

Prevalence and risk factors of *Theileria equi* infection in horses in Minas Gerais, Brazil



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ABSTRACT

Theileria equi, a protozoon in the phylum Apicomplexa, is the causative agent of equine theileriosis. The aim of this cross-sectional study was to determine the prevalence of IgG antibodies against *T. equi*, by using the indirect fluorescent antibody test (IFAT) reaction and correlating the serostatus with some epidemiological variables relating to the way in which Mangalarga Marchador horses are raised in southern Minas Gerais, Brazil. In this study, 506 horses were used, all clinically healthy, on 53 horse farms distributed across 27 municipalities in southern Minas Gerais. The statistical tests, comprising the chi-square test and generalized estimating equations (GEE), were performed in the SPSS Statistics 20.0 software. The true seroprevalence of *T. equi* among the horses was 57.0% (288 out of 506; ranging from 52.1 to 62.0%) and among horse farms, 98.1% (52 out of 53; ranging from 90.3 to 99.9%). The horse farms on which the majority of the horses presented good body condition and which did not use chemical products to kill ticks when new horses were introduced, had horses over the age of 5 years, comprised <100 ha in area and did not use embryo transfer showed greater seroprevalence of *T. equi* ($p < 0.05$). The results from this study make it possible to state that infection with *T. equi* is endemic in the south of Minas Gerais and is widely distributed among horse farms raising Mangalarga Marchador horses. However, these infections are subclinical or chronic.

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1. Introduction

Theileria equi, a protozoon in the phylum Apicomplexa (known initially as *Babesia equi*), parasitizes equine blood cells. It is one of the causative agents of equine piroplasmosis, which is a disease without pathognomonic clinical signs (Rothschild, 2013). In its acute phase, it can cause fever, apathy, hemolytic anemia, hemoglobinuria, jaundice and, in some severe cases, death (Schein, 1998). The infection is generally subclinical or chronic, and the horses can maintain the protozoon for several years and probably throughout their lives. Thus, infected horses act as a reservoir for the vector (De Waal, 1992). This protozoon can be transmitted by different species of ixodid ticks of the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus* (Mehlhorn and Schein, 1998). In Brazil, the tick *Rhipicephalus (Boophilus) microplus* is its vector (Guimarães et al., 1998a, 1998b; Heuchert et al., 1999; Battsetseg et al., 2002).

T. equi has wide geographic distribution and infects horses in Europe, Asia, Africa and Central/South America, but equine theileriosis is

considered to be endemic in tropical and subtropical areas (Schein, 1998). This disease has worldwide impact and causes economic losses through affecting trade and transportation of these animals. It is considered to be the most important cause of restrictions on exportation of serologically positive horses or participation in international events, such as fairs or sports events, with regard to the need to transport horses to countries that are considered to be free from *T. equi* (Friedhoff et al., 1990; Schein, 1998).

The indirect fluorescent antibody test (IFAT) and the competitive enzyme immunosorbent assay (cELISA) are the main tests used in serological surveys validated by the World Organization for Animal Health (OIE, 2014) for diagnosing *T. equi*. In Brazil, previous studies using serological methods have shown that infection by *T. equi* is endemic among horses, especially in the southeastern and southern regions, with high seroprevalence in the states of Rio de Janeiro (81%; Santos et al., 2011) and Parana (61%; Prochno et al., 2014).

Although serological surveys are important for characterizing areas that are endemic for equine theileriosis, few studies have aimed to identify the risk factors relating to seropositivity for *T. equi* in Brazil. One prerequisite for controlling the disease is to study its epidemiology, in order to ascertain its distribution and seasonality. This information is

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important for developing and improving disease control measures, especially given than prevention of this disease basically consists of combating the tick vector. It also needs to be borne in mind that the epidemiological characteristics associated with seropositivity for *T. equi* among equids can vary between the different regions of the country, according to the geographic area, altitude, farming conditions and area of origin of the equids (Santos et al., 2011).

Therefore, because of the importance of this disease and the lack of studies on the epidemiology of equine theileriosis in southern Minas Gerais, which is considered to be the place of origin of the Mangalarga Marchador breed, a Brazilian breed admired all over the world, this study had the aims of determining the prevalence of IgG antibodies against *T. equi* among clinically healthy horses, by using IFAT, and correlating their serostatus with some epidemiological variables relating to the systems used for raising horses of the Mangalarga Marchador breed.

2. Materials and methods

2.1. Horses and serological samples

This cross-section study was conducted in order to assess the seroprevalence of IgG antibodies against *T. equi* among 506 horses, all clinically healthy, on 53 horse farms that were raising the Mangalarga Marchador breed. These farms were distributed across 27 municipalities located in the microregions of Andrelândia, Lavras and São Lourenço, in the southern region of the state of Minas Gerais (Fig. 1). This study was approved by the Bioethics Committee for Utilization of Animals of the Federal University of Lavras, Minas Gerais, Brazil (023/11).

