Ciência Animal Brasileira

DOI: 10.1590/1809-6891v21e-59173

Seropositivity for Maedi-Visna virus in sheep in Porto Acre city – Western Amazon, Brazil

Soropositividade para o vírus Maedi-Visna em ovinos da cidade de Porto Acre – Amazônia Ocidental, Brasil

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Section: Medicina Veterinária

Received
June 24, 2019.
Accepted
March 3, 2020.
Published
June 16, 2020.

www.revistas.ufg.br/vet
visit the website to get the how to cite in the article page.

Abstract
Lentivirosis of small ruminants (LVPR) are chronic and degenerative infectious diseases, caused by Lentivirus, associated with numerous losses such as: drop in meat and milk production, predisposition to secondary infections, expenses with veterinary assistance and, even, early disposal of animals. In the northern region of Brazil, the epidemiological situation is poorly understood. Thus, this study aimed to determine the seropositivity of sheep for Lentivirus in Porto Acre city, Western Amazon, Brazil. 122 blood samples from sheep were collected and as a diagnostic method, agarose gel immunodiffusion was used, using the p28 protein of the capsid as antigen. The seropositivity of the sheep to the test was 8.2% (10/122). In 80% (4/5) of the investigated properties, the presence of seropositive animals was detected. It is worth noting that the acquisition of small ruminants from other states likely represented a risk to sheep health in the municipality of Porto Acre, Western Amazon, Brazil. It is concluded that there is a need for more systematic investigations on the prevalence of LVPR in the state of Acre.
Keywords: Lentivirus; Small ruminants; Amazon region; Serology.

Resumo
As lentiviroses de pequenos ruminantes (LVPR) são enfermidades infecciosas crônicas e degenerativas, causadas por Lentivírus, associadas a inúmeros prejuízos como: queda na produção de carne e leite, predisposição a infecções secundárias, gastos com assistência veterinária e, até mesmo, descarte precoce dos animais. Na região norte do Brasil, a situação epidemiológica é pouco elucidada. Objetivou-se, assim, por meio deste estudo, determinar a soropositividade de ovinos para Lentivírus no município de Porto Acre, Amazônia Ocidental, Brasil. Foram coletadas 122 amostras de sangue de ovinos e como método diagnóstico foi empregada a imunodifusão em gel de agarose, utilizando a proteína p28 do capsídeo como antígeno. A soropositividade dos ovinos ao teste foi de 8,2% (10/122). Em 80% (4/5) das propriedades investigadas, detectou-se a presença de animais soropositivos. É válido ressaltar ainda que a aquisição de pequenos ruminantes advindos de outros estados

provavelmente representou um risco à sanidade ovina no município de Porto Acre, Amazônia Ocidental, Brasil. Conclui-se que existe a necessidade de mais investigações sistemáticas sobre a prevalência de LVPR no estado do Acre.
Palavras-chave: Lentivírus; Pequenos ruminantes; Região amazônica; Sorologia.

Introduction

The first sheep introduced in the state of Acre came from the Northeast in the second half of the 19th century and at the beginning of the 20th century, during the period of rubber exploitation [1]. Since 2004, sheep farming has grown constantly due to the intensification of meat production for self-consumption and the diversification of income in small and medium farms, with the effective number of sheep in the state at 52,559 animals [2,3].

However, the sheep production chain in the state of Acre has not yet been consolidated, as the degree of technification in the properties is precarious, the extensive breeding system is prevalent and for the replacement of animals the uncontrolled natural breeding season is carried out. Product marketing logistics are also hampered by poor road infrastructure [2]. In addition, infectious and parasitic diseases associated with insufficient technical assistance represent an important obstacle to health and productivity in herds [4].

Lentivirosis of small ruminants (LVPR) are infectious diseases caused by Lentiviruses [5], which result in economic losses in sheep farming and slow the growth of the production chain [6], however, due to their chronic evolution, they may go unnoticed by the producer [7]. The disease triggers inflammatory, degenerative and immune-mediated lesions in the neurological, respiratory, mammary gland and joints [8,9,10] of the animals. Lentiviruses are classified into five phylogenetic groups and their viral prototypes for sheep and goats are the maedi-visna virus (MVV) and the caprine arthritis encephalitis virus (CAEV), respectively, although the possibility of interspecies transmission has already been demonstrated [11, 12.13].

In the northern region, the lentivirosis of small ruminants and the impacts on production are only described in the states of Amazonas [14] and Tocantins [15,16]. It is believed that the survey of epidemiological data in a region can bring relevant information about viral circulation and possible risk factors, allowing for better planning of prophylactic measures and control of lentivirosis in herds, and minimizing impacts by decreasing the production of meat and milk, birth of weak offspring, the predisposition to secondary infections, early slaughter and commercial embargoes [17].

