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Seroprevalence and risk factors associated with *Babesia caballi* and *Theileria equi* infection in equids

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ABSTRACT

A cross-sectional study was carried out on equids (horses, mules and donkeys) in Andalusia, Southern Spain, to assess the level of exposure to equine piroplasmosis and to investigate risk factors associated with these infections. At least one animal seropositive for *Theileria equi* and/or *Babesia caballi* was detected in 222/380 (58.4%) herds sampled by competitive inhibition ELISAs. The seroprevalences for *B. caballi* and *T. equi* were 13.2% and 56.1%, respectively; there was serological evidence of co-circulation of both piroplasms in 10.8% of herds. Antibodies against equine piroplasms were detected in 286/537 (53.3%) animals; 61 (11.4%) were seropositive for *B. caballi*, 270 (50.3%) were seropositive for *T. equi* and 24 (8.4%) were seropositive for both *T. equi* and *B. caballi*.

There was a significantly higher seroprevalence of *B. caballi* in mules (32.1%) compared with donkeys (17.0%) and horses (7.9%), and a significantly higher seroprevalence of *T. equi* in mules (66.1%) in comparison with horses (48.6%), but not donkeys (47.2%). There were significant differences in prevalence of both piroplasms among locations; the seroprevalence of *B. caballi* ranged from 0 to 22.5%, while the seropositivity to *T. equi* ranged from 26.7 to 63.3%. A multiple logistic regression model indicated that the risk factors associated with a higher *T. equi* seroprevalence were increased age, presence of ticks and vaccination against other diseases. Risk factors associated with a higher seroprevalence of *B. caballi* were species (mules compared to horses), entry of horses in the last 6 months, presence of ticks and presence of shelter. The findings indicate widespread exposure to equine piroplasmosis in Southern Spain.

Introduction

Equine piroplasmosis is a tick-borne disease caused by the obligatory intraerythrocytic protozoa *Babesia caballi* and *Theileria equi* (Mehlhorn and Schein, 1998). Both haemoprotozoan parasites are mainly transmitted by ticks of the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus* (de Waal, 1992). The distribution of piroplasmosis depends on the presence of vectors and the disease is endemic in tropical and subtropical regions (Brüning, 1996). The international movement of horses has led to the spread of equine piroplasmosis from endemic to non-endemic areas; control measures, including serological testing, have been implemented to prevent the introduction of *B. caballi* and *T. equi* into disease-free areas (Friedhoff et al., 1990).

The clinical manifestations of equine piroplasmosis include fever, icterus, anaemia, haemoglobinuria, bilirubinuria and, occasionally, death (Knowles, 1996). Although babesiosis/theileriosis in equids can be acute, subacute or chronic (Rampersad et al., 2003; Uilenberg, 2006), disease in endemic areas frequently is subclinical and animals may recover from the disease and become long term carriers (de Waal and Van Heerden, 1994). 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.

Diagnosis of equine piroplasmosis can be performed by direct and indirect methods (OIE, 2008). Direct diagnosis includes demonstration of intraerythrocytic forms in Giemsa stained blood or organ smears, or by molecular techniques (Nagore et al., 2004; Criado et al., 2006; Alhassan et al., 2007; Adaszek and Winiarczyk, 2008). Indirect methods currently are the prescribed methods to carry out large scale epidemiological studies and to evaluate herd prevalence (number of positive herds = herds with at least one positive animal/total number of sampled herds) in an area. Serological methods include the complement fixation test (CFT), indirect

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fluorescent antibody test (IFAT), immunochromatographic tests, indirect enzyme-linked immunosorbent assay (iELISA) and competitive ELISA (cELISA) (Hirata et al., 2002; Asgarali et al., 2007; Acici et al., 2008; Sigg et al., 2010; Mujica et al., 2011; Seo et al., 2011). The cELISA is the test of choice and is recommended by the World Organization for Animal Health (OIE, 2008). This serological test distinguishes between *T. equi* and *B. caballi* and has been shown to have higher specificity than CFT, IFAT or iELISA (Knowles et al., 1992; Brüning et al., 1997; Shkap et al., 1998; Kappmeyer et al., 1999; Katz et al., 2000; Xuan et al., 2002).

Equine piroplasmosis is endemic in many regions of Asia, America, Africa and Europe. In Europe, the disease has been reported in horses in the UK, France, Germany, Switzerland, Italy, Greece, the Czech Republic, Hungary, Poland, Portugal and Turkey (Joyner et al., 1981; Leblong et al., 2005; Karatepe et al., 2009; Fritz, 2010; Kouam et al., 2010; Moretti et al., 2010; Sigg et al., 2010; Adaszek et al., 2011; Grandi et al., 2011). Equine piroplasmosis has been reported previously in Spain (Habela et al., 2000; Camacho et al., 2005; Criado et al., 2006; Barrera et al., 2010), but epidemiological information on *T. equi* and *B. caballi* is limited and no studies on equine piroplasmosis in Spanish donkeys or mules have been conducted. The aims of the present study were to analyse the seroprevalence of *T. equi* and *B. caballi* in equids (horses, mules and donkeys) in Southern Spain and to identify risk factors associated with these infections.

