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Original Research

Seroprevalence and Molecular Analysis of *Babesia caballi* and *Theileria equi* in Horses From Central Italy During a 10-Year Period

Micaela Sgorbini [a,*], Francesca Bonelli [a], Simona Nardoni [b], Guido Rocchigiani [b], Michele Corazza [a], Francesca Mancianti [b]

[a] Department of Veterinary Sciences, Veterinary Teaching Hospital, University of Pisa, Pisa, Italy
[b] Department of Veterinary Sciences, University of Pisa, Pisa, Italy

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ABSTRACT

The aim of the present article was to retrospectively analyze serologic data about *Babesia caballi* and *Theileria equi* infection in horses from Central Italy in a 10-year period. In addition, part of sera samples was examined by polymerase chain reaction (PCR) to evaluate the concordance between the two tests. A total of 1,441 serum specimens were examined. Each sample was examined by immune fluorescent antibody test (IFAT) for both *B. caballi* and *T. equi*, starting from 1/80 dilution. During 2012, blood samples (n, 117) were also checked by PCR. Differences in the prevalence values between *T. equi* and *B. caballi* were evaluated by means of chi square. Agreement between IFAT and PCR results was calculated by Cohen kappa. Five hundred of 1,441 horses tested seropositive for piroplasms. Differences in prevalence values between *T. equi* and *B. caballi* were statistically significant ($P < .001$). Fifty-six of 117 sera examined in 2012, scored positive to at least one test; IFAT results showed 38 of 117 sera positive to *T. equi*, whereas PCR results scored positive for 48 of 117. *B. caballi* was detected neither by IFAT nor PCR. Cohen kappa index was 0.599 indicating a moderate agreement. Prevalence values observed are consistent with data available from literature in which an extremely wide range is reported. The low and occasional occurrence of antibodies against *B. caballi* is in line with some studies and has been confirmed by PCR. The agreement between IFAT and PCR found is moderate allowing us to recommend the utility of performing both tests to achieve a correct diagnosis.

1. Introduction

Babesia are tick-transmitted protozoan hemoparasites of economic, veterinary, and medical impact worldwide [1]. They are considered to be the second most commonly found parasites in the blood of mammals after trypanosomes. In their vertebrate hosts, they reproduce asexually inside erythrocytes, and together with *Theileria* spp., they are referred to as piroplasms or piroplasmids. The sexual phase of such parasites' life cycle typically takes place in Ixodid ticks, which acquire and transmit the parasites during their blood meals [2].

There is currently only one recognized *Babesia*, *Babesia caballi*, and a *Theileria*, *Theileria equi*, species that cause equine piroplasmosis. They are both transmitted by *Hyalomma*, *Dermacentor*, and *Rhipicephalus* tick species [3].

It has been estimated that only 10% of horses around the world are raised in piroplasmosis-free regions, while the disease is endemic in vast tropical and subtropical regions [1]. The disease has been reported in horses throughout Europe [4].

* Corresponding author at: Micaela Sgorbini, Department of Veterinary Sciences, Veterinary Teaching Hospital, Via Livornese snc, San Piero a Grado, PI 56122 Italy.
E-mail address: micaela.sgorbini@unipi.it (M. Sgorbini).

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In general, such infections showed various degrees of severity that can often be associated to the host's age, immunologic status, concurrent infections with other pathogens, and/or genetic factors. The clinical manifestations of equine piroplasmosis include fever, icterus, anemia, hemoglobinuria, bilirubinuria, and, occasionally, death [5,6] even if disease in endemic areas frequently is subclinical and animals may recover and become long-term carriers [6,7]. Disease due to infection with *B. caballi* usually is less severe than infection with *T. equi*, but the causative agents cannot be differentiated on the basis of clinical signs alone [4].

Because of the threat posed to the pure-breed and racehorse industry, testing for equine piroplasmosis is a mandatory requirement for the international movement of horses to prevent the introduction of *B. caballi* and *T. equi* into disease-free areas [8].

Diagnosis of equine piroplasmosis can be performed by direct and indirect methods. Direct diagnosis includes demonstration of intraerythrocytic forms in Giemsa-stained blood or organ smears, or by molecular techniques [9]. Indirect methods currently are the prescribed methods to carry out large-scale epidemiologic studies and to evaluate herd prevalence in an area.

The aim of the present article was to retrospectively analyze serologic data about *B. caballi* and *T. equi* infection in horses from Central Italy in a 10-year period. In addition, part of samples was examined by polymerase chain reaction (PCR) to evaluate the concordance between the two tests.

2. Materials and Methods

A total of 1,441 equine serum specimens were examined between January 2002 and December 2012. Samples were sent refrigerated to the Department of Veterinary Sciences of Pisa by practitioners. Inclusion criteria for the present survey were (1) horses living in Central Italy; (2) apparently healthy subjects with no symptoms of piroplasmosis or poor performance; and (3) samples collected for serologic surveillance and not for diagnostic and therapeutic purposes.

Horses were of various breed and aged 3 to 18 years; 395 of 1441 males (27.4%), 453 of 1,441 females (31.4%), and 593 of 1,441 geldings (41.2%). Management practices (feeding or housing) were not determined.

Each sample was examined by immune fluorescent antibody test (IFAT) for both *B. caballi* and *T. equi*, using multiwell slides (Fuller Labarotories, Fullerton CA). Sera were twofold diluted, starting from 1/80, following the manufacturer's instructions as described by Moretti et al [5].

During 2012, blood samples (n = 117) were also sent by veterinarians in EDTA Vacutainer tubes, and the whole blood specimens were stored at −20°C until used for molecular analysis (PCR). DNA was extracted using the QIAamp DNA blood minikit (Qiagen) as described in the manufacturer's protocol.

Piroplasms were detected using the primers Cab-R and Equi-R in combination with the forward primer Bec-UF2 to amplify fragments of the 18S rRNA gene of 392 and 540 bp specific for *T. equi* and *B. caballi*, respectively. The reaction was carried out as described by Alhassan et al [10].

Table 1
Seroprevalence per year of IFAT-positive horses for *Theileria equi*, *Babesia caballi*, and both.

Parasite Species	Year of Sampling, n (%)										
	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
T. equi	5/13 (38.5)	5/32 (15.6)	17/100 (17)	53/112 (47.3)	117/349 (33.5)	118/307 (38.4)	73/199 (36.7)	9/110 (8.2)	14/57 (24.5)	6/45 (13.3)	38/117 (32.5)
B. caballi	0	0	11 (11)	4/112 (3.6)	6/349 (1.7)	3/307 (0.9)	1/199 (0.5)	2/110 (1.8)	0	0	0
Mixed infections	0	0	1 (1)	0	5/349 (1.4)	3/307 (0.9)	0	0	0	0	0
Total	5/13 (38.5)	5/32 (15.6)	29/100 (29)	57/112 (50.9)	128/349 (36.7)	124/307 (40.3)	74/199 (37.2)	11/110 (10)	14/57 (24.6)	6/45 (13.3)	38/117 (32.5)

Abbreviation: IFAT, immune fluorescent antibody test.