Ticks and Tick-borne Diseases 8 (2017) 936–941

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Original article

Molecular, epidemiological, haematological and biochemical evaluation in asymptomatic *Theileria annulata* infected cattle from an endemic region in Spain

Lydia Calleja-Bueno*, Ángel Sainz, Mercedes García-Sancho, Fernando Rodríguez-Franco, Juan V. González-Martín, Alejandra Villaescusa

Department of Animal Medicine and Surgery, College of Veterinary Medicine, Complutense University of Madrid, Avda. Puerta de Hierro s/n, 28040, Madrid, Spain

ARTICLE INFO

Keywords:
Theileria annulata
Cattle
Prevalence
PCR
Central Spain

ABSTRACT

Mediterranean theileriosis is one of the most fatal theilerioses, with considerable economic impact on livestock production. The potential consequences of asymptomatic infection on the animal's health and on the epidemiology in endemic regions are still unclear. The objectives of this study were to determine the molecular prevalence of *T. annulata* in a representative population of asymptomatic cattle in extensive management in Madrid, Central Spain, an area where practitioners frequently report cases of clinical theileriosis, and to evaluate the existence of associations between infection by this pathogen and haematological, biochemical and epidemiological data. *T. annulata* DNA was detected in 22.4% of the study population. The age was statistically associated with *T. annulata* prevalence rates, with a higher prevalence in cows older than 8 years (26.1%). Introduction of new cattle to the farm, grazing on pastures with other herds of cattle and previous history of clinical cases were statistically related to a higher prevalence of *T. annulata* infection. Herds with more than one ectoparasiticide treatment per year and that used more than one drug had significantly lower prevalences of infection with *T. annulata*. The location of farms in areas with a mean temperature higher than 17.1° C and mean altitude lower than 962 m was statistically associated with the presence of *T. annulata*. In our study, the mean values of haematological parameters were within the normal adult range, but it is noteworthy that some *T. annulata*-infected animals presented low values for red blood cell parameters.

1. Introduction

Tick-borne diseases affect 80% of the world's cattle population (Marcelino et al., 2012). Tropical or Mediterranean theileriosis, caused by *Theileria annulata* and transmitted by several species of ticks of the genus *Hyalomma*, is one of the most fatal theilerioses in Europe, North Africa and Asia. It has also considerable economic impact on livestock production due to the high morbidity and loss of productivity in autochthonous breeds and mortality in imported cattle (Brown, 1997; Dolan, 1989). The disease is characterized by an early lymphoproliferative phase that is accompanied by enlargement of lymph nodes. On development of pyrexia, a lymphodestructive phase, which is associated with a pronounced leukopenia, is initiated. The disease is further characterized by a marked anaemia (Tait and Hall, 1990).

Veterinarian practitioners frequently describe clinical theileriosis cases in bovine production in extensive management in the area of Madrid, Spain. This is a region of 8,030.1 km^2 located in the centre of the country, between the coordinates 39.82 and 41.30 latitude and −2.91 and −4.70 longitude (Catálogo de Información Territorial de la Comunidad de Madrid). The climate is Mediterranean, with variations depending mainly on the altitude. There are a mountain climate area and a continental Mediterranean climate area with two subtypes. The subtype warm-summer continental Mediterranean climate has an average annual temperature of 11 °C, and the subtype hot-summer continental Mediterranean climate has an average annual temperature above 14 °C (Consejería de Medio Ambiente, Administración Local y Ordenación del Territorio −Secretaría General Técnica, 2007). The bovine population in Madrid represents 1.8% of the national population in extensive grazing systems, with approximately 33,717 cows (SITRAN, 2014). A study of 1989 showed a seroprevalence of *T. annulata* in Madrid and in Castilla La Mancha (Central Spain) of 90% by testing more than 500 animals (Brandau et al., 1989). Ten years later, another study determined the seroprevalence of this disease in 51.5% in Madrid by testing 297 bovines (Viseras and García-Fernández, 1999).

* Corresponding author at: Department of Animal Medicine and Surgery, College of Veterinary Medicine, Complutense University of Madrid, Avda. Puerta de Hierro s/n, 28040, Madrid, Spain.
E-mail address: l.calleja@ucm.es (L. Calleja-Bueno).

http://dx.doi.org/10.1016/j.ttbdis.2017.08.006
Received 22 February 2017; Received in revised form 24 July 2017; Accepted 17 August 2017
Available online 25 August 2017
1877-959X/

Nevertheless, a study of molecular prevalence of this tick-borne disease has not been performed in the area.

Besides, it has been described in endemic regions that recovery from acute disease caused by *T. annulata* infection can result in the development of a persistent piroplasm carrier state that plays an important role in the maintenance of the parasite life cycle and that can affect cattle production (d'Oliveira et al., 1995). However, little is known about the consequences that *T. annulata* asymptomatic infections could have on reservoir's health.

The aim of this study was therefore to report the molecular prevalence of *T. annulata* infection in cattle in Central Spain (Madrid) and to evaluate the existence of associations between DNA amplification of this pathogen and haematological, biochemical and epidemiological data.

2. Materials and methods

2.1. Animals

In order to assess the current situation of *T. annulata* asymptomatic infections, a representative cattle population that can reflect the entire population of this region was analysed. Four hundred and ninety six cattle exposed to ticks in field conditions and without clinical signs were included in this study. Blood samples and epidemiological data were collected from each animal from April to October 2015 during the Spanish national brucellosis, tuberculosis, and bovine leukosis eradication program. Spanish ethical guidelines and animal welfare regulations (Spanish Royal Decree Law 53/2013) were strictly respected and herd owners consent was also obtained. The study was conducted in 34 farms of 24 different municipalities and from 3 different climate areas in the Community of Madrid: a mountain climate area (M), and a continental Mediterranean climate area, subdivided in warm-summers (W) and hot-summers (H) (Fig. 1). A stratified sampling was performed to ensure a similar proportion between the number of sampled animals and the cattle population from the different regions. Besides, the sample size of each farm was 25% of the total number of animals in the farm. The minimum and maximum size of herds examined were eight (W16, with two animals sampled) and 200 (H2, with 50 animals sampled), respectively (Table 1).

