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Review Article

Equine Piroplasmosis: Current status

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Abstract

Equine piroplasmosis (EP) caused by apicomplexan haemoprotozoan parasites**,** *Babesia caballi* and *Theileria equi* poses a threat to intercontinental maneuver of equids. It is diffused by infected ixodid ticks, contaminated blood sources needlesand also by transplacental route. For potent chemotherapy to be effective, timely confirmation is required along with a swift test to provide an early diagnosis. Affirmation of EP in blood smear or by *in vitro* technique is an utter method of confirmation, but for this well-furnished laboratory, experienced person and more time is required. So, many different serodiagnostic tests such as the complement fixation test (CFT), indirect fluorescent antibody test (IFAT), enzyme linked immuneosorbent assay (ELISA) has been recommended for detecting the presence of antibodies to *Babesia* spp. These have several disadvantages, including low sensitivity for detecting latent infections and false-positive results. To increase sensitivity and differentiate between current and past infection ELISA may be an alternative, but its main demerit is its poor specificity and limited antigen supply. Now a day's more specific and accurate techniques are available for the direct detection of the parasites, i.e. Polymerase chain reaction (PCR) targeting different genes of DNA probes. Since there is no vaccine available for EP, thus, the control must include effective tick control, serological, molecular monitoring of equids and the application of antiprotozoal drugs.

Keywords: Babesia caballi,diagnosis, Equine Piroplasmosis, Theleria equi, Tick transmission.

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Introduction

Equine Piroplasmosis (EP), also called as babesiosis, is a notifiable disease of equines (horses, donkeys, mules, and zebras) caused by blood-borne protozoan parasites including *Babesia caballi* and *B. equi*. These apicomplexan parasites are naturally transmitted from one animal to another via mainly ticks. The infected animals remain carriers of these parasites for long periods and remain source of infection to the tick vectors. Dual infection with both the organisms has also been reported, thus resting the chances of cross immunity. Other reason for occurrence of both the infections in the same populations could be attributed to the sharing of the same vectors by both the parasites [1].Some group of researchers proposed that *B. equi* can be classified as *Theileria equi* [2], based on genomic analysis it belongs to a lineage between *Babesia* and *Theileria*,so this topic is yet under discussion. The only country made free from EP is United States as a result of a joint USDA (United state department of agriculture)-APHIS (Animal and Plant Health Inspection Services) and State of Florida eradication program for *B. caballi* that ran from 1962 to 1988 [3]. If an EP outbreak occurs then the cost of responding to the situation vary depending upon the extent of response necessary, as well as movement and testing requirements.

The geographic distributions of both the piroplasms include most of the world's tropical and subtropical regions, but *B. caballi* infections are reported more from northern part of the globe [4]. The disease conditions are endemic throughout Asia except in some countries like Siberia and Japan [1]. In regions where EP is endemic, *B. equi* infections are more prevalent than *B. caballi* infections [5]. The concurrent infection of both *B. caballi* and *B. equi* were detected in zebras from two national parks in South Africa by serological and culture methods [6]. Equine infections with *B. caballi* and *B. equi* are also prevalent in the Middle East and Asia [5], and both *B. caballi* and *B. equi* are widespread in China and Mongolia [7]. Neither *B. caballi* nor *B. equi* has become established in Australia or New Zealand [5]. Africa, Europe, Asia, and middle-east are endemic for EP [4]. The recent reports of equine piroplasmosis were received from France (endemic), United Arab Emirates (endemic, sporadic clinical cases), and the US (Florida) [8]. The US equine association reported that 231,664 horses had been tested since November 2009, of which 215 were positive either for *Theileria equi* (205) or *Babesia caballi* [10].

In early nineties, EP was not recognized as a different disease and was often confused with other diseases of equines. First recorded case of EP was in South Africa in 1901 and the disease condition was also called as biliary fever, anthrax fever, equine malaria and bilious fever or bilious form of horse sickness [9]. EP was referred to as equine malaria because the clinical signs observed in EP infected

equids in South Africa (Pretoria) were similar to malaria infection (Plasmodiidae) found in humans [10]. The first case in US of *B. caballi* was identified in a horse in 1961 in southern Florida. Though the source of infection was never definitively determined; it was assumed to be attributable to importation of horses from Cuba [11, 12].

