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ORIGINAL RESEARCH

Clinical and Laboratory Findings in Equine Piroplasmosis

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ABSTRACT

The objective of this study was to evaluate equine piroplasmosis (EP) as a cause of morbidity in horses in Sardinia (Italy), describe the clinical signs and altered hematologic and biochemical parameters, and illustrate response to different treatments. Among 44 horses suspected of tick-borne disease, 38 were polymerase chain reaction (PCR) positive for *Theileria equi* (n = 27) or *Babesia caballi* (n = 6), whereas five were positive for both protozoans. Typical clinical features of piroplasmosis were seen in some of the horses, whereas others had nonspecific mild symptoms. Hematologic findings revealed involvement of the three blood cell lineages (anemia, leukopenia or leukocytosis, thrombocytopenia), and biochemical variations were related to increased bilirubin, alteration of serum phosphorus, and hypoalbuminemia. We suggest that the two protozoans are the most important causative agents of equine tick-borne disease in this geographic area, and we observe that different clinical features are associated with the disease; in addition to the typical aspects of piroplasmosis, characterized by fever, pale mucous membranes, and icterus, we can signal other nonspecific mild signs such as weight loss, weight loss associated with an insignificant leukopenia, or weight loss associated with depression, anorexia, and mild hyperbilirubin. The study is intended as a practical contribution for veterinary practitioners because it describes different clinical presentations and laboratory findings of EP, suggests diagnostic and therapeutic approaches to the disease, and shows diffusion of the disease in a Mediterranean region.

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INTRODUCTION

Equine piroplasmosis (EP) is one of the most important tick-borne diseases, with an economic worldwide impact on the horse industry. The disease is caused by *Theileria equi* and *Babesia caballi*, two hemoprotozoan parasites of red blood cells, which belong to the phylum *Apicomplexa*, order *Piroplasmida*.[1] Approximately 14 species of Ixodid ticks of the genera *Dermacentor*, *Rhipicephalus,* and *Hyalomma* are able to transmit both parasites.[2] Recently it has been hypothesized that *Haemaphysalis longicornis* may play a role in the transmission of *T. equi*.[3]

The clinical signs of piroplasmosis are variable and often nonspecific.[4] In rare hyperacute cases, animals may be found dead or dying. More often, piroplasmosis presents as an acute infection with fever usually exceeding 40°C, depression, inappetence, pale mucous membranes or icterus, dyspnea, and increased respiratory and heart rates. Additional features that may be seen include sweating, congested mucous membranes, petechial hemorrhages or ecchymoses on the conjunctiva, colic, edema of the distal limbs around the head and eyelids, and incoordination. Massive intravascular destruction of parasitized erythrocytes could result in hemoglobinuria. The subacute symptoms are less severe but may resemble the symptoms of acute form. Chronic infections typically result in variable clinical presentations involving mild inappetence with weight loss, transient fever, poor exercise tolerance, weakness, mild anemia, subicterus, and enlarged spleen.[5]

The most frequent hematologic alterations are reduction of the number of red blood cells, platelet counts, and hemoglobin concentration.[2,6] Acute infections are also characterized by neutropenia and lymphopenia[7]; furthermore, decreased plasma fibrinogen, serum iron, phosphorus, and elevated bilirubin concentrations have been reported.[8]

Especially in endemic areas, the infection may assume a subclinical course and such animals could become carriers of the protozoans.[4] It would seem that horses infected with *B. caballi* might spontaneously clear the organism after 12 to 42 months, whereas spontaneous clearance of *T. equi* organisms does not appear to occur.[9] In carrier horses, which

may act as a reservoir of infection, a delicate equilibrium between the parasite and the immune mechanisms of the host is present. Parasites are in very low numbers in the blood and generally may not be detected in Giemsa-stained blood smears.[10] A breakdown in homeostasis resulting in clinical babesiosis may develop after immunosuppressive events or after strenuous exercise.[11] As a result of the increased movement of horses worldwide, animals that are low-level carriers represent a risk of introduction of these parasites into disease-free areas.

