 days after tick feeding and usually last 6 to 9 days. Kinetes penetrate into salivary gland cells 7 to 8 days after attachment. In these cells, sporonts, sporoblasts, and then sporozoites are formed (sporogony). Sporozoite development is typically complete between day 6 and 24 after completion of tick feeding (Rothschild and Knowles, 2007).

Conclusions

Though making the effort to be as concise as possible with this comment on Equine Piroplasmosis, so far we only occupied ourselves with a basic historical perspective and the most important aspects of the etiology, the pathogenesis and the life cycles of the involved parasites. This fact is due to the very special and complicated nature of EP, and so far, we made reference to only some pieces of the pretty demanding puzzle. But nevertheless, it surely became obvious, that EP can be associated with significant economic losses e.g. as abortions, loss of performance, death and restrictions in meeting international requirements related to exportation or participation in sporting events. Therefore, a further newsletter will point out issues relating to epidemiology, diagnosis, therapy and prevention.

Further and binding informations are also available at the OIE, easy to find with google using the keywords "Equine Piroplasomosis" and "OIE".

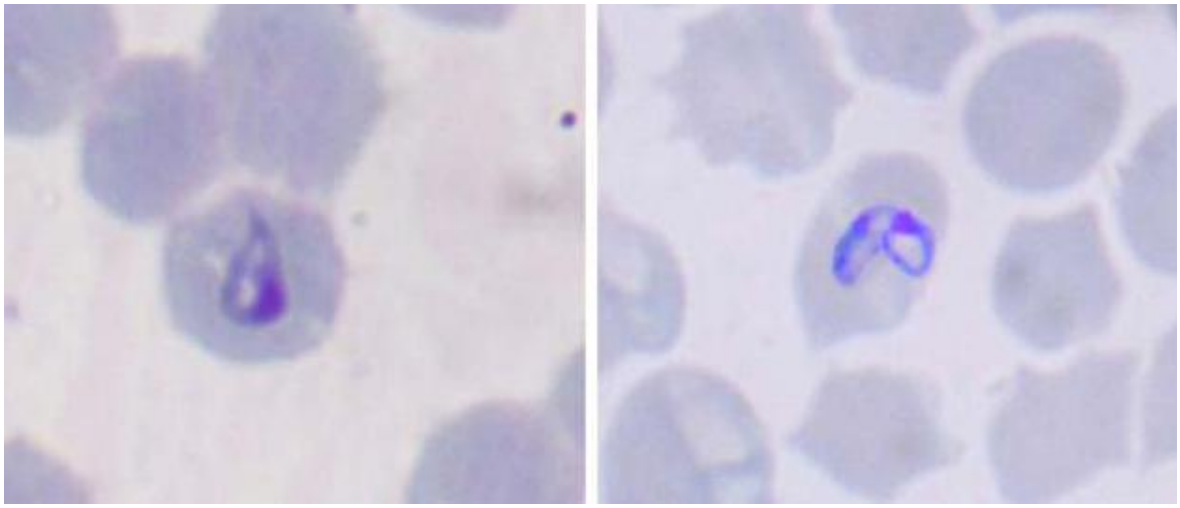


Fig. 1 *Babesia caballi* (Giemsa-stained, 1000-fold magnification)
On the left, the pear-shaped (piriform) configuration of the parasite within a red blood-cell
On the right, the large, paired merozoites - joined at the posterior ends - in the equine erythrocytes as a diagnostic feature of *B. caballi* infection

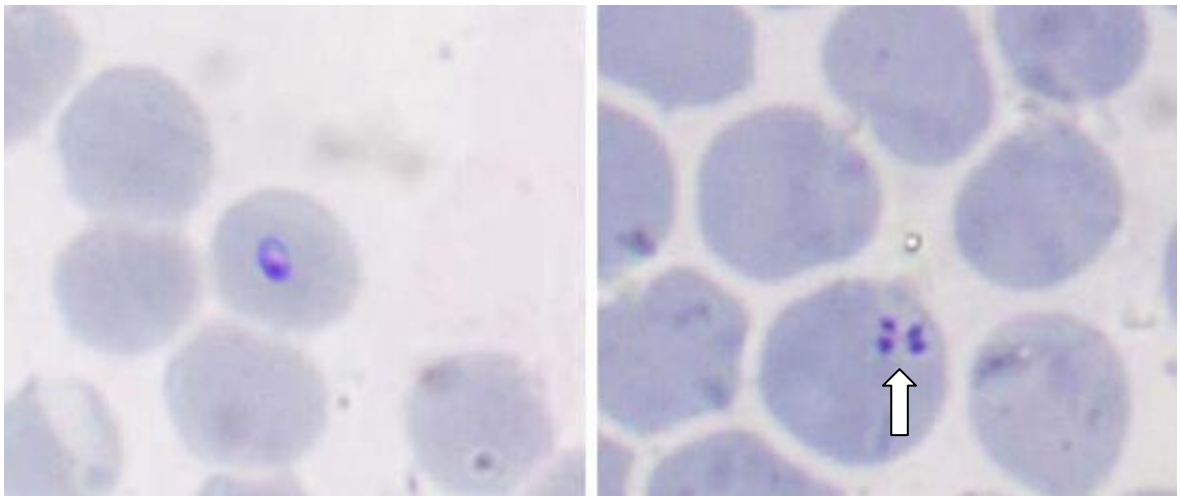
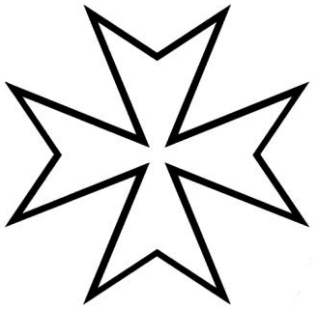