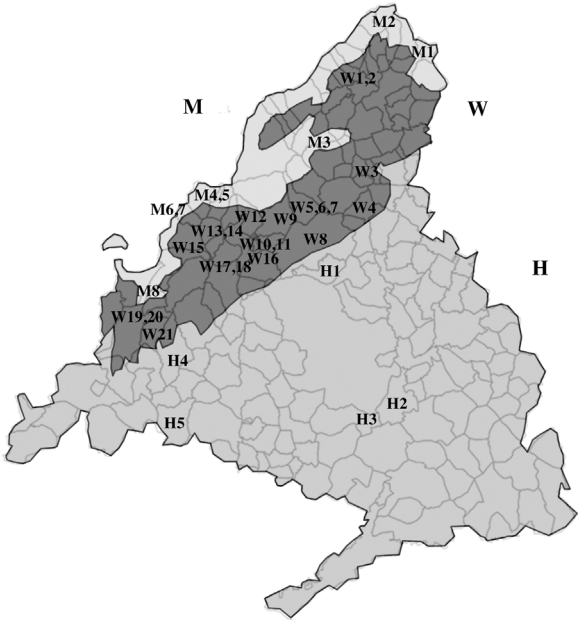

Fig. 1. Map of Madrid with 24 municipalities of the study (Table 1) and the 3 climate areas: a mountain climate area (M), a warm-summer continental Mediterranean climate area (W) and a hot-summer continental Mediterranean climate area (H).

Table 1
Municipalities of Fig. 1 and *T. annulata* PCR results.

Municipality	N° Farm	Neg	Pos	N	% pos
Prádena del Rincón	**M1**	25	0	25	0
Somosierra	**M2**	7	0	7	0
Bustarviejo	**M3**	19	2	21	9.5
Cercedilla	**M4, 5**	14	0	14	0
Los Molinos	**M6, 7**	6	10	16	62.5
Robledo de Chavela	**M8**	5	1	6	16.7
Gascones	**W1, 2**	44	1	45	2.2
Cabanillas	**W3**	25	5	30	16.7
Pedrezuela	**W4**	1	19	20	95
Soto El Real	**W5, 6, 7**	52	0	52	0
Colmenar Viejo	**W8**	23	2	25	8
Manzanares El Real	**W9**	20	0	20	0
El Boalo	**W10, 11**	17	2	19	10.5
Mataelpino	**W12**	3	0	3	0
Becerril de la Sierra	**W13, 14**	6	5	11	45.4
Guadarrama	**W15**	7	3	10	30
Moralzarzal	**W16**	2	0	2	0
Villalba	**W17, 18**	16	5	21	23.8
Robledo de Chavela	**W19, 20**	28	3	31	9.7
Fresnedillas de la Oliva	**W21**	0	4	4	100
Tres Cantos	**H1**	1	9	10	90
Rivas-Vaciamadrid	**H2**	50	0	50	0
Perales del Río	**H3**	0	15	15	100
Navalagamella	**H4**	0	25	25	100
Villamantilla	**H5**	14	0	14	0
	Total	385	111	496	22.4

Different epidemiological and clinical data were collected through questionnaires. Temperature, relative humidity and altitude were registered at the moment of sampling. The sampling time was, approximately, at the same hour. EDTA and non-anticoagulated blood were collected from the coccygeal vein of each animal for haematological and biochemical profiles whenever possible (in 351 cows) and for DNA extraction for polymerase chain reaction (PCR) in all the animals of the study.

2.2. DNA extraction

DNA was extracted from 200 µl of each blood sample using the UltraClean® BloodSpin® DNA Isolation Kit (Mo Bio Laboratories, CA) following manufacturer instructions. DNA concentration was quantified and the quality assessed by absorbance ratios 260/280 nm and 260/230 nm using spectrophotometry (NanoDrop™, Thermo Scientific).

2.3. Polymerase chain reaction (PCR)

PCR analysis was performed on the samples to detect *T. annulata* DNA. The forward primer N516 (5′-GTAACCTTTAAAAACGT-3′) and reverse primer N517 (5′- GTTACGAACATGGGTTT-3′) were derived from the *Tams*-1 gene encoding the 30-kDa major merozoite surface antigen of *T. annulata* (d'Oliveira et al., 1995). PCR conditions were the previously described by d'Oliveira et al. (1995) for *T. annulata* with a modification of the number of cycles, as described by Almería et al. (2001). The reaction mixture, with a total volume of 25 µl, contained 5 µl of genomic DNA, 12.5 µl of DNA AmpliTools HotSplit Master Mix (Biotools B & M Labs, S.A., Spain) and 0.25 µl of each primer (50 µM). Negative and positive samples were included with each run.

The reactions were performed in an automatic DNA thermal cycler MasterCycler® ep Gradient (Eppendorf, Germany). The *T. annulata* PCR products were visualized by electrophoresis on 1.2% agarose gel containing ethidium bromide (10 mg/ml) run at 90 V for 30 min.

Presence of PCR inhibitors in negative samples was assessed by the amplification of a fragment of the constitutive gene for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein (Barber et al., 2005; Birkenheuer et al., 2003).