EP is endemic in many parts of Europe, Africa, Asia, and America where suitable tick vectors are present. In parts of southern Europe, infections caused by *T. equi* are more frequent than infections caused by *B. caballi*.[4] In Italy, *T. equi* is the most frequent. Both parasites are widespread throughout the southern part of the country, from Latium to Sicily, including Sardinia.[12]

Piroplasmosis may be the most frequent tick-borne disease of horses in Sardinia: (1) The existing ecosystems are excellent for the survival of many tick genera and in particular *Rhipicephalus*, *Dermacentor,* and *Hyalomma*, which are known vectors of piroplasmosis and are well represented in the island, contrary to the genus *Ixodes*, vector of *Anaplasma phagocytophilum* (*Ehrlichia equi*), which is found only sporadically.[13-15] (2) Recently, Alberti et al[16] diagnosed only three cases of *A. phagocytophilum* among 20 horses with typical symptoms, and the other 17 horses could well be affected by other pathogens, such as piroplasms. (3) The elevated seroprevalence found by Scala et al[17] and Pinna Parpaglia et al[18] and the high number of polymerase chain reaction (PCR) positives for piroplasms in asymptomatic horses found by Pinna Parpaglia et al[18] are contrary to the low seroprevalence and negative PCR for *A. phagocytophilum* found by Pinna Parpaglia (unpublished data). The last author has described a wide circulation of *T. equi* and *B. caballi*, showing the highest percentages (82.8%) of seroprevalence (by immunofluorescence assay test [IFAT]) against *T. equi* among Italian regions.[18] These reports illustrate the presence of piroplasmosis in asymptomatic horses,[17,18] whereas studies about its incidence as a cause of clinical disease in Sardinia are lacking. Every year, in particular during the warm season, many veterinarians working in Sardinia find in horses clinical forms compatible with tick-borne diseases that cause important economic losses.

In the current study, the authors evaluate EP as a cause of morbidity in Sardinia, describe clinical signs, alteration of hematologic and biochemical parameters, and illustrate the response to different treatments as a practical contribution for veterinary practitioners.

MATERIALS AND METHODS

Between June 2003 and February 2007, 44 horses from various areas of Northern Sardinia (Italy) were examined because of a suspicion of tick-borne disease. There were 17 males and 27 females of various breeds (with a predominance of Thoroughbred and Anglo-Arab), between 1 month and 18 years of age (4 foals and 40 adults). The animals were raised and lived in various habitats (riding schools, hippodromes, farms, in box or paddock) and were racehorses or breeders. A clinical examination, including specific PCR studies for *Theileria equi* and *Babesia caballi,* and blood smears were performed on every horse, and hematologic and biochemical analyses were performed on some samples. Two different therapeutic protocols were used.

PCR

For DNA extraction, blood samples were collected into sterile tubes containing K_3 ethylenediaminetetra-acetic acid (EDTA) and stored at –20°C until used. Blood was washed repeatedly by adding phosphate-buffered saline and centrifuging at 11,000*g* for 5 minutes at 4°C until a white pellet was obtained. The pellet was resuspended in 500 µL DNA extraction buffer (10 mM Tris-HCl pH 8, 2 mM EDTA, 0.1% sodium dodecyl sulfate, 500 µg proteinase K/mL) and incubated at 40°C overnight. After extraction with phenol and chloroform according to Sambrook et al,[19] the DNA was precipitated with 0.1 volume of absolute ethanol and 2 volumes of 3M CH3COONa (sodium acetate) then centrifuged at 15,000*g* for 60 minutes at 4°C. The pellet was washed with cold 70% ethanol. The ethanol was removed by centrifugation and the DNA was dissolved in 100 µl: Tris-EDTA. The DNA concentration was determined photometrically at 260 nm and stored at –20°C until used.

Two PCRs specific for *T. equi* and *B. caballi* were performed as previously described by Bashiruddin et al.[4] The primers specific for *T. equi* (BEQF, forward primer: catcgttgcggcttggttgg; BEQR, reverse primer: ccaagtctcacaccctattt) and *B. caballi* (BCAF, forward primer: ttcgcttcgctttttgtttttact; BCAR, reverse primer: gtccctctaagaagcaaacccaa), that amplify DNA targets of 664 and 659 bp, were chosen from areas of sequence variability of 16S rRNA, designed not to react with each other or with the other piroplasms. The final 50-µL PCR mixture contained 150 ng DNA, 1 µM forward primer, 1 µM reverse primer, 0.2 mM deoxyribonucleotide triphosphate mixture and HotMaster *Taq* polymerase with its buffer (Eppendorf) using the manufacturer's recommendations. For *T. equi*, amplification conditions were: 5 minutes at 94°C, 31 cycles each of 94°C/30 seconds, 58°C/30 seconds, 68°C/40 seconds. For *B. caballi*, the amplification was altered to 30 cycles each of 94°C/30 seconds, 55°C/30 seconds, 68°C/40 seconds. The final extension period was 5 minutes at 68°C. Reactions were performed in an automated DNA thermal cycler and PCR products were submitted to electrophoresis on 1.5% agarose gels to