RELACIÓN ENTRE INFECCIONES ASINTOMÁTICAS CON *Anaplasma marginale, Babesia* spp. y *Trypanosoma vivax* EN TOROS Y NIVELES DE TESTOSTERONA

Relationship between asymptomatic infections with *Anaplasma marginale, Babesia* spp. and *Trypanosoma vivax* in bulls and testosterone levels

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ABSTRACT

Some bovine hemotropic infections are associated with decreased reproductive potential in bulls, but the underlying mechanisms involved are poorly understood. The main objective of this transversal study was to determine *Anaplasma marginale, Babesia* spp., and *Trypanosoma vivax* infections in asymptomatic bulls (n=85) of various breeds and ages, and to compare serum average testosterone levels in infected and non-infected animals. The highest prevalence by Polymerase Chain Reaction (PCR) was 46% for *A. marginale*, 34% for *Babesia* spp. and 60% for *T. vivax.* The greatest percentages of bulls infected with *A. marginale* and *Babesia* spp. were found in the Holstein breed (75.0 and 66.7%, respectively), and the lowest in the Carora breed (35.7 and 14.2%, respectively) with values of P<0.001. The percentage of bulls infected with *T. vivax* was higher in the Holstein, Carora and Mixed Breeds (75.0, 71.4 and 76.5%) as compared to the Brahman breed (38.1%). The highest percentages of infection with A. *marginale* and *Babesia* spp. were found in animals 10-19 months old and less than 29 months old, respectively, while the highest percentage of infection with *T. vivax* was observed in 40-49 months old animals. These results show the importance of the breed and age in the selection of individuals for studies of prevalence, treatment and epidemiological control. Hematocrit values were statistically lower in animals infected with *A. marginale* as compared to the non-infected group (P<0.02). Bulls infected with *T. vivax* presented lower levels of serum testosterone, while bulls either infected with *A. marginale or Babesia* spp. or non-infected, showed no differences in this hormone. The decrease in testosterone levels in bulls appears to correlate with *T. vivax* chronic infection, suggesting a link between the presence of this pathogen and hormonal levels.

Key words: Hemotropics; testosterone; age; breed; cattle.

RESUMEN

Algunas infecciones causadas por hemotrópicos están asociadas con una disminución del potencial reproductivo en toros; sin embargo, los mecanismos involucrados han sido poco estudiados. El principal objetivo de este estudio transversal fue determinar la infección con *Anaplasma marginale*, *Babesia* spp. y/o *Trypanosoma vivax* en toros asintomáticos de distintas razas y edades (n=85), y comparar niveles séricos promedios de testosterona en animales infectados y no-infectados. La mayor prevalencia obtenida por la reacción en cadena de la polimerasa (PCR) fue 46% para *A. marginale,* 34% para *Babesia* spp. y 60% para *T. vivax.* El porcentaje más alto de toros infectados con *A. marginale* y *Babesia* spp. fue encontrado en la raza Holstein (75,0 y 66,7%, respectivamente) y el más bajo en la raza Carora (35,7 y 14,2%, respectivamente) con valores de P<0,001. El porcentaje de toros infectados con *T. vivax* fue más alto en las razas Holstein, Carora y en los mestizos (75,0; 71,4 y 76,5%) que en la raza Brahman (38,1%). Se encontró un porcentaje mayor de infección con *A. marginale* y con *Babesia* spp. en el grupo etario de 10-19 meses y en los toros menores de 29 meses de edad, respectivamente, mientras que el mayor porcentaje de infección con *T. vivax* fue observado en el grupo etario de 40-49 meses. Estos resultados mostraron la importancia de la raza y la edad en la selección de individuos para estudios de prevalencia, tratamientos y control epidemiológico. Los hematocritos fueron estadísticamente menores en los animales infectados con *A. marginale* comparado con los no-infectados (P<0,02). Los toros infectados con *T. vivax* presentaron niveles inferiores de testosterone sérica, mientras que los toros asintomáticos bien sea con *A. marginale* o con *Babesia* spp. como los no infectados, no mostraron diferencias significativas en los niveles en esta hormona. La disminución en los niveles de testosterona en toros parece correlacionarse con la infección crónica con *T. vivax*, lo cual sugiere una conexión entre la presencia de este patógeno y los niveles de esta hormona.