Based upon morphology, the haemoparasite was named as *Piroplasma equi* and was first recognized in South Africa as the causative agent of disease in 1901. A few years later, it was discovered that 2 separate and distinct parasites could infect equine erythrocytes, one significantly larger than the other [13]. The larger of the parasites was termed *Piroplasma caballi*, only to be later reclassified as *B. caballi*and the smaller one was termed as *Piroplasma equi. B. caballi* is considered as a classic '*Babesia*' species, the taxonomy of *B. equi* remains controversial [2,14]. Based on finding of an extra-erythrocytic stage within equine lymphocyte, *B. equi* was reclassified as *T. equi* in 1998 [2]. Molecular phylogenetic investigations indicated that the organism possesses characteristics of both *Babesia* and *Theileria* lineages, possibly placing it between the two [15- 18]. Recent genomic analysis supports the concept of a new genus for *T. (B.) equi* [14]. Additional data are needed to determine the final placement of this parasite. In India EP outbreak was reported from Hissar (Haryana) due to imported horses [19].

The points behind redefining *B. equi* into *T. equi* are:

- *B. equi* sporozoites enter lymphocytes and initiate a schizogonic phase resulting in motile merozoites (20).
- Development of *B.equi* in the salivary glands is same as in *Theileri*a species [21, 22].
- No reproductive development of *B. equi* occur inside the organs of ticks, including the ovaries [23,24], but in genus *Babesia*, repeated kinete/sporokinete formation occurs in many cells
- The formation and shape of sexual stages of *B. equi* differs from that of typical *Babesia* species [24, 25].
- Kappmeyer *et al* [26] found a surface protein in *B. equi* (and its encoding gene) that is homologous that of *Theileria* species.
- Stages of *B. equi* inside the lymphocytes are very susceptible to halofuginone and parvaquone, which are effective against theilerial schizonts [27].

The disease is encountered more frequently in summer, especially during the rainy season. The infected cells are lighter in weight than normal cells. The method described as under may be used to concentrate the infected erythrocytes. Mix equal parts of 2% sodium citrate with the blood to be tested, then centrifuge at 500 to 700 rpm for 3 to 5 min. The supernatant fluid can then be decanted and remaining fluid and the sediment was centrifuged again at 1,500 to 2,000 rpm for 15 to 20 min, now with the centrifuged material smears were prepared and observed under microscope for piroplasmosis [28].After the initial discovery of the causative agents for EP, it was confirmed that the disease was transmitted by ticks. It was reported that the de-ticked horses were susceptible to repeat infections as they did not develop a lasting immunity to the disease agent(s) [29]. Premunity to EP in horses depends on the presence of the parasite in the body and lasts 6 months to 1 year. However, if an immune horse gets infection by another infected tick during this period, a further period of premunity is conferred, and the horses can become lifelong pre-immune carriers.

Equine piroplasmosis results from infection by the haemoprotozoa, *Babesia caballi* or *Theileria equi* (formerly *Babesia equi*) belonging to phylum Apicomplexa, order Piroplasmida. When Ramanow sky's stain (Giemsa's, Leishmann's, or Wright's stains) are used for staining, the merozoites of *B. caballi* inside the RBCs appear as pear-shaped basophilic-staining bodies approximately, 2–5 µm long and 1.3–3.0 µm in diameter [30]. The paired merozoites joined at their posterior ends are considered to be a diagnostic feature of *B. caballi* infection [31]. In case of *T. equi,* intraerythrocytic forms of merozoites are relatively small, less than 2–3 µm long [28], and are pyriform, round or ovoid in shape. A characteristic arrangement of four pear-shaped merozoites, measuring about 2 µm in length, forming a tetrad known as the 'Maltese cross' arrangement is seen in *T. equi.* They can be amoeboid, or signet-ring shaped with a clear halo shape also [32]. Equids are natural hosts of *B. caballi* and *T. equi*, and ixodid ticks are natural vectors [1, 4, 33]. Multiple ixodid tick species have been identified as either natural or experimental vectors of piroplasmosis. *Babesia caballi* is transmitted by 15 separate species (*Dermacentor* [*Anocenter*](7 species), *Hyalomma*(6 species), and *Rhiphicephalus*(2 species) and *T. equi* by 14 species (*Dermacentor*(4 species), *Hyalomma*(4 species), *Rhiphicephalus (Boophilus)*(5 species), and *Amblyomma cajennense*). Though *B. caballi* and *T. equi* parasites have been detected in dogs; however, the epidemiologic significance of these detections is unknown [34, 35].

