

Microbiological and molecular evaluation of *Streptococcus equi* subspecies *equi* and risk factors associated to equine adenitis in rural properties of Rio Branco Micro-Region, Acre, Brazil

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ABSTRACT

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 16SrRNA 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.

Avaliação microbiológica e molecular do *Streptococcus equi* subespécie *equi* e fatores de risco associados à adenite equina em propriedades rurais da microrregião de Rio Branco, Acre, Brasil

RESUMO

Objetivou-se realizar uma pesquisa microbiológica e molecular do *Streptococcus equi* subesp. *equi* na microrregião de Rio Branco, Acre, bem como avaliar os fatores de risco. Foram submetidos à avaliação clínica 64 equinos, dos quais se colheu amostras de secreção nasal para cultivo bacteriológico. Os isolados foram encaminhados à extração de DNA e PCR. Os fatores de risco foram verificados por meio de questionários epidemiológicos. Verificou-se que 32,8% (21/64) dos animais apresentavam sinais clínicos sugestivos. Na PCR, 50 (78,1%) amostras foram compatíveis fenotipicamente ao gênero, mas nenhum destes apresentou amplificação do gene 16SrRNA. A ausência de desinfecção de instalações e vacinação foram os fatores de risco de maior frequência. Conclui-se que o *Streptococcus equi* subesp. *equi* não está presente na população estudada, porém, esta pode estar suscetível à doença, devido à falta de critérios sanitários nas criações.

Palavras-chave: Garrotinho, estudo epidemiológico, Amazônia Ocidental.

Brazil's equine herd is the largest one in Latin America and the third one in the world, totaling about 5 million horses, which generates a profit of 16 billion Reais for the country, in addition to direct and indirect jobs (MAPA, 2016). In the state of Acre, 94.821 horses are estimated and currently, the local equideoculture is under development (IBGE, 2016), which makes it essential to control the health situation of the herds (REED; BAYLY, 2000).

In this context, equine adenitis is an acute contagious infectious disease with a worldwide distribution, caused by *Streptococcus equi* subspecies *equi*, which generates significant damage to equine breeding (KIRINUS, 2010; LIBARDONI, 2015). This disease is characterized by mucopurulent inflammation of the upper respiratory tract, manifested by nasal discharge, cough, fever, anorexia, depression and abscess lymphadenitis, mainly of submandibular and retropharyngeal lymph nodes (SILVA; VARGAS, 2006; MORAES et al., 2009; FLOCK et al., 2012).

The transmission of the disease occurs by direct or indirect contact. Risk factors such as stress, overwork, overcrowding, malnutrition, inappropriate transportation, parasitism and concomitant diseases make the animals susceptible to the pathogen (AMARAL et al., 2008).

In the state of Acre, there are no reports on the occurrence of equine adenitis, generating the need for a preliminary epidemiological analysis of the state herd (SILVA; VARGAS, 2006).

Thus, the objective of this study was to carry out a microbiological and molecular research of *Streptococcus equi* subspecies *equi* in rural properties of Rio Branco micro-region, state of Acre, Brazil, as well as to assess the risk factors to which the equines are exposed.

This study was approved by the Committee on Ethics in the Use of Animals (CEUA) of the Federal University of Acre (UFAC), Rio Branco campus, under license N. 09/2016. The sample population was composed of male and female equines, between six months and six years old, submitted to semi-intensive breeding systems and belonging to the municipalities of Rio Branco, Senador Guiomard, Plácido de Castro and Porto Acre, in the state of Acre, Brazil.

Six rural properties were selected by non-probabilistic convenience and 64 horses were randomly selected. These were submitted to clinical evaluation to record the following variables: age, gender, weight, respiratory rate, heart rate, temperature, nutritional status, degree of hydration, vaccination and presence of clinical signs (RADOSTITS et al., 2014).

The survey of risk factors was carried out by applying an epidemiological questionnaire to the owners (SWEENEY et al., 2005). The variables analyzed were: type of exploration, age, body score, type of installation, feeders and drinking fountains, cleaning utensils, veterinary assistance, animal acquisition, quarantine, disinfection of facilities and vaccination (MORAES et al., 2009).

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.



Figure 1. Collection of equine nasal secretion.

The biological material was kept in a 5% sheep blood agar medium and incubated at 37° C for 24 hours, for later macro and 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 *ependorfs* 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 (THRUSFIELD, 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.

Clinical signs	Frequency
Weight Loss	18.7% - 12/64
Dehydration	12.5% - 8/64
Nasal secretion	7.8% - 5/64
Epistaxis	4.6% - 3/64
Cough	3.1% - 2/64
Lymph node hypertrophy	1.5% - 1/64
Arrhythmia	1.5% - 1/64
Total	32.8% - 21/64

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.

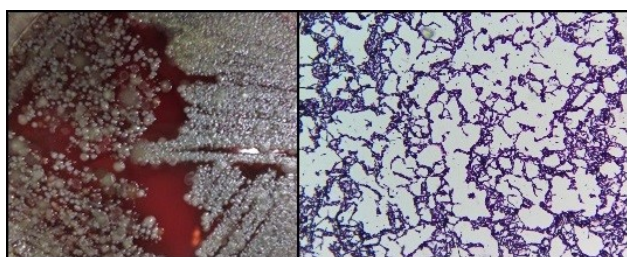


Figure 2. Isolates phenotypically compatible with *Streptococcus* genus.

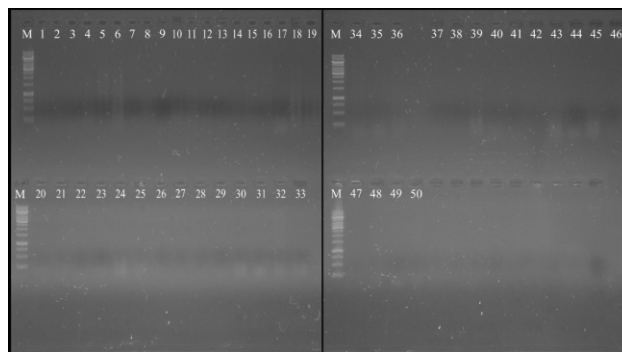


Figure 3. Molecular analysis of the isolates by polymerase chain reaction (Note M - molecular marker).

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 microbiota (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 individuals 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; LIBARDONI, 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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