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Original article

Seroepidemiological survey of *Theileria equi* and *Babesia caballi* in horses from a rural and from urban areas of Paraná State, southern Brazil

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ABSTRACT

The objective of this study was to evaluate the seroepidemiological data of *Babesia caballi* and *Theileria equi* in horses from a rural settlement and carthorses from urban areas of Paraná State, southern Brazil. A total of 198 horses, including 32 from the rural settlement and 166 carthorses from Colombo ($n = 48$), Pinhais ($n = 76$), Londrina ($n = 24$), and Curitiba city ($n = 18$) was sampled and tested using a commercial competitive inhibition ELISA (cELISA) test. Out of the 198 horses, 193 (97.5%) were seropositive for at least one piroplasm species. Antibodies to *T. equi* were detected in 155/198 horses (78.3%), antibodies to *B. caballi* were detected in 137/198 horses (69.2%), and antibodies to both were detected in 99/198 (50.0%) horses. Horses living in the rural settlement and Colombo were more likely to be seropositive to *T. equi* than those in Curitiba ($p < 0.05$). Horses older than 5 years were more likely to be seropositive for *T. equi* than those younger than 5 years ($p < 0.05$). No significant association was found between gender or the presence of ticks and seropositivity to *T. equi* ($p > 0.05$). In conclusion, the high seroprevalences to *B. caballi* and *T. equi* observed in this study emphasize that active surveillance programs are critical for monitoring animal health status, particularly because carthorses may act as urban disseminators of these piroplasms.

Introduction

Equine piroplasmosis (EP) caused by *Theileria equi* and *Babesia caballi* is an important tick-borne disease that affects equids worldwide and significantly impacts the equine industry (Friedhoff et al., 1990; Kerber et al., 1999; Schnittger et al., 2012; Short et al., 2012). The severity of clinical signs varies and may include fever, weight loss, anemia, hemoglobinuria, and jaundice in susceptible horses (Schein, 1998). Horses infected with *B. caballi* may spontaneously clear the organism, whereas this does not occur with *T. equi* (De Waal and Van Heerden, 1994). In endemic areas, horses with subclinical infection or those that recover from a primary infection may become life-long carriers of the organism (De Waal, 1992); therefore, they may introduce these organisms into disease-free areas. Alternatively, carrier horses may allow these parasites to adapt and develop in new hosts because of changes in distribution, population dynamics of ticks, and vertebrate host contact (Chauvin et al., 2009).

In Brazil, *B. caballi* is primarily transmitted by *Anocentor nitens* ticks (Roby and Anthony, 1963), whereas *T. equi* is transmitted by *Rhipicephalus* (*Boophilus*) *microplus* (Battsetseg et al., 2002). In addition, some evidence suggests that *Amblyomma cajennense* ticks are associated with *T. equi* infection in Brazil (Kerber et al., 2009). Serological surveys for *T. equi* and *B. caballi* have been conducted in horses from different countries with seroprevalence data of 21.6% and 54.1% in Israel (Steinman et al., 2012), 45.2% and 27.4% in Mexico (Cantú-Martínez et al., 2012), 8.2% and 0.3% in Italy (Grandi et al., 2011), and 50.3% and 70.6% in Venezuela, respectively (Mujica et al., 2011). However, the seroprevalence data of EP in Brazil remain scarce and are limited to a few regions of the country. Therefore, according to the recommendations adopted by the World Organization for Animal Health (OIE, 2012) to identify EP

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carriers, a commercial competitive enzyme-linked immunosorbent assay (cELISA) was used in this study to determine the seroepidemiological data of *T. equi* and *B. caballi* and possible associations with the presence of ticks and the age and gender of horses from a rural settlement and carthorses from urban areas of Paraná State, southern Brazil.

Materials and methods

This study was approved by the Ethics Committee in Animal Experimentation and Animal Welfare at the Universidade Estadual de Londrina (number 34/2011) and Universidade Federal do Paraná (number 027/2010), Paraná State, Brazil.

Study design

The study was performed in 5 cities located in 2 regions (northern and south-central) of Paraná State, southern Brazil (Fig. 1).

In the northern region, a rural settlement situated in Alvorada do Sul county (22°54′34.4″ S 51°13′49.1″ W) and an urban area located in the city of Londrina (23°08′47″ S 51°19′11″ W) were used. Both regions have a subtropical climate, with rainfall throughout the year; however, rain is more prevalent during the summer months. The average temperature is 25 °C (INMET, 2012). The rural settlement is subdivided into 60 homesteads with an area of approximately 12 hectares each, totaling 786 hectares. The area also comprises 20% of a native forest with a diverse fauna. In the selected urban area, the carthorses live, rest, and graze next to an abandoned square, which is covered by a mixed overgrowth pasture consisting of grass and undesired plants, such as bushes and scrubs. In both regions, a large number of ticks are found throughout the year. Dogs, horses, and humans share the same environment and are potentially exposed to common ticks.

In the south-central region, the following 3 urban areas were studied: Curitiba (25°25′47″ S 49°16′19″ O), Colombo (25°17′31″ S, 49°13′26″ W), and Pinhais (25°26′41″ S 49°11′33″ W). These cities are included in an extension project entitled 'CartHorses', which consists of educational activities for the prevention and control of zoonotic diseases. These horses are used to pull carts and move daily throughout the cities with their owners to collect recycling materials.