Other related protozoa such as *Babesia bovis* (the causative agent of bovine babesiosis) have been reported rarely in horses. *T. equi* is thought to have a wider distribution than *B. caballi.* The lifecycles of *B. caballi* and *T. equi* include sporozoites (an asexual infective stage), merozoites (an asexual blood stage), and gametocytes (a sexual blood stage). *Babesiacaballi* is transmitted transtadially and transovarially by its tick vectors [36] but *T. equi* is generally transmitted through intrastadial transmission [37]. Transovarial transmission of *T. equi* occurs, but its precise role in epidemiology has not been known in details [38, 39]. Differences in these parasites, replication cycles can affect their methods of transmission. Inside the tick, *Babesia* zygotes multiply as 'vermicules,' which invade many of the tick's organs including the ovaries, and *Babesia* species are readily passed to the next generation of ticks in the egg (transovarial transmission). When an infected larval, nymphal or adult tick of the next generation attaches to a new host, the parasite is stimulated to undergo its final maturation, thus infecting the host.

In contrast, *Theileria* zygotes do not multiply in the ovary of tick and transovarial transmission of *T. equi* is uncertain or absent, it has been shown that sporozoites inoculated into horses via a tick bite invade the lymphocytes [19]. The sporozoites undergo development in the cytoplasm of these lymphocytes and eventually form *Theileria*-like schizonts. Merozoites released from these schizonts enter RBCs. Ticks that transmit this organism can become infected as larvae and transmit the infection as nymphs, or they can become infected as nymphs and transmit the infection as adults.

Like *B. caballi*, *T. equi* parasites are only stimulated to complete their maturation after the tick attaches to feed. For this reason, a tick infected with either organism must remain attached to the host for a time before it becomes infective. Thus often, *B. caballi* and *T. equi* are transmitted after the tick has been attached for a few days. EP can also be transmitted by iatrogenic transmission directly between animals by contaminated needles and syringes, or by blood transfusions from untested, infected donor horses. Sharing equipment, such as dental or tattoo equipment, those may be contaminated with blood may also act as potential pathway for transmission. After recovery, horses may become carriers for long periods. Animals infected with *B. caballi* can remain carriers for up to 4 years, but might be able to clear the organism eventually. Equids infected with *T. equi* appear to remain permanently infected. Transplacental transmission of *B. caballi* has rarely been reported, and some sources consider the evidence for this route to be unreliable. The incubation period for equine piroplasmosis is 12 to 19 days when it is caused by *T. equi,* and 10 to 30 days when it is caused by *B. caballi* [28, 31].

Equine piroplasmosis causes systemic inflammatory response syndrome, subsequent multi organ system dysfunction, and hypercoagulability [40]. In *B. caballi*due to clumping of infected red blood cells (RBC) microthrombi formation in small vessels, resulting to venous stasis and vasculitis [41, 42]. Prolonged clotting times and thrombocytopenia have been reported during EP [43]. Causes of these alterations may include immune-mediated destruction, splenic sequestration and/or excess consumption. Piroplasmic form causes lysis of RBC resulting in varying degrees of hemolytic anemia. Erthrophagocytic destruction of RBC from the circulation by macrophages enhances the chances of anemia. In *T. equi* infection the biochemical structure of erythrocyte membranes changes. This may cause decreased deformability of the red cells, resulting in reduction of micro vascular blood flow. Moreover there is an accumulation of oxidative ions which lead to destruction of RBC [44].Placental transmission of *T. equi* from infected carrier mares to their fetuses has been reported [16, 45- 47]. It causes abortion (most commonly in late gestation), stillbirth, or neonatal infection. The influence of prevalence causing placental transmission can be affected by geographical or genetic isolate/strain of equines [48]. Blood contamination during breeding practices could also present a transmission risk for EP [49]. In most cases, the horses become lifelong carriers due to immune evasion strategies of parasite. In asymptomatic horses it has been reported that parasite were present including bone marrow, capillaries, and central nervous system vasculature [50-52]. Some studied showed that, horses infected with *B. caballi* can undergo self-clearance of the parasite without treatment [53, 54].

Both the infections result in a variety of clinical signs. The incubation period ranges between 12 to 19 days and 10 to 30 days, in case of *T.* equi and *B. caballi*, respectively [1]. Thus, the clinical disease can manifest in different forms. It may be per-acute, acute, sub-acute, or chronic [55]. In per acute disease condition, there is abrupt onset of signs, which lead to

collapse and sudden death. In *T. equi* infection, due to hemolysis there is anemia. *B. caballi* infected horses do become anemic as well, but death mainly occurs due to multiple organ dysfunction, which is related to systemic intravascular coagulation [40]. Hemolytic anemia results in icteric or pale mucous membranes, tachycardia, tachypnea, weakness and pigmenturia [44, 56]. In acute infection, equines initially develop signs such as high fevers [104°F], weight loss, peripheral edema, lethargyand anorexia [50]. Petechial hemorrhages caused by thrombocytopenia are mainly observed on mucous membranes, including the nictitating membrane [54]. Some equines show signs of gastrointestinal complications such as colic or impactions followed by diarrhea. Other sings include secondary development of cardiac arrhythmias, catarrhal enteritis, laminitis, pneumonia, pulmonary edema, and central nervous system disease characterized by ataxia, seizures and myalgia [11,54, 56, 57]. Hemoglobin- induced pigment nephropathy and systemic responses to severe inflammation (hypotension) result in acute renal failure [54].

