

ARTÍCULO ORIGINAL

Study on seasonal activity in dogs and ehrlichial infection in *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) from southern Uruguay

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ABSTRACT

*The seasonal activity on dogs of **Rhipicephalus sanguineus** in southern Uruguay has been studied from samples collected in veterinary clinics of the country. From a total of 744 specimens, adults (92%) were active on dogs between August and March (peaking in October) being absent between April and July. Immatures represent more than 80% of captures on dogs in summer. This pattern is well correlated with the climate records in the zone. The search for ehrlichial DNA from tick-derived extracts (36 pools from 180 ticks) was negative in all the cases. While **R. sanguineus** has been incriminated as vector of these pathogens, additional studies are required to understand the role of this tick in the transmission of ehrlichial organisms.*

Key words: *Rhipicephalus sanguineus*, seasonal activity, dogs, absence of ehrlichial DNA, Uruguay.

INTRODUCTION

About 74 species are currently recognized in the tick genus *Rhipicephalus* Koch, 1844¹. *Rhipicephalus sanguineus* is probably the most widely distributed tick in the World. It is found circumglobally approximately between the latitudes 50°N and 42°S. Its preferences for dogs as main host have facilitated its worldwide distribution^{2,3}. *R. sanguineus* is an important vector of pathogens, like *Babesia canis*, *B. gibsoni* and *Ehrlichia canis*. The tick is also involved in the transmission of several species

of *Rickettsia* that affect humans, being the primary vector of *Rickettsia conorii*¹. *R. sanguineus* has been introduced and is widely distributed in the Neotropical Region, where high prevalence and parasitic intensity are common in dogs.

While accurate specific status of some populations still remains to be done⁴, *R. sanguineus* should be considered the most important ectoparasite on dogs throughout the Neotropics⁵.

Uruguay is located in south-eastern South America (30°-35°S, 53°-58°W). The country

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belongs to the Uruguayensis District of Pampean Province⁶, which is characterized by the dominance of subtropical prairies with forests associated to water courses and highlands, with bush lands and hydrophilic communities in some areas⁷. Most parts of the country are below 300 m altitude. Climate is subtropical-subhumid, with an average yearly temperature of 17° C and 1,300 mm of rainfall. The vegetation consists mainly of diversified grasslands. Nineteen tick species have been recognized in Uruguay. One of these, *Rhipicephalus (Boophilus) microplus* has enormous implications in animal production, while others like *Amblyomma triste* are involved in human health aspects^{8,9}. *R. sanguineus* has been identified as a potential vector of diseases to both animals and humans in Uruguay but no studies have been reported detailing its ecology and potential to transmit pathogens⁸. The aim of this paper is to draw the seasonal dynamics of *R. sanguineus* on dogs in Uruguay, bearing in mind the lack of studies about this tick in the country, as well as to perform a systematic search of ehrlichial DNA on tick samples. In the absence of a longitudinal design, the study is performed around samples collected in veterinary clinics for a period of 5 years.

MATERIAL AND METHODS

Ticks were collected on dogs in urban and suburban areas from November 2000 until February 2005, in the counties of Montevideo (34°54'S, 56°09'W) and Canelones (34°32'S, 56°17'W) located in southern Uruguay (Figure 1). The southern portion of the country was selected because the highest population of urban and peri-urban dogs concentrates in this part of the country. This material was obtained after the examination of dogs in private veterinary clinics and from material for diagnosis remitted to the Department of Parasitology, Veterinary Faculty, Montevideo. The material was fixed and stored in tubes containing ethanol 70°. They were determined to the species and stage level, then larvae and nymphs grouped together as immatures. Climatic data (temperature, relative humidity and rainfall) were collected from the ground recording station of Prado (34°51'S 56°12'W) in Montevideo County. Collections were grouped according to months and seasons

to show the general picture of the seasonal activity pattern.

PCR procedures were performed on tick samples as follows. Ethanol was eliminated using distilled water for 10 minutes. Each tick was longitudinally sectioned and separately stored at -20°C. DNA was individually extracted from 180 tick samples, grouped into 36 lots, using lyses with ammonium hydroxide 0,7 M. The DNA was quantified, pooled in groups of 5 samples and adjusted to 150 ng for the PCR reactions. Primers EHR16SD (52 -GGT-ACC-YAC-AGA-AGA-AGT-CC-32) and EHR16SR (52 -TAG-CAC-TCA-TCG-TTT-ACA-GC-32), were used for detection of ehrlichial DNA¹⁰. This set detects all *Ehrlichia* species. An initial 5 min denaturation cycle at 95°C was followed by 34 cycles of denaturation (95°C for 30 s), annealing (55°C for 30 s), and extension (72°C for 90 s) with a final 5 min extension at 72°C. Distilled water and DNA from *E. canis* (kindly supplied by A. Sainz, Veterinary Faculty, Madrid, Spain) were used as negative and positive controls, respectively. The amplification products were visualized on 1% agarose gels after electrophoresis of 10 µl of amplified material.

RESULTS

Rhipicephalus sanguineus ticks were detected on dogs from all localities with overall prevalences of 83.5 % and 23.2 % for adult and subadult ticks, respectively. Additionally, *A. triste* and *A. tigrinum* were detected on 11% of dogs.

A. triste were determined in 7 localities for Montevideo: Urban area (34°53'S, 56°08'W), Villa García (34°46'S, 56°02'W). Canelones: La Paz (34°46'S, 56°13'W), Progreso (34°40'S, 56°12'W), Ta0pia (34°33'S, 55°42'W), Toledo Chico (34°44'S, 56°06'W), Santa Rosa (34°31'S, 56°02'W) and *A. tigrinum* in 1 locality for Canelones: Santa Rosa. A total of 744 *R. sanguineus* were collected from dogs (400 males, 287 females and 57 subadults).