The population sample was composed of 167 males (33%) and 339 females (67%), among which 115 were foals (23%) and 391 were adults (77%). Blood samples were collected by means of venipuncture of the jugular vein and were placed in Vacutainer tubes containing EDTA. After separation of individually identified plasma samples in cryotubes, these were kept frozen at -20°C until the serological analysis was performed. The samples were collected between April 2012 and October 2013.

The farms were randomly selected from a list provided by the Mangalarga Marchador Horse Breeders' Association (Associação de Criadores de Cavalos Mangalarga Marchador, ACCMM). The inclusion criteria used were as follows: in addition to rearing the Mangalarga Marchador breed, the horse farm needed to have at least 10 horses for the prevalence calculation, excluding foals <6 months old and pregnant mares.

The number of horses (n) needed for estimating the seroprevalence was defined based on the formula provided by the Pan-American Zoonosis Center (Cepanço, 1973), which is: $n = [p \times (100 - p) \times Z^2] / (d \times p / 100)^2$. Considering the estimated prevalence (p) to be 50%, with a 95% degree of confidence (z) of 1.96 and an admissible margin of error (d) of 10%, a minimum number (n) of 384 samples was obtained.

Among the samples from the different farms, we tried to maintain the ratio between the mesoregions assessed and the size and structure of the farms, thereby seeking balanced representation. Approximately 10 horses were sampled per farm, which resulted in a total of 53 farms. At the time of visiting the horse farms, interviews based on semi-structured pretested forms were conducted, with the aims of gathering information (independent variables) about the possible factors relating to occurrences of *T. equi* and characterizing the Mangalarga Marchador horse farms (Supplementary file).

2.2. Serological tests

IgG antibodies against *T. equi* were detected by means of the indirect fluorescent antibody test reaction, in accordance with the technique described by the Inter-American Agricultural Cooperation Institute (IICA, 1987), using slides of the antigen made with red blood cells infected by *T. equi*. These slides had been produced previously in the Parasitic Diseases Laboratory of the Federal University of Lavras (UFLA), Brazil, and were kept frozen at -20°C until they were used. The slides prepared for IFAT comprised not only the test serum, but also positive and negative controls and anti-equine IgG conjugate (SIGMA, St. Louis, MO, USA). The serological samples were considered positive for *T. equi* when they presented a reaction at a dilution of 1:80 or higher (Heim et al., 2007).

2.3. Statistical analysis

To calculate the true seroprevalence on each horse farm, the sensitivity and specificity of the tests were adjusted from the individual to the horse farm level using the Herdacc software (Jordan and McEwen, 1998). Horse farms were considered to be positive when they had at least one seropositive horse. In the true prevalence calculations for IgG antibodies against *T. equi*, the values of 89.2% and 99.0% were used respectively for sensitivity (SE) and specificity (SP), in accordance with Ogunremi et al. (2007). Following this, the sensitivity and specificity were used to calculate the true prevalence at the herd level (Noordhuizen et al., 2001) using the epidemiological calculator at

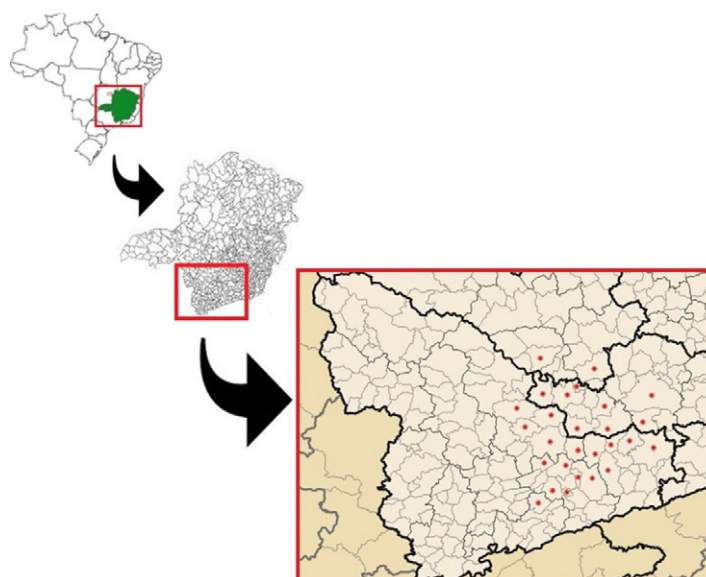


Fig. 1. Location map of the municipalities located in the microregions of Andrelândia, Lavras and São Lourenço, southern Minas Gerais, Brazil.

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