Ticks and Tick-borne Diseases 7 (2016) 462–469

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Babesia caballi and *Theileria equi* infections in horses in Central-Southern Italy: Sero-molecular survey and associated risk factors

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ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form 12 January 2016
Accepted 19 January 2016
Available online 21 January 2016

Keywords:
Theileria equi
Babesia caballi
Italy
Horse
sero-molecular prevalence
Risk-factor

ABSTRACT

Babesia caballi and *Theileria equi* are tick-borne pathogens, etiological agents of equine piroplasmosis that affect different species of Equidae causing relevantly important direct and indirect losses.

A field study was conducted to evaluate the distribution of the equine piroplasms in an area of Central-Southern Italy and to identify correlated risk factors. Serum samples of 673 asymptomatic horses were collected during spring-summer of 2013 to estimate the seroprevalence of the parasites within the study area using *T. equi* and *B. caballi* Antibody test kit (VMRD®, Inc, Pullman, WA, USA). The 273 seropositive samples were subsequently tested by real time PCR to verify the presence of the genome of the piroplasms, indicative of the carrier status of the subjects. The variables chosen to identify which were the risk factors associated with the serological and PCR-positivity for each of the equine piroplasms were the following: gender, age, breed, access to pasture, altitude, land cover, climatic zone, soil type and province location (coastal/inland).

The resulting overall seroprevalence for *T. equi* was 39.8% (268/673) and for *B. caballi* was 8.9% (60/673) while 70.3% of the PCR tested samples (185/263) were positive for *T. equi* and 10.3% (27/263) for *B. caballi*. The univariate and multiple logistic regression models were used to assess the association of the risk factors with the different outcomes. The risk factors found to be associated with *T. equi* seropositivity were gender, age, breed, access to pasture, land cover, soil type and province location, while those associated with PCR-positivity were age, soil type and province location. As the number of *B. caballi* seropositive subjects was limited, the multiple logistic regression model was performed only for the PCR-positive status, identifying climatic zone and soil type as the sole risk factors. In the study area, a major diffusion of *T. equi*, in terms of seroprevalence and PCR-positivity was present when compared to that of *B. caballi*, probably related to the cumulative effect of the life-long infection of the former protozoan. The identification of risk factors relative to each piroplasm infection, specific to a study area, is important in the development and improvement of tailored control and prevention programmes aimed at containing health and economic consequences.

1. Introduction

Equine piroplasmosis (EP) is a disease caused by two species of intra-erythrocytic protozoa, namely *Babesia caballi* and *Theileria equi* that affects horses, mules, donkeys and zebras. Both parasites are transmitted by ticks of genera *Dermacentor*, *Hyalomma*, and *Rhipicephalus* (Scoles and Ueti, 2015). EP is endemic in tropical and temperate areas and occurs in acute, sub acute and chronic forms. Typical clinical signs of EP are fever, depression, anaemia, icterus, oedema, anorexia and, occasionally, mucosal petechiae and ecchymoses. Horses surviving the acute phase may remain seropositive, inapparent carriers with low levels of parasitaemia, condition that occurs more frequently in *T. equi* infections (De Waal, 1992). While disease due to *B. caballi* is reported as less severe than that induced

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http://dx.doi.org/10.1016/j.ttbdis.2016.01.011
1877-959X/

by *T. equi*, clinical signs are common to both protozoa (De Waal, 1992). For this, differential diagnosis on clinical basis is unreliable and is therefore performed using laboratory methods represented by stained blood smears, serological tests, such as complement fixation test, indirect fluorescent antibody test (IFAT), ELISA, and PCR methods (Sumbria et al., 2015). EP is a major constraint to the international movement of horses causing important economical losses to the horse industry (Friedhoff et al., 1990). Prevalence studies conducted in other areas of Italy reported different levels of seropositivity when using IFAT, ranging from 0.3% (Grandi et al., 2011) to 56.0% (Moretti et al., 2010) for *B. caballi* and from 8.2% (Grandi et al., 2011) to 50.5% (Moretti et al., 2010) for *T. equi*. Using PCR, different levels of positivity were also described, ranging from 0% (Grandi et al., 2011) to 6.0% (Laus et al., 2013) for *B. caballi* and from 11.7% (Laus et al., 2013) to 33.0% (Grandi et al., 2011) for *T. equi*. The aims of this paper were to determine the prevalence of both parasites, serologically and using PCR assays in asymptomatic horses of Central-Southern Italy and to identify the associated risk factors, not yet investigated for the specific area and species.