Palabras clave: Hemotrópicos; testosterona; edad; raza; bovinos.

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INTRODUCTION

Hemotropic cattle infections in Venezuela are mainly caused by *Anaplasma* (*Anaplasma marginale*), *Babesia* (*B. bigemina* and *B. bovis*) and *Trypanosoma* (*T. vivax*). These diseases cause anemia, fever, weight loss, low milk and meat production, difficulties in reproduction and, in some cases, death of the animal, which generate economic losses in cattle production. These arthopod-borne infections are characterized by long-lasting interactions with cattle, which result in the induction of reservoirs [3, 4, 12, 19, 27].

Prevalence of blood parasitic infections in cattle have been traditionally determined by parasitological and serological techniques, the latter ones based on the evaluation of antibodies in the animal serum (ELISA, immunofluorescence antibodies test, card agglutination test). On the other hand, molecular techniques such as the Polymerase Chain Reaction (PCR) have been used to determine the presence of parasites in cattle and to identify asymptomatic animals in carrier status [7, 19, 34, 43].

Previous studies indicate that the seroprevalence of *A. marginale* and *Babesia bovis* may differ according to the age of the animal [13, 35].However, there are no previous studies using PCR to determine prevalence of these three hemotropic agents in some of the predominant breeds found in Venezuela, including Holstein and Jersey (*Bos taurus*), Brahman (*Bos indicus*) and in animals well adapted to the tropics, with increased milk and meat yields, such as the Carora breed [24].

Previous studies have reported that *T. vivax* infections are associated with reduced reproductive capacity in bulls, with damage in both testis and epididymis, decreased sperm quality [1, 38, 39] and decreased serum testosterone levels [44]. However, no studies have been reported on the possible link between *A. marginale* and/or *Babesia* spp. infections and testosterone levels.

The aim of this investigation was to evaluate the prevalence of *A. marginale*, *Babesia* spp. and *T. vivax* in bulls of different breeds and ages and to correlate serum testosterone levels with anemia and hemotropic infections.

MATERIALS AND METHODS

Animals and blood samples

Blood samples were taken from 85 apparently healthy male cattle used for reproductive purposes, between July 2010 and September 2011, in a farm located at 450 meters above sea level, in the José Tadeo Monagas Municipality, northern region of Guárico State, Venezuela. These animals were from the following breeds: Holstein (n=24), Carora (n=14), Brahman (n=22), mixed (n=17), Brown Swiss (n=5) and Jersey (n=3). A group of 56 male cattle, 4-65 months old, was used to analyze the relationship between age and the prevalence of *A. marginale, Babesia* spp and *T. vivax.* This was a transversal study and only one sample was collected and evaluated from each animal. Blood samples were collected from the jugular vein using BD Vacutainer® tubes. Sera were obtained by centrifugation and used for testosterone determination. Whole blood samples with ethylenediaminetetraacetic acid disodium salt (EDTA) were used for determination of hematocrit and to obtain genomic DNA for *Anaplasma marginale, Babesia* spp. and *T. vivax* PCR assays.

The investigation project was approved by the Research Ethics Committee of the Simón Bolívar University. The study was conducted following the guidelines established in the Code of Bioethics and Biosafety “Fondo Nacional para la Ciencia y Tecnología” (FONACIT) [31].

DNA was extracted from whole blood samples using a commercial kit (Wizard DNA Purification Promega®, USA) according to the manufacturer instructions and stored in a Philco vertical freezer, Model 1472, (Philadelphia Stg. Batt. Co.; USA) at -20 °C, until use.