Thus, the objective of this study was to determine the seropositivity of sheep for maedi-visna virus in the municipality of Porto Acre, Western Amazon, Brazil, given that the situation in the state of Acre is still unknown.

Material and methods

This study was approved by the Animal Use Ethics Committee of the Federal University of Acre, under authorization No. 29/2017.

The state of Acre is located in the southwest of the northern region of Brazil in the Western Amazon, and is bordered by the Amazon to the north, Rondônia to the east, Bolivia to the southeast and Peru to the south and west. The region has an equatorial climate, with temperatures ranging between 25 and 40 ° C and forest vegetation in the Amazon. The state's territorial extension is 152,581,388 km², comprising 22 municipalities.

According to the last agricultural census, the population of sheep in the state is 52,557 heads, of which 5,544 animals are allocated in the municipality of Porto Acre (09 ° 35'18 "S; 67 ° 31'57" W) [3]; area selected for the study because it is provided with a program to strengthen the sheep chain, in addition to factors such as access to the property, the infrastructure of the place and the availability of producers to participate in the study.

The sample size calculation was based on descriptive epidemiological studies (18,19). The estimated prevalence was 1.62% [14], with a 95% confidence interval and statistical error of 5%, requiring at least 118 samples. For non-probabilistic convenience, five properties were selected in the municipality of Porto Acre, of which 20 to 25 animals were randomly assigned to the study, totaling 122 sheep. The profile of the studied population was then constituted by animals with aptitude for cutting, being 88 females and 34 males, of the Dorper races (4.1% - 5/122), Santa Inês (25.4% - 12/31) or without defined race (70.5% - 86/122), with a variable age range between two months to five years and an average weight of 25kg.

In a technical visit to the properties from July to October 2017, an evaluation form of the general characteristics of the creation (type of creation, degree of technification, origin of the animals and sanitary management) was completed through 15 objective questions [20]. The sheep were submitted to blood collection by puncture of the jugular vein, with a single sample per animal, which was placed in sterile tubes without anticoagulant (Vacutainer® tube) and transported under refrigeration to the Laboratory for Infectious Diseases of Animals of the Unit of Teaching and Research in Veterinary Medicine at the Federal University of Acre, Rio Branco-AC. The serum, extracted by centrifugation at 5,000 rpm at room temperature, was stored in 1.0 mL aliquots per sample and frozen at -20 ° C until serological tests were performed.

As a diagnostic method, agarose gel immunodiffusion (IDGA) was used to detect anti-MVV antibodies, using capsid p28 protein in a commercial kit (Biovetech Indústria e Comércio de Produtos Biotecnológicas LTDA, Recife, PE, Brazil), licensed by Ministry of Agriculture, Livestock and Supply (MAPA) under number 9050/2005. Positive samples were characterized by the formation of antigen-antibody precipitation lines on the gel, observed in 48 hours [21]. The results were analyzed using descriptive statistics, through relative and absolute distribution [18].

Results and discussion

According to the results of the IDGA, 8.19% (10/122) of the tested sheep were reactive for Lentivirus, being these three males (30% - 3/10) and seven females (70% - 7/10), all of them breed undefined and aged between two to three years. In 80% (4/5) of the properties investigated in the municipality of Porto Acre, the presence of seropositive animals was detected. The seropositivity rate in these creations, individually, varied between 4.0 and 14.2%.

Of the properties where a focus of lentivirosis was identified, 75% (3/4) were under extensive farming systems, without a balanced diet plan and without the adoption of sanitary control measures. The introduction of animals without quarantine was practiced in all (100% - 4/4) of the creations. As for the origin of the sheep, 75% (3/4) came from commercialization with neighboring creations of the municipality itself, however, in a property (25% - 1/4), the breeders and breeders were acquired through the Strengthening Program of the Sheep Productive Chain, implemented by the government of the state of Acre in 2010.

The animals identified as seropositive did not present an evident clinical picture. It is known that LVPRs develop through a progressive immune-mediated inflammatory response, with a long incubation period and chronic evolution, restricting the clinical diagnosis and promoting the perpetuation of inapparent carriers in the herd (6), which, in turn, serve as source of infection to the others susceptible [10, 22]. Thus, the use of laboratory tests is necessary to identify the infection, segregate and dispose of animals [23].