2. Materials and methods

2.1. Study area and sampling method

This study involved the horse population of an area of Central-Southern Italy as represented in Fig. 1. Sample size was defined on an expected prevalence of 50% of an infinite population, a confidence interval of 95% (95% CI) and an absolute accuracy of 5% that resulted in 384 samples. Although other studies report higher equine piroplasmosis prevalence levels (Moretti et al., 2010), the sample size definition criteria were selected to maximise the accuracy of the prevalence estimation. Qualified veterinarians randomly collected blood samples, with and without EDTA, during spring-summer 2013 from the long-term resident horse population of the study area. The serum was obtained by centrifugation for 10 min at 358 g and stored at −20 °C while, uncoagulated blood was stored at −80 °C. All operations on the horses were performed with the owner's consent and according to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

To identify equine piroplasm related risk factors, data on the following variables were registered at blood collection: gender (gelding; male; female); age (young ≤ 6 years; adult between 7 and 12 years; senior > 12 years); breed (foreign breed; Italian breed; mixed breed); access to pasture (yes/no) province location of stable (coastal/inland). Furthermore, using the Global Positioning System, the geographic location of the animals included in the study was established allowing other variables to be evaluated. These were altitude (≤150 meters above sea level (m asl); 151–600 m asl; > 600 m asl); land cover (> 75% forest; crops 50–75%; 50–75% forest; mixed, with no dominant land cover); climatic zone, based on length of growing period (LGP) which is number of days during a

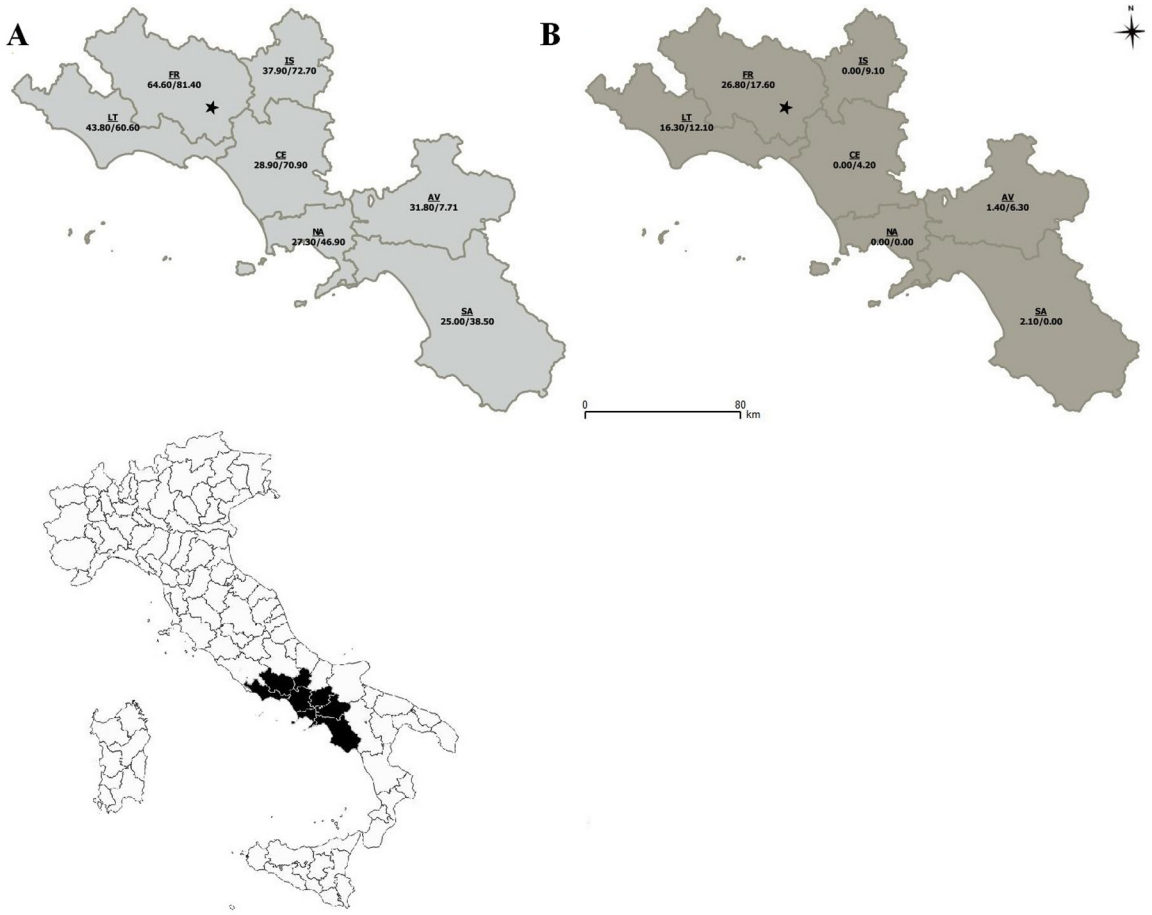

Fig. 1. Serological and PCR-positivity prevalences for *T. equi* (A) and *B. caballi* (B) for each province investigated. First number is the serological prevalence, second number is the PCR-positivity prevalence. FR = Frosinone and LT = Latina belong to Latium Region; IS = Isernia to Molise Region; AV = Avellino; CE = Caserta; NA = Naples and SA = Salerno to Campania Region. CE, LT, NA, SA are coastal provinces, the others are inland. Province location in Italy is shown at bottom left. ⋆ in figure represents location of Aurunci Mountains.