Detection of *A. marginale*, *Babesia* spp. and *Trypanosoma* spp. through PCR

Detection of *A. marginale*, *Babesia* spp. and *Trypanosoma* spp. in blood samples from male cattle was performed through PCR using the following primers and conditions: *A. marginale* was evaluated using primers based on the Major Surface Protein 5 gene (*msp5*) [37] and primers PiroA/PiroB were used for the determination of *Babesia* spp. [36]. Primers based on the sequence of a *T. vivax* specific antigen recognized by the Tv27 monoclonal antibody were used to determine trypanosome infection, as described [32]. The PCR products were analyzed by 1.5% agarose gel electrophoresis followed by staining with 0,001 % SYRSafe. The bands were visualized using UV light and a digital image capture system for gels (FOTO/Analyst® Investigator).

Determination of serum testosterone

Testosterone was determined in the serum samples from 85 male cattle, using the DRG® Testosterone ELISA commercial kit (DRG International, Inc., USA) following the manufacturer instructions.

Statistical analyses

The statistical analyses were performed using the IBM-SPSS statistical software version 10, available on line (Chicago, IL, USA). P values <0.05 were considered statistically significant [29]. The comparison of testosterone levels between positive or negative animals to each hemotropic was performed by Student’s t test, the comparison between *Anaplasma marginale*, *Babesia* spp. or *T. vivax* prevalence based on the breed was evaluated by Chi square test, and the comparison between *Anaplasma marginale*, *Babesia* spp. or *T. vivax* prevalence in cattle based on age groups was evaluated by ANOVA.

RESULTS AND DISCUSSION

The prevalence of hemotropics in the male cattle population was assessed in a dairy farm that maintains bulls. The prevalence of *A. marginale*, *Babesia* spp. and *T. vivax* was determined through PCR assays that detect the presence of parasite DNA.

Molecular diagnosis has a significant advantage over serological techniques that detect antibodies in sera from animals that have been previously exposed to the parasite, but do not discriminate between present and past parasite infections. On the other hand, parasite detection by blood smears has very low sensitivity and the microhematocrit tube technique can be used for trypanosome detection only, with low sensitivity [49].

TABLE I shows that the overall prevalence through PCR was 46% for *A. marginale*, 34% for *Babesia* spp., and 60% for *T. vivax*. Co-infection with *A. marginale* and *Babesia* spp. was 25% and co-infections involving *T. vivax* varied between 7 and 15%. The highest prevalence of *A. marginale* appeared in the Holstein breed (75%) and the lowest in the Carora breed (35.7%), with statistically significant differences (P<0.0001), while Brahman and mixed breeds both showed prevalence values of approximately 53%. In relation to the prevalence of *Babesia* spp., the highest percentage was observed in the Holstein breed (66.7%) and the lowest in Carora (14.2%). The highest prevalence for *T. vivax* was detected in the Holstein breed (75.0%) and the lowest in Brahman (38.1%). In addition, the Brahman, Carora and mixed breeds presented statistically significant differences for this protozoan, when compared to Holstein (P<0.04). Co-infections with *A. marginale* and *Babesia* spp. were mainly observed in the Holstein breed, followed by mixed, Brahman and Carora. Triple infections were highest in Holstein bulls (33.3%). The Jersey breed was excluded from the statistical analysis since it was represented only by three animals. These data showed that the highest prevalence of these infections was observed in the Holstein breed (*Bos taurus*), while the lowest was observed in Carora. Moreover, Brahman and mixed breed showed similar results. The Carora breed, a hybrid resulting from the cross between Brown Swiss and Criollo Amarillo de Quebrada Arriba (Lara State) is considered one of the most important local breeds in terms of the cattle farming industry in Venezuela [48]. Genetic studies, using microsatellites, have shown the evolution of Carora to an autonomous, independent mixed breed. This research showed that the Carora breed had the lowest prevalence of *A. marginale* and *Babesia* spp infections. In contrast, the European Holstein breed appears to be the most

