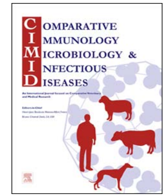
Comparative Immunology, Microbiology and Infectious Diseases 55 (2017) 1–5

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

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



Mycoplasma ovis infection in goat farms from northeastern Brazil

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ARTICLE INFO

Keywords:
Hemotropic mycoplasma
Hemoplasma
Mycoplasma spp.
Small ruminants
Anemia

ABSTRACT

Although *Mycoplasma ovis* (formerly *Eperythrozoon ovis*) has been described in small ruminants worldwide, data on *M. ovis* in goats remain scarce. Accordingly, the aims of the present study were to i) determine the prevalence of hemoplasmas in goats, ii) identify the tick species parasitizing the animals, and iii) determine factors associated with infection in five dairy and three beef goat farms from the Paraíba State, northeastern Brazil. Blood samples were obtained from 402 goats. Samples were screened for hemoplasmas using a pan-hemoplasma PCR. The positive samples were confirmed by sequencing. An epidemiological questionnaire was given to each farm owner addressing age, gender, and presence of ticks. A total of 158/402 (39.3%) goats were positive for *M. ovis* by PCR. Sequencing of PCR positive samples has shown ≥99% identity with multiple *M. ovis* 16S rDNA sequences deposited in GenBank, including *M. ovis* isolates from humans. Dairy (OR = 2.15; 95% CI: 1.40–3.32%; $P = 0.0004$) and anemic goats (OR = 2.33; 95% CI: 1.51–3.71%; $P = 0.0001$) were more likely to be infected than beef and non-anemic animals, respectively. *Amblyomma parvum* (49/52, 94.23%) and *Rhipicephalus microplus* (3/52, 5.77%) were the tick species found parasitizing the animals, with no significant association between the presence of ticks and infection by *M. ovis* ($P = 0.1164$). This is the first reportedly molecular detection of *M. ovis* infection in goats from South America. In conclusion, *M. ovis* is highly prevalent in goats from northeastern Brazil, mainly in dairy animals.

1. Introduction

Hemotropic mycoplasmas (hemoplasmas) are small pleomorphic bacteria, which attach to the surface of erythrocytes and may cause hemolytic anemia in a wide variety of mammals worldwide [1]. Based on the detection of 16S rDNA gene fragments, two hemoplasma species have been initially found infecting small ruminants, *Mycoplasma ovis* (formerly *Eperythrozoon ovis*) and '*Candidatus* Mycoplasma haemovis' [2]. However, the complete genome sequence of *M. ovis* strain Michigan contains two copies of the 16S rDNA genes, corresponding to the previously reported sequences for *M. ovis* and '*Ca.* M. haemovis' [3]. Thus, whether these hemoplasmas are a single species or separate strains/species remains to be fully established.

While *M. ovis* has been identified as a cause of hemolytic anemia crisis in sheep [4], goats may develop shorter and less severe bacteremia, and may act as chronic and latently infected carriers [5]. Data on *M. ovis* in goats have been scarcely reported, with prevalence ranging from absent in Australia, Tunisia and Morocco [5–8] to 20% or 94% in Hungary and Malaysia, respectively [2,9]. In South America, to the authorś knowledge, no study has been performed on prevalence and factors associated with *M. ovis* infection in goats to date. However, *M. ovis* has already been detected in deer from Brazil [10,11] and in sheep from Argentina [12], showing highly likelihood of infection in domestic goats.

Mycoplasma ovis can be transmitted by several blood-sucking arthropods, including ticks (*Haemaphysalis plumbeum* and *Rhipicephalus bursa*) and mosquitoes (*Aedes camptorhynchus*) [4]. In Brazil, nymphs of *Amblyomma sculptum* [13] and adult forms of *Amblyomma parvum* [14,15] and *Rhipicephalus microplus* [15] ticks have been identified parasitizing goats. Considering that neither *H. plumbeum* nor *R. bursa* have not been described in this country, studies regarding *M. ovis* infection and related tick species in goats are needed.

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http://dx.doi.org/10.1016/j.cimid.2017.08.004
Received 6 July 2017; Received in revised form 23 August 2017; Accepted 23 August 2017
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Goat production has been of great socioeconomic importance in northeastern Brazil, which concentrates 91% of goat herds [16]. Characterized by a tropical climate and limited economical resources, goat farming in this region has been mostly for sustaining livelihoods [16]. Considering that *M. ovis* has already been found infecting human beings [17,18], close and frequent human-animal contact on local goat production associated with the presence of arthropod vectors may represent a risk for public health. Accordingly, the aims of the present study were to i) determine the prevalence of hemoplasmas in goats, ii) identify the tick species parasitizing the animals, and iii) determine factors associated with infection in five dairy and three beef goat farms from the Paraíba State, northeastern Brazil.

2. Material and methods

2.1. Ethical approval

This study was approved by the Ethics Committee for Animal Experimentation and Animal Welfare at Universidade Federal da Paraíba (protocol 3305/14), and conducted according to the ethical principles of animal experimentation, adopted by the Brazilian College of Animal Experimentation.

