The objective of this study was to carry out a microbiological and molecular research of Streptococcus equi subspecies equi in the Rio Branco micro-region, Acre, Brazil, as well as to assess the risk factors. Sixty-four horses were submitted to clinical evaluation, from which nasal secretion samples were collected for bacteriological culture. The isolates were submitted to DNA extraction and PCR. The risk factors were verified through epidemiological questionnaires. It was verified that 32.8% (21/64) of the animals presented clinical signs suggestive. In the PCR, 50 (78.1%) samples were phenotypically compatible to genus, but none of them showed amplification of the 16S rRNA gene. The lack of disinfection of facilities and vaccination were the most frequent risk factors. It was concluded that Streptococcus equi subspecies equi is not present in the studied population, however, it may be susceptible to the disease due to the lack of sanitary criteria in the breeding.

Keywords: Strangles; epidemiological study; Western Amazonia.
In addition, nasal secretion samples were collected with *swab* by scrolling movements through the nostrils of the animals (Figure 1), being conditioned in *Stuart* medium and transported under refrigeration (MCVEY et al., 2016) to the Laboratory of Infectious Diseases of Animals of the Federal University of Acre.

The biological material was kept in a 5% sheep blood agar medium and incubated at 37°C for 24 hours, for later macroand microscopic analysis. Small-sized and whitish-cream colonies, formed by coccoid-type bacteria of the type Gram positive, aerobic, catalase negative and β-hemolytic from group C of Lancefield (TRABULSI, 2008) were considered.

Phenotypically compatible isolates were transferred to *eppendorf* type tubes, with 1ml of distilled water; and frozen at -20°C (MCVEY et al., 2016), which were sent to DNA extraction (MANIATIS et al., 1989). The extraction product was submitted to the polymerase chain reaction, aiming at the amplification of the 16SrRNA gene, with expected fragment size of 435 base pairs (BAVERUD, 2007).

The data collected were organized in an Excel spreadsheet and the results were expressed by descriptive statistics in absolute and relative frequency, being presented in the form of tables (THRUSSFIELD, 2004). In the clinical evaluation of the horses under study, it was verified that 32.8% (21/64) of the animals presented clinical signs suggestive of equine adenitis, such as weight loss, dehydration and nasal secretion (Table 1). Changes in respiratory rate and temperature, as well as lung noise and abscess head injuries, were not observed.

Table 1. Frequency of clinical signs suggestive of equine adenitis in animals of the Rio Branco-AC/Brazil micro-region.

<table>
<thead>
<tr>
<th>Clinical Signs</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Loss</td>
<td>18.7% - 12/64</td>
</tr>
<tr>
<td>Dehydration</td>
<td>12.5% - 7/64</td>
</tr>
<tr>
<td>Nasal secretion</td>
<td>7.8% - 5/64</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>4.6% - 3/64</td>
</tr>
<tr>
<td>Cough</td>
<td>3.1% - 2/64</td>
</tr>
<tr>
<td>Lymph node hypertrophy</td>
<td>1.5% - 1/64</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>1.5% - 1/64</td>
</tr>
<tr>
<td>Total</td>
<td>32.8% - 21/64</td>
</tr>
</tbody>
</table>

In the microbiological analysis of the isolates, 50 (78.1%) samples were phenotypically compatible to the *Streptococcus* genus (Figure 2). However, none of these presented amplification for the 16SrRNA gene of *S. equi* subspecies *equi* (Figure 3), demonstrating that the horses evaluated in this study were not carriers of the pathogen.

Regarding the risk factors, in all the properties (100% - 6/6) there was no disinfection of facilities and vaccination against equine adenitis; in 83.3% (5/6) there was no quarantine and disinfection of utensils; in 66.6% (4/6), these instruments of work were shared; and 33.3% (2/6) had no veterinary assistance, had shared drinking fountains and feeders, and had recently purchased animals. Regarding the type of animal exploitation, 66.6% (4/6) used the animals for work, 16.6% (1/6) for leisure and 16.6% (1/6) for reproduction.

According to the results obtained, it is believed that the absence of biochemical tests (fermentation of sugars) is a factor that directly influenced in the inadequate selection of samples, bringing limitations to the PCR result, as bacteria of the *Streptococcus* genus are part of the natural equidae microflora (FONSECA, 2010).

Bacterial isolation is a widely used method of diagnosis for the disease, but it is a slow technique with low sensitivity. In turn, molecular tests have high specificity, reducing the detection of false positive animals, which gives greater credibility to the results (CORDONI et al., 2015).

Although no equines positive for *S. equi* subspecies *equi* were identified in this study, the clinical-epidemiological evaluation allows us to state that the animals may be at risk of getting the disease later. Their physical conditions and the lack of more stringent sanitary criteria for the acquisition and introduction of new animals, as well as the inefficient procedures of disinfection in the breeding, are characterized as predisposing factors (MORAES et al., 2009).

According to Panzini & Carneiro (2008), the transmission of equine adenitis occurs through direct contact or shared use of utensils, as well as feeders and drinking fountains, observed in 66.6% and 50% of the studied properties, respectively. The pathogen can survive for weeks in the environment, depending on the climatic characteristics of the region, and the fomites are extremely relevant in the epidemiology of the disease (MORAES et al., 2009).

None of the evaluated properties performed disinfection of facilities and vaccination of the herd against equine adenitis. Thus, a focus of the disease could quickly become an outbreak. Although not fully effective, the vaccine provides immunization in about 50% of vaccinated individual and, in case of outbreaks, vaccinated animals tend to develop the milder form of the disease. In addition, other aspects such as absence of quarantine (83.3%), recent acquisition of animals (33.3%) and lack of veterinary assistance (33.3%) also allow the entry of the disease into a healthy herd (KIRINUS, 2010; LIBARDO, 2015).

It was concluded that the *Streptococcus equi* subspecies *equi* is not present in the studied population. However, it may be susceptible to the disease due to the lack of strict sanitary criteria in the breeding. Therefore, further studies in broader areas would be of great importance in order to demonstrate the real incidence and prevalence of the disease in the state of Acre.
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