TABLE I

PREVALENCE OF *Anaplasma marginale, Babesia* spp. AND *T. vivax* IN A HERD OF 4-65 MONTH-OLD BULLS

Breed	Total N°	*A. marginale* Positive n(%)	*Babesia* spp. Positive n(%)	*T. vivax* Positive n(%)	*A. marginale and Babesia* spp. Positive n(%)	*T. vivax. and A. marginale* Positive n(%)	*T. vivax and Babesia* spp. Positive n(%)	*T. vivax, Babesia* spp. *and A. marginale* Positive n(%)
Holstein	24	18 (75.0)	16 (66.7)	18 (75.0)	15 (62.5)	1(4.2)	1 (4.2)	8 (33.3)
Carora	14	5 (35.7)*	2 (14.2)*	10 (71.4)*	1 (7.1)*	1(7.1)*	1 (7.1)*	0 (0)*
Brahman	22	11 (53.4)†,‡	8 (38.1)†,‡	7 (38.1)+	3 (14.3)†	0 (0)†	7(31.3)†	0 (0)†
Mixed breed	17	9 (52.9)§,ϕ	7 (41.2)§ϕ	13 (76.5)	6 (35.3)§,ϕ	0 (0) §, ϕ	1 (5.9)§,ϕ	1 (5.9)§,ϕ
Brown Swiss	5	2	1	w/d	w/d	w/d	w/d	w/d
Jersey	3	1	0	2	w/d	w/d	w/d	w/d
Total	85	46%	34%	60%	25 %	7 %	15 %	13 %

Data from Holstein, Carora, Brahman and mixed breed were evaluated through Chi square test. *P< 0.0001 Carora vs Holstein; †P< 0.0001 Brahman vs Holstein; ‡P< 0.001 Brahman vs Carora; §,P< 0.001 Mixed breed vs Holstein; ϕP< 0.001 Mixed breed vs both Carora and Brahman. Brahman vs Mixed breed +P< 0.05. w/d: without data

susceptible to infections by *T. vivax*, *A. marginale*, and *Babesia* spp. [8, 9, 18, 38]. Duangjinda et al. [18] showed that Zebuine (*Bos indicus*) has a higher degree of tolerance to tick-borne diseases caused by *A. marginale*, *B. bigemina* and *B. bovis*, as compared to the high Holstein fraction crossbreed (≥ 87.5% Holstein). These authors identified different allelic associations related to tick-borne disease tolerance caused by *A. marginale* (DRB3*14 and *41), *B. bovis* (*14), and *B. bigemina* (*10 and *51). Similar results were reported by Bock et al. [8], who found that pure *Bos taurus* breeds were more susceptible and more affected by *B. bovis* and *A. marginale* infections. Studies carried out by Florio-Luis et al. [21] characterized the Criollo Limonero breed, a close relative of the Carora ancestor, Criollo Amarillo de Quebrada Arriba as a 100% Trypanotolerant mixed breed. They reported lower levels of trypanotolerance in other mixed breeds, including Holstein (84.62%) and Brown Swiss (72.73%) crossbreeds.

Although no studies on prevalence of these hemotropic agents through PCR have been previously reported in Venezuela, several seroprevalence studies have been carried out on bovine hemotropic agents, using immunofluorescence antibodies test (IFAT) and ELISA. For this reason, some hemotropics prevalence data from Venezuela and other countries, using serological or molecular techniques will be presented.

