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Serological survey of *Anaplasma* sp. in sheep from State of Alagoas, Brazil

Levantamento sorológico para "Anaplasma" sp. em ovinos do Estado de Alagoas, Brasil

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SUMMARY

In Brazil, information concerning anaplasmosis in small ruminants is scarce. In this paper, it is shown an epidemiological survey of *Anaplasma* sp. in sheep from three micro-regions (Sertão, Zona da Mata and Agreste) of Alagoas, Northeast of Brazil, by enzyme linked immunosorbent assay - ELISA with recombinant major surface protein - MSP5 of *Anaplasma marginale*. The frequency of seropositive sheep was 8.92% (20/224). Among regions, frequencies were: Sertão - 6.89% (2/29), Zona da Mata – 10.46% (18/172), and Agreste – 0% (0/23). Differences in the frequencies of seropositive sheep were not statistically significant. However, the lower frequencies were detected in the semi-arid micro-regions (Sertão and Agreste), possibly, due to the unsuitable climatic conditions for the development of tick vectors. These findings point to the need of further studies, to elucidate epidemiological and economic aspects of sheep anaplasmosis in the region.

Keywords: epidemiology, MSP5, northeast, small ruminant

RESUMO

No Brasil, informações acerca da anaplasmose em pequenos ruminantes são escassas. Neste artigo, é apresentado um *levantamento* epidemiológico para *Anaplasma* sp., em ovinos provenientes de três micro-regiões (Sertão, Zona da Mata e Agreste) do estado de Alagoas, Nordeste do Brasil, por ELISA com MSP5 recombinante de *Anaplasma marginale*. A frequência de ovinos soropositivos foi de 8,92% (20/224). Entre as regiões, as frequências foram: Sertão – 6,89% (2/29), Zona da Mata – 10,46% (18/172) e Agreste – 0% (0/23). Diferenças entre as ocorrências de ovinos soropositivos não foram estatisticamente significativas. No entanto, a baixa frequência detectada na região do Sertão e Agreste em relação à Zona da Mata possivelmente ocorreu devido à influência das condições climáticas sobre o desenvolvimento dos carrapatos vetores. Esses achados pontuais necessitam de futuros estudos para elucidar os aspectos epidemiológicos e econômicos da anaplasmose ovina na região.

Palavras chave: epidemiologia, MSP5, nordeste, pequenos ruminantes

INTRODUCTION

Anaplasmosis is caused by intraerythrocytic rickettsias of the genus *Anaplasma* (Rickettsiales: Anaplasmataceae) (DUMLER et al., 2001), and ticks are invertebrate hosts of *Anaplasma* sp. A number of vertebrate species can be infected by this genus, including ruminants. Cattle are infected by *A. marginale* (including the subspecies *centrale*), *A. bovis*, and *A. phagocytophilum*, while sheep and goat are infected by *A. ovis* and *A. phagocytophilum* (DUMLER et al., 2001). *Anaplasma marginale* was also described, experimentally, infecting sheep (SHARMA, 1988).

Bovine anaplasmosis is endemic in most of the Brazilian territory (SOUZA et al., 2000; SANTOS et al., 2001; SOUZA et al., 2001; ARAÚJO et al., 2005; BARROS et al., 2005). Nevertheless, little information is available, concerning ovine/caprine anaplasmosis, in despite of the expressive number of sheep, goat and the expansion of small ruminants herds in this country (ANUALPEC, 2006; RAMOS et al., 2008).

Diagnosis of anaplasmosis in small ruminants is based, mainly, on the identification of the rickettsia in stained blood smears. However, rickettsemias below 0.1%, in chronic carriers, are not detected by this method (PALMER, 1992). Serological assays, based on Major Surface Protein 5 (MSP5) of *A. marginale* have been successfully used, for the detection of antibodies against *Anaplasma* sp., as this protein is highly conserved (DREHER et al., 2005; ALLEMAN et al., 2006; STRIK et al., 2007). The amino acid sequence of MSP5 of *A. marginale* has similarities of 63% to 96%, among different species of *Anaplasma*, and similarities of 47% to 54%, with MAP2 (*major antigenic protein* 2) of *Ehrlichia* (DREHER et al., 2005; STRIK et al., 2007). Serological cross-reactions have been observed between MSP5 of *A. marginale*, *A. centrale*, *A. phagocytophilum* and *A. ovis* (NDUNG'U et al., 1995; DREHER et al., 2005), as well as between MSP5 of *A. phagocytophilum* and *Anaplasma platys*, *Ehrlichia canis*, and *Ehlichia chaffeensis* (STRIK et al., 2007).