Abortions or neonatal infections can occur in pregnant carrier mares [16, 56]. Acute, severe signs develop in neonatal foals infected *in-utero* with *T. equi* [27, 45, 46, 58-61].These foals can exhibit clinical signs at birth or can become ill at 2–3 days of age. Clinical signs in foals are nonspecific, such as weakness and decreased suckling, but progress to resemble as those of an infected adult, including icterus, fever, and anemia. Chronic infections lead to liver failure or disseminated intravascular coagulation [39]. In Chronic EP infection can also result in lethargy, anorexia, weight loss, and poor performance, mild anemia and enlarged spleen which are nonspecific signs. Splenic enlargement is caused by the increased rate of extravascular hemolysis that occurs within the spleen [33, 43, 54].

In profound anemia, the level of packed cell volumes [PCV] is as low as 10%, but seldom falls below 20%. Thrombocytopenia is also commonly identified (32, 53, 54). Depending on chronicity of the disease, hydration status, and associated conditions the fibrinogen concentration can be elevated and albumin concentration can also vary. Hyperbilirubinemia is often observed and the liver enzyme activities, ALP, AST, and GGT can be elevated because of reduced blood flow to the liver [55]. Hypophosphatemia and hypoferremia are common, due to altered erythrocytic metabolism [62]. Gross examination might demonstrate evidence of anemia as well as varying degrees of icterus, splenomegaly, edema, pulmonary edema, congestion, cardiac hemorrhages, hydro-pericardium, hydrothorax, hepatomegaly, ascites, enlarged discolored kidneys, and lymphadenopathy [54]. Examination of pulmonary tissue can demonstrate edema, congestion, and hemosiderin-laden macrophages within the pulmonary alveolar walls. Histopathologic findings include renal tubular necrosis with hemoglobin casts, centrilobular necrosis of the liver and micro thrombi within the liver and lungs [56].

There is no cross immunity between *B. caballi* and *T. equi*as horses can be infected with both parasites simultaneously [11,50]. Immune response is unable to eliminate *T. equi*, and infected horses are reported to remain infected for life [19, 6]. Infections with *B. caballi* are self-limiting, lasting up to 4 years after infection [54]. Splenectomized equines invariably succumb to disease (80% parasitemia) but equines with a spleen are typically able to overcome acute *T. equi*-induced disease [44, 63,64]. *B. caballi* inoculated splenectomized equines may or may not develop disease, but death caused by infection has been documented [65]. Nitric oxide (NO) production by macrophages is an essential effector mechanism of immune control against*T. equi* infection.*T. equi* infected horses produce antibodies against immunodominant merozoite proteins termed as equine merozoite antigens (EMAs), which are present on surface of merozoites [66]. Recently the nomenclature of equine immunoglobulin G has been adjusted to accommodate the 7 unique IgG heavy chain genes discovered in the equine genome [67]. In the acute stages of *T. equi* infection, high IgG1 and IgG4 and 7 correlate with control, but in chronic phase of infection IgG5 and to a lesser extent IgG3 increase after resolution of parasitemia [68]. Donkeys vaccinated with *T. equi* immunogen were able to mount a protective response characterized by high anti-merozoite antibody titers and merozoite-specific lymphocyte proliferation [69]. Again in *B. caballi* infection less is known about protective immune responses. Equine produce antibodies to rhoptry associated protein-1 (RAP-1), this remains partially uncharacterized in *B. caballi* infections, but in infections due to *B. bovis* plays a pivotal role in induction of humoral immunity [70].

Future directions

Important areas of future research in babesiosis include improving diagnostic tests, development and trials new babesial agents and critically assessing the taxonomic relationships of the piroplasms [71]. Development and standardization of serologic and molecular diagnostic tests is particularly important for the diagnosis of subclinical, chronically infected animals. Development of an accurate and rapid ELISA would allow more samples to be tested. Molecular, antigenic, morphological and biological characteristics of the parasites need to be considered. On the basis of the recent emergence of tick-borne babesiosis, the development of a vaccine against equine babesiosis is the need of the hour.

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