Seroprevalence of anaplasmosis and babesiosis in the midwestern region of Venezuela using IFAT was 55.7 % for *A. marginale*, 78.2% for *B. bigemina* and 38.8% to *B. bovis* [25]. In Guárico State, Toro [47] reported an average seroprevalence of 81.8 % for *B. bigemina* and 73.4 % for *B. bovis* through IFAT. Subsequently, Guillén et al., [23] conducted a retrospective study of 15 years and reported seroprevalences of 58.8 % for *B. bigemina*, 47.5% for *B. bovis,* and 6.42 % for mixed infections. Reyna-Bello et al. [37] found a 47% seroprevalence for *A. marginale* in different regions of Venezuela, through indirect ELISA using recombinant MSP5 as antigen. *Trypanosoma vivax* outbreaks have been reported in Monagas State, in the eastern region of Venezuela, where 27 and 50 % of the animals were

parasitologically and serologically positive, respectively. Co-infection with *A. marginale* was observed with parasitological and serological prevalence values of 9 and 68.9%, respectively [22]. Suarez et al. [45] found a 5.9% of *T. vivax* infections in bovines (n_T=1572), determined by the microhematocrit technique and 33.1% seropositivity through IFAT and ELISA (n_T=1675) in forty-nine Venezuelan farms. Epizootic outbreaks of *T. vivax* have been reported in Zulia State [42] with a 77% parasitological prevalence and a mortality rate of 15.15%. Tamasaukas et al. [46] found an overall 60% seroprevalence for *T. vivax* in bovines in Guárico state through IFAT, 56.9% in the rainy season and 45.7% in the dry season.

The present study showed a prevalence of 46% for *A. marginale*, 34% for *Babesia* spp., 60% for *T. vivax*, and 25% of co-infections in male cattle through PCR. In the Pantanal region, in Brazil, prevalence of *T. vivax*, determined through PCR ranged from 34.8% in buffaloes (*Bubalus bubalis*) to 44.7% in bovines [14], similar to those found in the present study. In Costa Rica, using real-time PCR, the prevalence of *A. marginale* was 56.9; 1.3 % for *B. bigemina*, and 0.45% for *B. bovis* in carrier cattle [41]. Prevalence has been also assessed in several countries in Africa, in Angola, 38 % positivity was detected for *A. marginale* (PCR) [28]. In Sudan, the prevalence of *B. bigemina*, *B. bovis*, and *A. marginale* was estimated at 4.0, 1.9, and 6.1%, respectively (PCR) [5]. In Mozambique, using PCR (semi-nested hot-start) positivity for *B. bigemina* was between 30 and 89 %, while for *B. bovis* ranged from 27- 83 % [30]. In Asia, the prevalence of *A. marginale* was 8.7 % in Mongolia (Msp5 nested PCR) [50], in Thailand, 1.9 to 39.2 % of the bovines were positive in BboSBP2 PCR, depending on the province [11] and in India, the prevalence for *A. marginale* through PCR in carrier bulls was 45.2-73.1 % [43]. In summary, the prevalence found in this group of bulls was similar to that reported in various countries in Africa and Asia using PCR with different molecular markers.

Average serum testosterone and the percentage of bulls infected with *A. marginale*, *Babesia* spp. and *T. vivax* were plotted on seven age intervals, ranging from 4 to 65 months old (FIG. 1). In the 4-9 months group, 50% of the male cattle were infected with *A. marginale* or *Babesia* spp., and 75% with *T. vivax*. In the 10-19 months old group, 100% of the animals were infected with *A. marginale*, 55.6% with *Babesia* spp. and no infections with *T. vivax* were observed. The lowest prevalence for *A. marginale*, *Babesia* spp. and *T. vivax* was observed in 30-39 month old animals. The prevalence for these infections increased in 40-49 month old and 50-59 month groups. Statistical analysis by Chi square test showed that anaplasmosis presented a significant increase in the 10-19 month group as compared to all others (P<0.004). Significant differences in babesiosis were observed only between the 20-29 month and the 30-39 month groups (P<0.05). Prevalence of *T. vivax* in the 30-39 month interval was lower than those in the following groups: 4-9, 20-29, 40-49, 50-59, and 60-65 (P<0.003). These results indicate that the susceptibility to hemotropics could be related to the age of the animal.