This work reports a survey of *Anaplasma* sp. in sheep from Alagoas State, Northeastern region of Brazil, using an ELISA based on MSP5 recombinant of *A. marginale*.

MATHERIAL AND METHODS

Serum samples were obtained by non-probabilistic convenience, according to Costa Neto (1977), from 224 sheep, from three micro-regions of the State of Alagoas, Brazil: Zona da Mata (172), Agreste (23), and Sertão (29). Negative control sera were obtained of sheep clinical healthy, tick free and negative to *Anaplasma sp*. by blood smear examination and PCR for *msp4*. The positive sera were obtained from two sheep inoculated subcutaneously with approximately 1μg of initial bodies lisate of *A. marginale* for three times at seven days intervals. The first inoculation was made with MPL + TDM + CWS adjuvant (Sigma, M-6661) and others with Freund's incomplete adjuvant (Sigma, F-5506), following the manufacture's instructions.

The detection of antibodies against *Anaplasma* sp. was done by ELISA with recombinant MSP5 of *A. marginale*, conforming described by

Melo et al. (2007) adapted for sheep (RAMOS et al., 2008). ELISA 96-wells plates (Greiner Bio One, Microlon, 65101) were adsorbed with 16 ηg/well of the recombinant protein, diluted in PBS with 0.01% tween-20 (PBST) and incubated at 4°C for 12 h. After that, plates were blocked with PBST, with 5% fat free milk, and incubated at 37°C for 60 min. After five washes with PBST, test and control sera, diluted 1:200 in PBST, were incubated at 37°C for 60 min. After five washes, as described, anti-sheep IgG peroxidase conjugated (Sigma), diluted 1:10,000 in PBST, was added. After 30 min, at 37°C, plates were washed as described, and chromogen Fast-OPD (Sigma) was added. Reactions were stopped after 20 min with 2.5N H_2SO_4, and absorbances were obtained on a Bio-Tek EL-800 ELISA reader, with a filter of 490 ηm. The cutoff was calculated for each plate, according to Frey et al. (1998).

The frequency differences of seropositive sheep among micro-regions were analyzed by Fischer's exact test, with a level of significance of 5%.

RESULTS AND DISCUSSION

The overall frequency of seropositive sheep was 8.9% (20/224). The frequencies in the three micro-regions were: Sertão – 6.89% (2/29), Zona da Mata – 10.46% (18/172), and Agreste – 0% (0/23). These frequencies were low, compared to the prevalences of seropositive sheep found by Hornok et al. (2007) (99.4%) in Hungary. Seroprevalences were found by De La Fuente et al. (2005) (75.0%), in Sicily, Italy, using competitive ELISA, based on recombinant MSP5 of *A. marginale* and monoclonal antibody ANAF16C1, and found by Ramos et al. (2008) (16.17%) in Ibimirim county, semi-arid region of Pernambuco State, Brazil.

The low frequency of seropositive sheep to *Anaplasma* sp. in Alagoas can be attributed to the reduced parasitism by ticks found in sheep in this study. Although, no counting was done, and ticks were not observed in sheep when blood samples were taken.

Differences in the frequencies of seropositive sheep among micro-regions were not statistically significant ($P > 0.05$). However, the lower frequencies were detected in the semi-arid micro-regions, possibly, due to the unsuitable climatic conditions of vectors development.

Due to the conservation between organisms of Anaplasmataceae family, MSP5 of *A. marginale* has been utilized as antigen for detection of Anaplasma in sheep, since 1995 (NDUNG'U et al., 1995), and, recently, a competitive ELISA based on this proteins have been validated for detection of antibodies for *Anaplasma* sp. in this species (SCOLES et al., 2008). Although, these are important tools for epidemiological study of infection by *Anaplasma* sp., ELISAs based on MSP5 recombinant of *A. marginale* no differentiate species (DE LA FUENTE et al., 2005).

This report represents the first description of antibodies for *Anaplasma* sp. in sheep from Alagoas State. More studies are necessary to understand the epidemiology of *Anaplasma* sp. infections in sheep, in Brazil, especially to define which species is involved, vectors and possible impacts in animal production and in public health.

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