Veterinary Parasitology 169 (2010) 273–278

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Seroprevalence of equine piroplasms and host-related factors associated with infection in Greece

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ARTICLE INFO

Article history:
Received 22 October 2009
Received in revised form 21 December 2009
Accepted 5 January 2010

Keywords:
Equine piroplasms
Theileria equi
Babesia caballi
Tick-borne disease
cELISA
Relative risk

ABSTRACT

Serum samples were collected from a total of 544 equids that included 524 horses, 13 mules, and 7 ponies from various regions of mainland Greece and were examined by competitive-inhibition ELISA (cELISA) to evaluate the level of exposure of Greek equids to *Theileria* (*Babesia*) *equi* and/or *Babesia caballi*, the causative agents of piroplasmosis. Association between seropositivity and host-related factors of species, gender, age, origin, activity and location were investigated. The overall seroprevalence was 11.6% (9.1–14.6%) with 95% confidence limit. The seroprevalence for *T. equi* and *B. caballi* was found to be 11% (8.6–14%) and 2.2% (1.2–3.9%), respectively. The animal-related factors significantly linked with seropositivity were the species, activities of farming, racing, recreation, and geographic location in Attica, Macedonia, Peloponnese and Thessaly region ($p < 0.05$). The relative risks for the presence of *T. equi*, *B. caballi* and mixed infection in mules compared to horses was 8.39, 33.58 and 40.31, respectively. The infection level for *T. equi*, *B. caballi* and mixed infection were significantly higher in farm equids than in racing equids ($p < 0.05$). Also, the rate of infection of *T. equi* was higher in farm equids than recreational equids ($p < 0.05$). The relative risk of *T. equi* infection between farming equids and equids used only for recreation activity was 3.25–1, while the relative risk of *B. caballi* infection was 0.14–1 for racing animals relative to recreation animals. The region with the highest level of infection to both parasites was Thessaly (38.8% *T. equi* and 6.1% B. *caballi*), followed by Peloponnese (10.4% *T. equi* and 3.9% *B. caballi*), Attica region (8.3% *T. equi* and 0.6% *B. caballi*) and finally Macedonia the region with the lowest prevalence (6.6% *T. equi* and 4.4% *B. caballi*). A higher seroprevalence rate was found among local animals compared to imported equids, indicating that equine piroplasm infection is enzootic in Greece. *T. equi* seroprevalence was significantly different and higher among increasing age groups of equids, suggesting persistent infections or lower transmission levels whereby animals may need to be exposed longer before acquiring the infection. Competent tick vectors *Rhipicephalus bursa* and *Rhipicephalus sanguineus* for the transmission of equine piroplasmosis were recovered from horses and dogs, respectively.

1. Introduction

Equine piroplasmosis (EP) is a tick-borne disease of equids, caused by two species of apicomplexan protozoa,

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doi:10.1016/j.vetpar.2010.01.011

Theileria (syn. *Babesia*) *equi* and *Babesia caballi*. *Babesia canis canis* of dogs has been reported in horses (Criado-Fornelio et al., 2003) but no clinical signs attributable to this parasite have been documented in equids. *T. equi* and *B. caballi* are intra-erythrocytic parasites that occur in most tropical, subtropical and temperate areas of the world and may be found together when a common tick vector is present (Mehlhorn and Schein, 1998). EP is endemic in many parts of Asia, Arabia, South and Central America, Africa and Europe. Within Europe, it is more prevalent in France (Leblong et al., 2005), Portugal (Bachiruddin et al., 1999), Spain (Camacho et al., 2005), Italy (Moretti et al., 2009) and Turkey (Karatepe et al., 2009). Ixodid ticks of the genera *Dermacentor, Rhipicephalus*, *Hyalomma, Boophilus* and *Haemaphysalis* transmit *T. equi* and *B. caballi*, which can result in infections characterized by fever, anaemia, icterus, haemoglobinuria, bilirubinuria and sometimes, death. In addition, intrauterine infections with *T. equi* may result in abortion and neonatal death (Potgieter et al., 1992). In some cases of acute or chronic disease, mortality can reach up to 50% (de Waal, 1992). Clinical signs of the infection are not pathognomonic, especially in endemic areas. Infected animals that recover from acute or primary infection of *T. equi* remain life-long carriers, whereas horses infected with *B. caballi* may remain carriers for up to 4 years (de Waal and Van Heerden, 1994). Carrier hosts maintain the life cycle of the parasites by serving as a source of transmission for ticks.