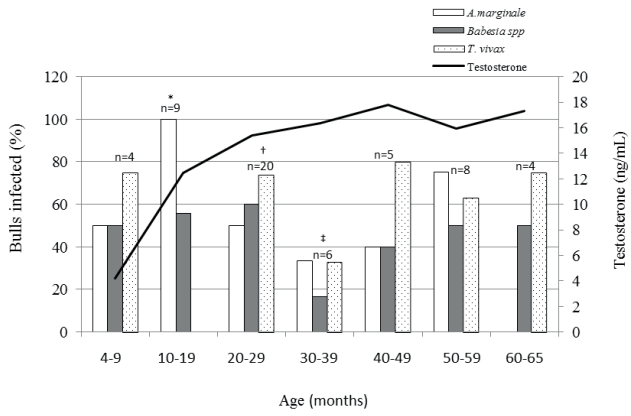

FIGURE 1. AVERAGE SERUM TESTOSTERONE AND *Anaplasma marginale, Babesia* spp. OR *T. vivax* INFECTION IN 4-65 MONTH-OLD MALE CATTLE FROM GUÁRICO STATE, VENEZUELA.

Testosterone levels were determined using an ELISA commercial kit. *Anaplasma marginale*, *Babesia* spp. *and T. vivax* prevalence was analyzed through PCR in a group of male cattle (n=56) from 4-65 months old, grouped in seven age intervals. *P<0.004 anaplasmosis in 10-19 months interval vs the following: 4-9, 20-29, 30-39, 40-49, 50-59, and 60-65. †P<0.05 babesiosis in the 20-29 months interval vs 30-39. ‡P<0.003 tripanosomosis in 30-39 months interval vs the following: 4-9, 20-29, 40-49, 50-59, and 60-65. Data were evaluated by ANOVA.

In the present study, the possible association between *A. marginale, Babesia* spp., and/or *Trypanosoma vivax* infection and serum testosterone levels was analyzed in a herd of bulls of different ages and breeds. A previous study indicated that bovine serum testosterone in males increases from puberty until 21 months, when it reached stability [26] and according to de Assumpção et al. [15], sexual maturity of male bovines from the Nelore breed is reached around 20 months, as confirmed by spermiogram. Similar results were found in the present investigation (FIG. 1). Therefore, only those sexually mature bulls that have reached stable levels of testosterone (>20 months) were included in the statistical analysis of bovine serum testosterone levels and single or mixed hemotropic infections. The analysis of serum testosterone levels and the percentage of hematocrit in the presence or absence of hemotropic infections are presented in TABLE II. Testosterone levels in bulls in the 20-65 month-old group ranged from 15.7 to 18.8 ng/mL and hematocrit values varied from 34.6 to 38.8%.

No statistically significant differences were observed in testosterone levels, relative to the presence or absence of *A. marginale* or *Babesia* spp.. However, testosterone levels were lower in the *T. vivax* infected bulls, as compared to the non-infected (P<0.01). No differences were observed in bulls co-infected with *A. marginale* and *Babesia* spp. as compared with their non- infected counterparts. However, co-infections involving *T. vivax,* with either *A. marginale* or *Babesia* spp. were accompanied with a decrease in serum testosterone levels (P<0.006). Triple infections with *A. marginale, Babesia* spp. and *T. vivax*, also produced a significant

TABLE II

SERUM TESTOSTERONE LEVELS AND HEMATOCRIT VALUES OF BULLS INFECTED OR NOT WITH *Anaplasma marginale, Babesia* spp. AND/OR *T. vivax*