Figure 2 shows the seasonal activity of the tick on dogs, together with details of the averaged climate values. Adults of *R. sanguineus* are evident in the second half of August through March, reaching a peak in October. Adults were not collected between April and July. Immatures were collected from August through February



Figure 1. Map of South America (A), Uruguay (B) and the sampling area (C). The squares in each map reflect the area subsequently magnified. Dots show the localities of captures.

with highest numbers on dogs in February. *Ehrlichia*-derived DNA was not detected in the 36 pools of ticks sampled.

DISCUSSION

Although both *A. triste* and *A. tigrinum* ticks were collected, these species were observed only on dogs wandering through sub-urban and rural areas. *R. sanguineus* is thus the most common tick on dogs living in urban areas in Uruguay. The pattern of the different stages of *R. sanguineus* is highly correlated with the recorded climate. The increase of temperature and rainfall in spring (after August in Southern Hemisphere) coincides with the maximum adult loads on dogs. This pattern of increase of adults on dogs may be originated by the synchronized moult of nymphs and/or beginning of activity in adults after winter diapause. This behaviour avoids the questing of the tick under cold winter conditions and facilitates a great number of adults in spring, as has been described in many places through the distribution range of the tick^{11,12}. Immatures concentrate in summer, in the period of maximum temperatures and low rainfall, as a result of the synchronized oviposition of adults

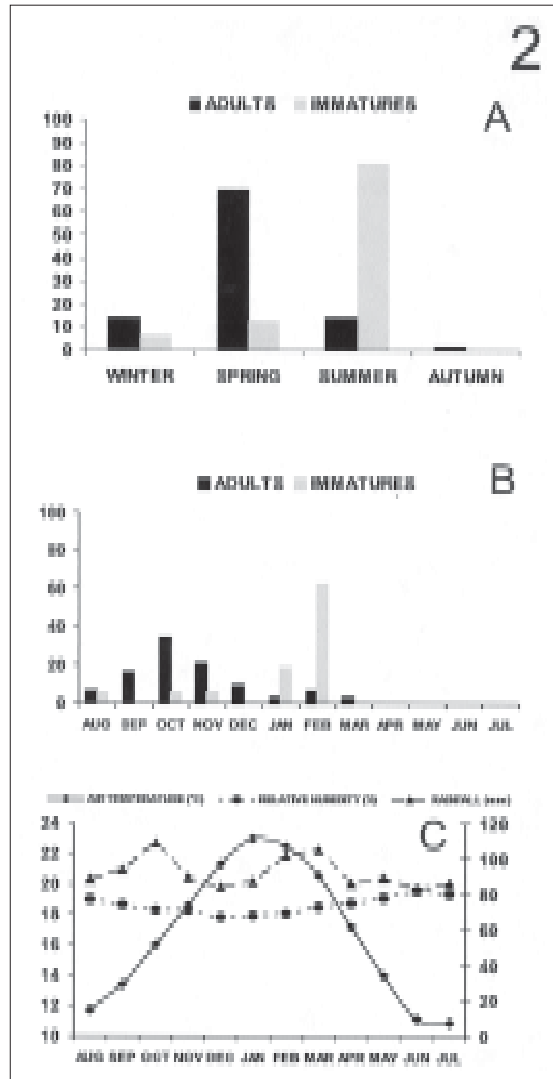


Figure 2. A. Monthly percentages of dogs parasitized with adults and immatures of *R. sanguineus*. B. Seasonal percentages of dogs parasitized with adults and immatures. C. Monthly averages of temperature, humidity and precipitations of the meteorological station of Prado, Montevideo.

and subsequent larval hatching. Autumn and winter are the periods of the year with the smallest numbers of ticks. These results show that *R. sanguineus* possesses a marked seasonality in the area of study, in agreement with the works carried out in Chile around the same latitude¹¹. In localities with a tropical climate, the tick has been found to have a continued pattern of activity¹³.

It is interesting to note that all the ticks examined by PCR were negative for the presence of *Ehrlichia* spp. The primers used herein have been already used to detect pathogens of the family Anaplasmataceae, including the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia* and *Wolbachia*^{10,14,15}. Bearing in mind the number of ticks used in this study (180) we should consider the hypothesis that the frequency of *Ehrlichia* spp. is low in the population of studied ticks, although is a pathogen common in dogs in other parts of South America^{16,17}.

Additional studies on the ecology of the tick are necessary to understand the many risk factors for both dogs and humans and to recognize the pathogens these ticks can transmit in the region of reference.

RESUMEN

La actividad estacional de *Rhipicephalus sanguineus* en perros del sur de Uruguay se ha estudiado mediante muestras colectadas en clínicas veterinarias. De un total de 744 especímenes, los adultos (92%) tienen su actividad en los perros entre agosto y marzo (alcanzando el máximo en octubre) estando ausentes entre abril y julio. Los inmaduros representan más de 80% de las capturas en los perros en verano. Este modelo se correlaciona con los datos climáticos de la zona. La búsqueda de ADN ehrlichial en el extracto de garrapatas (36 pools de 180 garrapatas) fue negativo en todos los casos. *R. sanguineus* se ha incriminado como vector de estos patógenos, por lo que se requieren estudios adicionales para entender el papel de esta garrapata en la transmisión de estos organismos ehrlichiales.

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