Traditionally, piroplasms are detected and identified by microscopic examination of thin blood smears collected from acutely infected animals. Several serological assays such as the complement fixation test (CFT), the indirect fluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA) as well as the competitive-inhibition ELISA (cELISA) have been developed mostly for large scale studies and to monitor infections during the latent stage characterized by microscopically undetectable parasitemia (Brüning et al., 1997; Shkap et al., 1998; Ikadai et al., 2000). The cELISA is currently the test of choice recommended by the World Organization for Animal Health (OIE, 2008). Direct detection methods using molecular tools have recently been developed and are considered reliable (Caccio et al., 2000; Nagore et al., 2004).

While reliable estimates of the numbers of cases of equine piroplasmosis and the related economic losses are not readily available, reports of disease and deaths at equestrian centres or stud farms are not uncommon when piroplasm-free adult horses are introduced into enzootic areas (Kuttler, 1988). The importance of EP lies in the constraints on travel of horses and its effect on the horse racing industry. EP is also a list B disease of the OIE, notifiable within 72 h of diagnosis. Only seronegative horses for both *T. equi* and *B. caballi* are qualified for importation to the United States, Canada, Australia and Japan (Friedhorff et al., 1990). Since testing of horses for EP is mandatory for the international movement of horses either for participation in international events or for export, the disease is important to Greece which has one of the largest equestrian centres in southern Europe, and moreover, hosted the 2004 equestrian Olympic Games.

Despite the importance of equine piroplasmosis, the literature on this disease in Greece is limited, with only one report of finding *B. caballi* and *T. equi* sporozoites in *Rhipicephalus sanguineus* and *Hyalomma plumbeum*, respectively (Haralabidis, 2001). Therefore, a cross-sectional, serological survey was conducted to evaluate the level of exposure of equids to piroplasms in various regions of Greece in terms of seroprevalence and the risk factors associated with the infections.

2. Materials and methods

2.1. Animals and sampled areas

Blood samples were collected at random from clinically healthy equids by venipuncture into sterile, anticoagulant-free tubes after obtaining the agreement of their owners in various regions of mainland Greece during 2007–2008. At the time of blood collection, the equids and the associated farm dogs and also the stray dogs wandering close to the equids were inspected for the presence of ticks which were collected for subsequent identification (Papadopoulos et al., 1996; Walker et al., 2000; Pavlidou et al., 2008). Data on the characteristics of the sampled animals (species, gender, age, origin, activity, location) were collected through questionnaires completed by the investigators on location during sample collection.

2.2. Sera preparation and cElisa

Sera were obtained from clotted blood samples by centrifugation and stored at $-20\,^{\circ}C$ until used. Serum samples were tested for the presence of antibodies to *T. equi* and *B. caballi* using a commercial cElisa test kit (VMRD Inc., Pullman, WA, USA) according to the manufacturer's instructions. The assay detects serum antibodies against EMA-1 surface protein on merozoites of *T. equi* (Knowles et al., 1992), and rhoptry-associated protein (RAP-1) of *B. caballi* (Kappmeyer et al., 1999). Samples associated with percent inhibition values $\geq$40 were considered positives. The sensitivity of *T. equi* and *B. caballi* cELISA is higher than that of the complement fixation text (CF) whereas the specificity of *T. equi* and *B. caballi* cELISA is 99.2–99.5% (OIE, 2008). The optical density values were obtained using an automatic plate reader (Infinite M200, Tecan).

2.3. Statistical analysis

The seroprevalence relative to the various characteristics (animal species, gender, age, origin, activity, and location) was calculated with an associated 95% confidence interval. Differences in prevalence between these various groups were assessed by the two-sided Chi-square and the Fischer's exact test. A p value of <0.05 was considered significant. The relative risk (RR) of the presence of infection was computed for the characteristics found significant. All the parameters were computed using Epi Info software (version 3.5.1). Each time an expected value in the Chi-square test was less than 5 as indicated by Epi-info software in an rxc contingency table, SISA-tables program was used to calculate the exact test p value.