	Nº	Testosterone (ng/ml)	Hematocrit (%)
Anaplasma marginale positive	20	15.7 ± 4.3	34.6 ± 4.0*
Anaplasma marginale negative	23	16.1± 5.2	38.2 ± 5.5
Babesia spp positive	21	15.7 ± 5.3	36.1 ± 4.9
Babesia spp negative	22	16.1 ± 4.3	36.9 ± 5.5
T. vivax positive	28	15.2 ± 5.5†	36.9± 5.2
T. vivax negative	13	17.5 ± 2.8	35.6± 4.5
Co-infection *A. marginale* and *Babesia* spp	14	16.7 ± 4.4	35.3 ± 4.2
A. marginale and Babesia spp negative	16	17.1 ± 4.4	38.4 ± 5.5
Co-infection *A. marginale* and *T vivax*	10	14.9 ± 5.5‡	34.8 ± 3.7
A. marginale and *T vivax* negative	5	18.8 ± 2.5	38.8 ± 5.5
Co-infection *Babesia* spp and *T. vivax*	13	14.9 ± 6.4§	36.9 ± 4.8
Babesia spp and *T. vivax* negative	7	17.6 ± 2.9	37.1 ± 5.3
Co-infection *A. marginale*, *Babesia* spp and *T. vivax*	6	16.3 ± 6.4ϕ	36.0 ± 3.2
A. marginale, Babesia spp and *T. vivax* negative	5	18.8 ± 2.5	38.8 ± 5.5

Results are expressed as average ± SD. Only 20- 65 month-old bulls were included. Infections were evaluated through PCR. *P<0.02 in comparison with the *A. marginale* negative group. †P<0.01 in comparison with the *T. vivax* negative group. ‡P<0.006 in comparison with the *A. marginale* and *T vivax* negative group. §P<0.006 in comparison with the co-infection *Babesia* spp. and *T. vivax* negative group. ΦP<0.02 in comparison with the triple negative for *A. marginale, Babesia* spp. and *T. vivax* group. Data were evaluated by Student's t test.

decrease in testosterone levels, as compared to the non-infected, negative group. In relation to hematocrit, bulls infected with *A. marginale* presented statistically lower levels as compared to the non-infected group (P<0.02).

In a study carried out by Boly et al. [10], the effects of *Trypanosoma congolense* infection on the pituitary gland of Baoulé bulls were evaluated through immunohistochemistry of LH- and FSH-secreting cells and response of plasma LH and testosterone to GnRH treatment. Based on the immunohistochemical study and the pituitary response to GnRH, they concluded that these parasites do not alter pituitary function, but affect testicular function.

On the other hand, a dromedary bull (*Camelus dromedarius*) experimentally infected with *Trypanosoma evansi* showed" decreasing serum testosterone levels [2] similar to male rats (*Rattus rattus*) infected with *T. evansi* [20]. These data agree with this study, where bulls infected with another species of trypanosome, *T. vivax* also presented lower levels of testosterone. In addition, previous studies reported that *T. vivax* infection reduced reproductive capacity, semen quality, with damage to both testis and epididymis, in bulls [1, 16, 17, 39, 40] and in sheep (*Ovis aries*) [6]. It is possible that the decrease of testosterone levels in the present research was due to dysfunction in Leydig cells in the testicular interstice or to reduced sensitivity of the Leydig cells to circulating LH [33]. Most studies on the relationship between trypanosomosis and serum testosterone are carried out in experimentally infected animals. Interestingly, the present study showed a decrease in testosterone levels in bulls naturally infected with *T. vivax*.

CONCLUSION

This study showed that *A. marginale*, *Babesia* spp., and *T. vivax,* assessed through PCR molecular diagnosis, showed the highest prevalence in Holstein bulls. Interestingly, the prevalence of infection seems to be affected by the age of the cohort. These findings show the influence and importance of breed and age in the selection of individuals for prevalence studies. Bulls infected with *T. vivax* presented lower levels of serum testosterone that could affect the fertility, while asymptomatic bulls, either infected or non-infected with *A. marginale* and *Babesia* spp. showed no differences in serum testosterone levels.

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