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Original Research

Tick-Borne Infections in Horses From Tuscany, Italy

Valentina Virginia Ebani*, Simona Nardoni, Fabrizio Bertelloni, Guido Rocchigiani, Francesca Mancianti

Department of Veterinary Science, University of Pisa, Pisa, Italy

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ABSTRACT

To estimate the prevalence of tick-borne pathogens in horses living in areas of Tuscany (Italy) with high risk of arthropod exposure, blood samples from 160 clinically healthy grazing horses were tested by polymerase chain reaction (PCR) for *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato (s.l.), *Coxiella burnetii*, *Babesia caballi*, and *Theileria equi*. Tick-borne infections were detected in 70 (43.75%) horses, in particular 43 animals (26.87%) resulted positive for *T. equi*, 41 (25.62%) for *A. phagocytophilum*, six (3.75%) for *C. burnetii*, and five (3.12%) for *B. burgdorferi* s.l. All horses scored negative for *B. caballi*. Twenty-five animals were coinfected: four horses (2.5%) were positive for *A. phagocytophilum* and *B. burgdorferi* s.l., four (2.5%) for *C. burnetii* and *T. equi*, and 17 (10.62%) for *A. phagocytophilum* and *T. equi*. Our results show the spread of tick-borne agents in equine population, including those able to infect humans. Detection of *C. burnetii* is of critical relevance because it is a severe zoonotic agent, that may be transmitted not only by tick bites, but also through feces, urine, and birth products of infected animals.

1. Introduction

Exposure to tick bites is considered to be a very high risk in horses, because of their frequent outdoor activity in rural and hilly or mountain areas. Equine tick-borne diseases are well known by veterinarians, because even if they often remain asymptomatic, in some cases, they can cause severe clinical forms as well.

Equine granulocytic anaplasmosis (EGA) is caused by *Anaplasma phagocytophilum*, an intracellular bacterium which infects granulocytes, particularly neutrophils, mainly transmitted by *Ixodes ricinus*. The disease is characterized by a wide range of clinical signs including lethargy, fever, limb edema, ataxia, petechiation, and thrombocytopenia [1]. Equine granulocytic anaplasmosis has been described for the first time in California and later in other regions of the United States, South America, and Europe. In Italy, the disease has been reported in ill horses, and serologic surveys have found prevalence values ranging from 9% to 17% [2–6].

Borrelia burgdorferi sensu lato (s.l.), a spirochetes mainly transmitted by ticks belonging to *Ixodes* genus, is the agent of Lyme borreliosis. The predominant clinical signs observed in horses include sporadic lameness, laminitis, swollen joints, muscle tenderness, and weight loss. Other manifestations, such as hepatitis, panuveitis, depression, facial paralysis, and encephalitis [7–9] have been reported. Seroprevalence values range from 6% up to 48% depending on the European country. In Italy, data about the spread of borreliosis in horses are scant; however, previous studies have detected 24.3% seroprevalence in Tuscany [10], 15.3% in a restricted area of Lazio [11], and 7% in an area including Umbria, Marche, and Lazio [6].

Babesia are tick-transmitted protozoan hemoparasites, of economic, veterinary, and medical impact worldwide [12]. They are considered to be the second most commonly

* Corresponding author at: Valentina Virginia Ebani, Department of Veterinary Science, University of Pisa, Viale delle Piagge, 2, 56124 Pisa, Italy.
E-mail address: valentina.virginia.ebani@unipi.it (V.V. Ebani).

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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. Currently, there is only one recognized *Babesia*, *Babesia caballi*, and a *Theileria*, *Theileria equi* species that causes equine piroplasmosis. They are both transmitted by *Hyalomma*, *Dermacentor*, and *Rhipicephalus* tick species [13].

It has been estimated that only 10% of horses around the world are raised in piroplasmosis-free regions, whereas the disease is endemic in vast tropical and subtropical regions [12].