Materials and methods

Study design

A cross-sectional survey was designed to analyse the seroprevalence of *T. equi* and *B. caballi* in equid herds from Andalusia, Southern Spain (36°N–38°60′N, 1°75′W–7°25′W). Andalusia has the highest number of equids in Spain; in the last census, there were more than 202,000 horses, around 13,200 donkeys and 18,500 mules in Andalusia out of 425,000 equids in Spain (CAP, 2011).

Based on the number of herds in Andalusia ($n \geqslant 10{,}000$), an estimated prevalence of 50%, a desired precision of ±5% and a confidence level of 95%, the aim was to sample 385 herds. A total of 380 herds were finally selected and the sampling was stratified by provinces according to the proportion of horses in each province. The herds in each province were selected by simple random sampling from the census of herds obtained from the Regional Government of Andalusia (CAP, 2011). The geographical distribution of the sampled herds in each of the eight provinces that constitute Andalusia is shown in Fig. 1. The size of the sampled herds ranged from 1 to 194 (median 13; mean 22.5). Within each herd, 1–3 animals were randomly selected. A total of 428 blood samples were collected from horses in 309 herds across eight provinces. In addition, blood samples were collected in Cádiz province from 56 mules in 35 herds and 53 donkeys in 36 herds. Permits for collection of sera were approved by the Regional Government of Andalusia (10 December

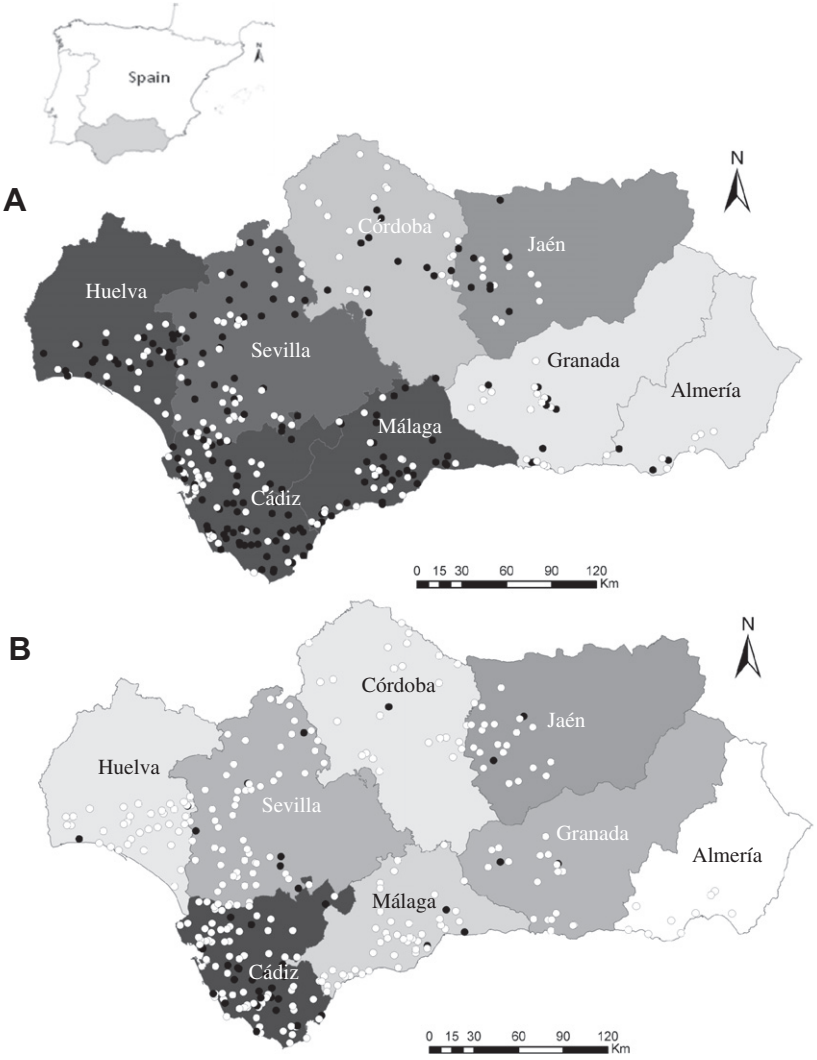

Fig. 1. Map of Andalusia (South Spain) showing the location of equids sampled. Black and white dots indicate seropositive and seronegative animals, respectively. The darker grey gradient represents the seroprevalence against to *T. equi* (A) and *B. caballi* (B) in the different regions sampled.