Fig. 2 *Theileria equi* (Giemsa-stained, 1000-fold magnification)
On the right, the division into four merozoites within an erythrocyte (forming the "Maltese cross")



Maltese cross

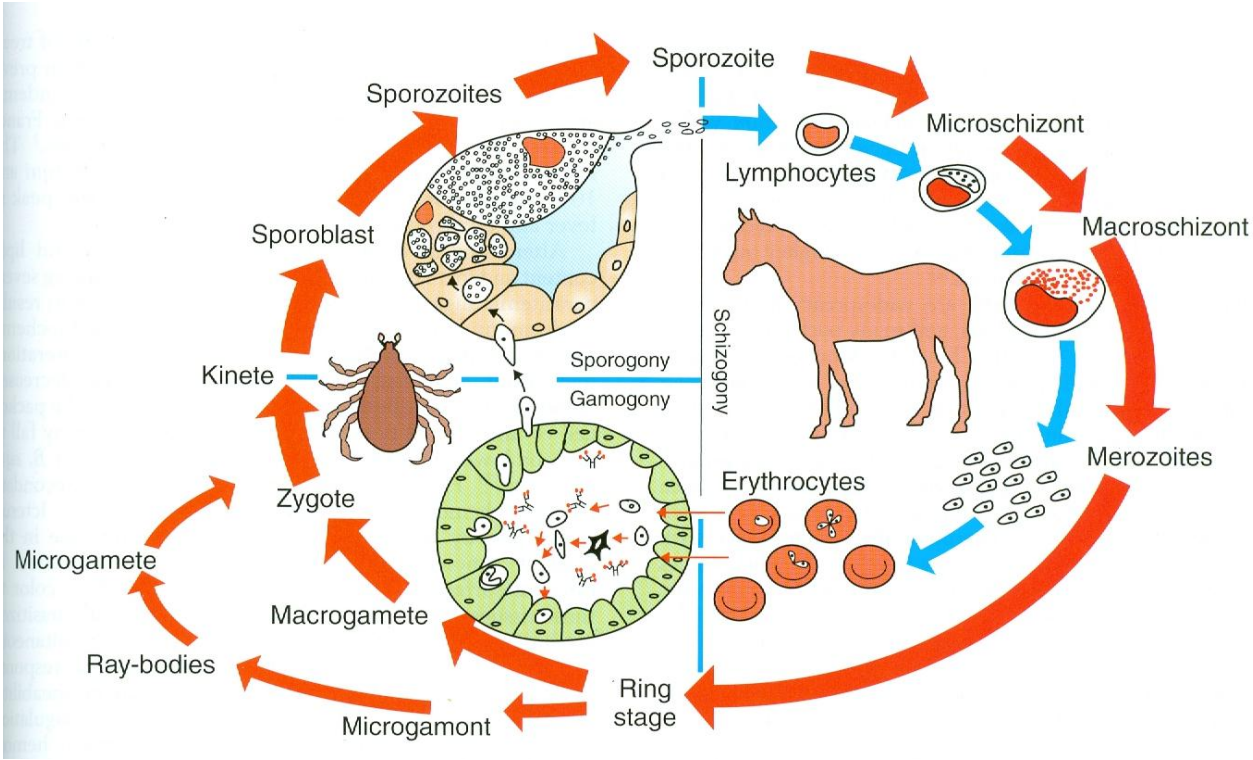


Fig. 3 Life cycle of ***Babesia caballi***.

T. equi life cycle is similar except for transovarial transmission within the tick and with the possible addition of pre-erythrocytic stage within lymphocytes (Rothschild and Knowles, 2007)

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