In general, such infections course with varying degrees of severity, which can often be associated with 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 [14]. Although babesiosis and/or theileriosis in equids can be acute, subacute, or chronic [15,16], disease in endemic areas is frequently subclinical. Animals may clinically recover from the disease and become long-term carriers [17]. Disease due to infection with *B. caballi* is usually less severe than infection with *T. equi*; however, the causative agents cannot be differentiated on the basis of clinical signs alone [18].

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, control measures, including serologic testing, have been implemented to prevent the introduction of *B. caballi* and *T. equi* into disease-free areas [19]. An extensive study dealing with equine piroplasms was carried out in northern and central Italy, indicating wide differences in prevalences among different examined areas [20], but to the best of our knowledge, Tuscany region has not recently investigated.

Coxiella burnetii may be considered an emerging tick-borne pathogen. It is an obligate intracellular bacterium which causes Q fever, a worldwide zoonosis transmitted by ticks of several species, even if the infection is usually acquired through inhalation of contaminated aerosol or ingestion of contaminated food, mainly raw milk and dairy products. *Coxiella burnetii* infects a wide range of animal species, including wild and domestic mammals and birds. Infected animals may excrete coxiellae in feces, urine, and vaginal discharge [21,22].

Serologic evidence of *C. burnetii* infection in horses has been previously reported, but the role of these animals as reservoirs is not clear. *Coxiella burnetii* is considered an important cause of abortion and infertility in ruminants [23]; moreover, equine genital disorders caused by this bacterium are reported by some authors [24].

As far as we know, the occurrence of these pathogens in horses from Tuscany (Italy) has never been investigated by molecular methods. Therefore, the aim of the present study was to evaluate the prevalence of the zoonotic agents *A. phagocytophilum*, *B. burgdorferi* s.l., and *C. burnetii*, as well as the prevalence of the equine pathogens *T. equi* and *B. caballi* in an area densely populated with the ticks.

2. Materials and Methods

2.1. Samples

Blood samples were collected from 160 grazing horses living in hilly areas of Central Italy. Animals were randomly chosen in various farms and horse centers. Owners reported past and/or recent ticks' exposure, but no signs of illness nor recent antibiotic or antiparasitic treatment.

Samples were drawn from the jugular vein in sterile tubes with EDTA and maintained at 4°C until DNA extraction, which was performed with the DNeasy Tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions.

DNA was stored at 4°C until used as template for polymerase chain reaction (PCR) assays.

2.2. Polymerase Chain Reaction

2.2.1. *Anaplasma phagocytophilum*

A primary amplification was carried out to amplify a 932-bp fragment of the 16S ribosmomal RNA (rRNA) gene of *A. phagocytophilum*, using the primers GE 3a and GE 10r. A nested PCR, with the primers GE 9f and GE 2, amplified a 546-bp fragment of the same gene; primary and secondary amplifications were performed with the same cycling conditions [25].

2.2.2. *Borrelia burgdorferi* sensu lato

Primers JS1 and JS2 were used to amplify a 261-bp fragment of the 23S rRNA gene of *B. burgdorferi* s.l. [26].

2.2.3. *Coxiella burnetii*

Coxiella burnetii was identified by amplifying a 687-bp fragment of the IS1111a gene using primers Trans-1 and Trans-2 as described by Berri et al [22].

2.2.4. *Babesia caballi and Theileria equi*

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 [27].

All primers sequences and PCR conditions are listed in Table 1.

Polymerase chain reaction amplifications were performed using the EconoTaq PLUS 2X Master Mix (Lucigen Corporation, Middleton, WI) and an automated thermal cycler (Gene-Amp PCR System 2700; Perkin Elmer, Norwalk, CT).

Polymerase chain reaction products were analyzed by electrophoresis on 1.5% agarose gel at 100 V for 45 minutes; gel was stained with ethidium bromide and observed. GelPilot 100 bp Plus Ladder (Qiagen) and SharpMass 100 Plus Ladder (Euroclone, Milano, Italy) were used as DNA markers.

Standard precautions were taken to avoid contamination of samples and reaction mixture, including strict separation of the areas for reagent preparation, DNA extraction, and amplification. Sterile distilled water instead of DNA was included as negative control to ensure the