2.2. Cross-sectional study

The sample size was calculated [19] based on an estimated number of 478,083 goats [16] with an expected prevalence of 50%. Thus, the minimum sample size required for detecting a difference with a 95% confidence level at 5% was estimated as n = 384. A total of 402 goat blood samples (35 males and 367 females) were collected from five dairy and three beef farms in the state of Paraíba (Fig. 1). As the only farm with certification for high-quality management practices by the Brazilian Ministry of Agriculture, the beef farm at Juarez Távora was used as the reference farm for statistical comparisons.

2.3. Sampling

Goat blood samples (up to 5 mL) were collected by venipuncture of the jugular vein using commercial tubes containing EDTA (BD Vacutainer®, Franklin Lakes, NJ, USA). An epidemiological questionnaire was given to each farm owner addressing age, gender, and presence of ticks. The age of the goats was stratified into groups of ≤ one year and > one year.

Ticks were removed using a commercial hook (ÓTom/Tick Twister®, H3D Inc., Lavancia, France), and kept in 70% ethanol-labeled tubes for further classification according to morphological taxonomic keys [13,20–22].

2.4. Packed cell volume and total plasma protein

The packed cell volume (PCV) and total plasma protein (TPP) were measured by routine centrifugation and refractometry techniques; a PCV of < 0.22 L/L and a TPP of > 75 g/L were used as indicators of anemia and hyperproteinemia, respectively [23]. Thereafter, blood aliquots were stored at −20 °C until molecular testing.

2.5. DNA extraction

DNA was extracted from 200 μL of whole blood using a commercial available kit (Illustra™ blood genomicPrep Mini Spin Kit, GE Healthcare, Little Chalfont, UK), according to the manufacturer's instructions. Negative control purifications using ultra-pure water were performed in parallel to monitor cross-contamination in each batch of 30 samples.

2.6. PCR assays

A conventional PCR for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed to ensure successful DNA extraction, as previously described [24].

Samples were screened for hemoplasmas using previously described pan-hemoplasma primers targeting the 16S rDNA gene (~900 bp) [25]. A goat-positive blood sample for *M. ovis* and ultrapure water were used as positive and negative controls, respectively. Specificity was evaluated using known positive samples for *Mycoplasma haemofelis*, *M. haemocanis*, *M. suis*, *M. wenyonii*, '*Candidatus* M. haemominutum', '*Ca.* M. turicensis', '*Ca.* M. haemobos', and *Anaplasma marginale*. The detection limit of the assay was measured by testing tenfold dilutions of the recombinant plasmid containing the 16S rRNA gene of *M. haemofelis* (from 10^9 to 1 copy of plasmid/reaction).

The amplified PCR products were subjected to gel electrophoresis in 1.5% agarose gels for one hour at 100 V, followed by SYBR safe staining

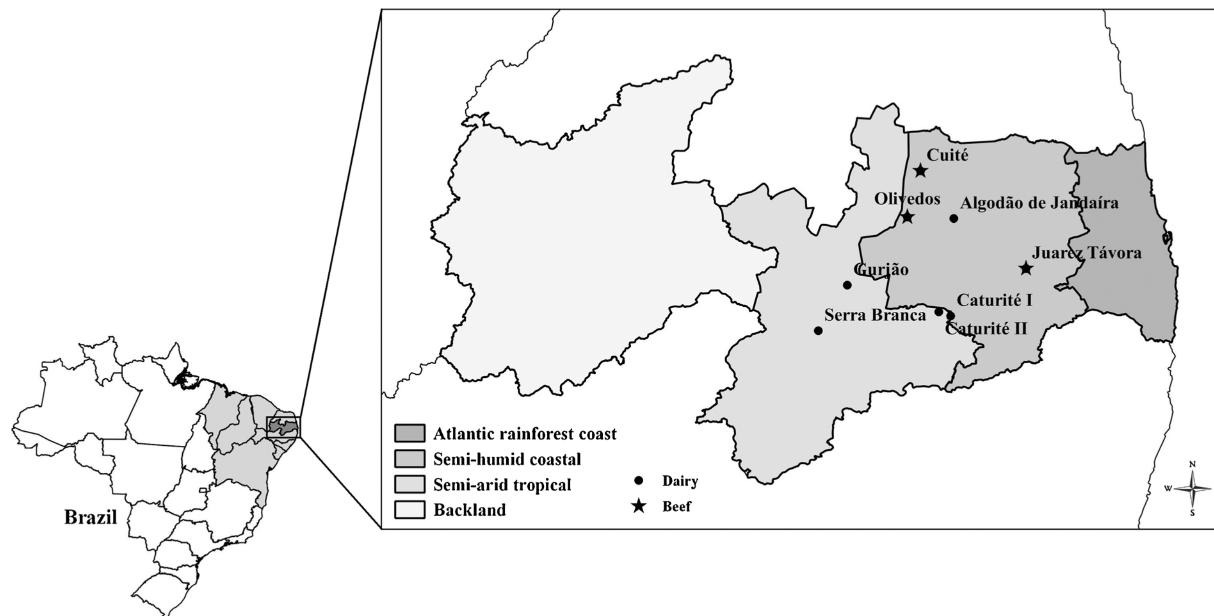

Fig. 1. Geographical locations of farms (dairy and beef) used in the study and limits of the climate in Paraíba, Northeast, Brazil.

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