The sampling period was chosen based on the seasonal dynamics of adult *Amblyomma* ticks (Toledo et al., 2008). Therefore, the samples were collected during the beginning of the spring and the end of the summer, with the exception of samples from Curitiba City, which were collected in the autumn.

Samples

A total of 198 blood samples was collected from apparently healthy horses of different breeds, ages, and gender. In the northern region, 32 horses from a rural settlement and 24 carthorses from Londrina were sampled. In the south-central region, 142 horses from urban areas were sampled as follows: 48 from Colombo, 76 from Pinhais, and 18 from Curitiba. During sampling, the owners responded to an epidemiological questionnaire addressing the horse breed, age, gender, and presence of ticks.

Blood samples (10 mL) were collected by venipuncture of the jugular vein using sterile vacuum tubes containing serum separator gel (BD Vacutainer®, Franklin Lakes, NJ, USA) and stored at room temperature (25 °C) until visible clot retraction. The samples were then centrifuged at 1500 × *g* for 5 min, and the serum was separated and stored at −20 °C until serological analysis.

Detection of antibodies against Theileria equi and Babesia caballi

All 198 horse serum samples were tested for *T. equi* and *B. caballi* using the commercial cELISA (VRMD Inc®, Pullman, WA, USA). The tests use the *T. equi* recombinant merozoite antigen (EMA-1) and the recombinant *B. caballi* rhoptry-associated protein 1 (RAP-1) as antigens, respectively. The optical density (OD) was measured using a wavelength of 630 nm, and samples with a percentage of inhibition (% *I*) ≥40% were considered positive. The % *I* was calculated as follow: % *I* = 100 [1 − sample OD/negative control OD)]. All procedures were performed according to the manufacturer's instructions.

Statistical analysis

Either the Chi-square or Fisher's exact test was used for the independent variables to determine the association between the individual factors with seropositivity to *T. equi* and *B. caballi*. The odds ratio (OR), 95% confidence interval, and *p* values were calculated separately for each variable. The results were considered significantly different when $p < 0.05$. The data were compiled and analyzed using the Epi Info™ Software (version 3.5.3).

Results

Of the 198 sampled horses, 108 were males and 90 females. The breeds included Appaloosa (1), Arabian (1), Crioulo (8), Mangalarga (13), Quarter Horse (2), Thoroughbred (2), and mixed breed (171) horses. The age of the horses was stratified into groups <5 years, 5–10 years, and >10 years.

A total of 193/198 (97.5%; 95% CI: 94.2–99.2%) horses was seropositive for at least one EP agent. Antibodies to *T. equi* and *B. caballi* were detected in 155/198 (78.3%; 95% CI: 71.9–83.8%) and 137/198 (69.2%; 95% CI: 62.3–75.5%) horses, respectively. Ninety-nine of 198 (50.0%; 95% CI: 42.8–57.2%) horses were seropositive for both hemoparasites. The seroprevalence in the rural settlement, in Londrina, Colombo, Pinhais, and Curitiba City for *T. equi* was 27/32 (84%; 95% CI: 67.2–94.7%), 22/24 (92%; 95% CI: 73.0–99.0%), 42/48 (87.5%; 95% CI: 74.8–95.3%), 53/76 (70%; 95% CI: 58.1–79.8), and 11/18 (61%; 95% CI: 35.7–82.7%), respectively. Antibodies against *B. caballi* were detected in 17/32 (53%; 95% CI: 34.7–70.9%) horses from the rural settlement, 21/24 (87.5%; 95% CI: 67.6–97.3%) from Londrina, 32/48 (67%; 95% CI: 51.6–79.6%) from Colombo, 53/76 (70%; 95%CI: 58.1–79.8%) from Pinhais, and 14/18 (78%; 95% CI: 52.4–93.6%) from Curitiba. Sixty-two of 198 (31.3%; 95% CI; 24.9–38.3%) horses showed the presence of ticks. In addition, horses living in Londrina showed the highest percentage of tick infestation (100%) compared to Curitiba (27.8%) ($p < 0.005$).

For the *T. equi*-seropositive horses, those living in Londrina and Colombo were 7 (95% CI: 1.24–39.49; $p = 0.0221$) and 4.45 times (95% CI: 1.24–15.97; $p = 0.0232$) more likely to be serologically positive for *T. equi*, respectively, than horses living in Curitiba City. Horses >10 years and those 5–10 years old were associated with seropositivity to *T. equi* ($p = 0.0014$). No significant association was found between gender ($p = 0.0970$) or the presence of ticks ($p = 0.8749$) and *T. equi* antibodies. Additionally, horses >10 years old ($p = 0.0118$) and the presence of ticks ($p = 0.0428$) were associated with seropositivity to *B. caballi*. The seroprevalence of *T. equi* and *B. caballi* in horses within each variable evaluated are summarized in Table 1.

Discussion

Serological data for EP have typically focused on horse populations involved in equestrian sports (Cunha et al., 1996), stud farms