To detect the disease, the techniques of agarose gel immunodiffusion (IDGA), immunoenzymatic assay (ELISA) and polymerase chain reaction (PCR) are commonly used, which are frequent targets of comparative studies to determine the best diagnostic strategy [23 , 24,13,6,25]. The IDGA is one of the techniques recommended by the OIE (2017), used in Brazil as a screening tool in epidemiological surveys, mainly because it has a practical and economically viable methodology [26].

CAEV and MVV are considered viral prototypes of Lentivirus in goats and sheep, respectively. However, using the IDGA serological test, even using the MV28 capsid protein p28 as an antigen, seropositive animals may have been exposed to both viral agents, therefore, there is a need for molecular tests for taxonomic differentiation [13].

IDGA, when compared to ELISA, has good specificity, but low sensitivity, bringing further limitations to its use, given the possibility of false-negative results considering the single sample test [23]. That is, the frequency found in the study area may still be underestimated.

In general, serological methods may fail due to certain factors, such as the period of immunological window, low antibody titers, late seroconversion or intermittent seropositivity and seronegativity reactions [27]. On the other hand, molecular techniques, such as PCR, although of high cost, show promising results, allowing the detection of Lentivirus infection before seroconversion [24,28].

It has been shown that the combined use of the aforementioned tests increases the sensitivity and specificity of the diagnosis, facilitating the detection of infected animals and contributing to the effectiveness of control and eradication campaigns [11,23].

VM is found throughout Europe [29]. The disease-free countries are New Zealand and Australia. The prevalence is much higher in developed countries, which seems to be related to the management system [11]. In Brazil, the occurrence of LVPR in sheep has been the object of study in several states [21,30,19], mainly due to the increase in sheep farming in the country and the recommendations of the Sanitary Code for Terrestrial Animals for the commercialization of small ruminants at the international level [26].

Thus, national seroprevalence is low to moderate, with rates ranging between 0.1% and 7.9% [21,30,19], with the exception of Ceará, where there was a variation between null detection at 50.43 % in different regions [31]. In the northern region, until now, only in the states of Amazonas [14] and Tocantins [16] epidemiological surveys of LVPRs have been carried out, showing zero occurrence and seropositivity of 1.62%, respectively. The seropositivity observed in the city of Porto Acre - AC (8.2%) was higher than the other notifications in the national territory.

The type of breeding has a direct influence on seropositivity rates. Intensive systems increase the possibility of transmission of the pathogen through direct contact, aerosols [29] or even by breastfeeding [10]. In the municipality of Porto Acre, most properties adopt a semi-intensive or extensive management system, with low agglomeration and population density in the farms. However, these animals now have free access to childbirth residues and contaminated urogenital secretions in pastures, mainly because maternity paddocks are not routinely introduced to establishments in the region, which can increase the risk of infection [19].

In addition, the presence of risk factors, such as failures in health management, intercurrent diseases, absence of quarantine, inefficient technical assistance, and, mainly, the lack of investment in the diagnosis of infectious diseases in sheep must also be considered [29,32], a reality observed in the municipality of Porto Acre. This critical situation determines the introduction of infected animals, as well as the spread and perpetuation of the virus in herds.

The origin of the animals' acquisition is also an important factor to be discussed. The emergence of LVPR in the state of Acre may be related to a governmental action to encourage small producers, which allowed the introduction of breeding stock and breeders from other states, without rigidity in sanitary control. In 2010, the State Secretariat of Agriculture and Livestock of Acre prepared and financed the Project to Strengthen the Sheep Production Chain for donation of sheep to local producers, mainly in the municipalities of Porto Acre and Cruzeiro do Sul. The guarantee offered was the purchase of lambs by a state slaughterhouse, improving the income and quality of life of families living in the countryside. However, the animals provided by the Project for the formation of the Acre herd came from Bahia and São Paulo, states in which the LVPR had already been detected in previous research in an endemic way [33,34].

As there is no efficient treatment and the development of vaccines is not possible due to

the high rate of viral mutation caused by the variety of strains in different geographical regions, discarding seropositive animals and replacing them with seronegative animals is the most efficient measure to reduce the occurrence LVPR in the herd, although economically expensive.

Thus, the practice of quarantine, as well as the cleaning and disinfection of drinking fountains and feeders, the isolation of sheep in the puerperium, the investigation of animals with respiratory problems in the last five years or recently introduced are relevant actions for the control of the disease [35 , 36,27,37,6]. A recent international study also deals with genome research, envisioning the possibility of identifying genes that are candidates for resistance to Lentivirus infections, which may later be a reality in the country [38].

However, it is known that the actions of the National Health Program for Goats and Sheep (PNSCO / MAPA), which establish guidelines for the epidemiological surveillance of certain infectious diseases, are still minimal in practically the entire national territory. It is believed, then, that the restructuring of the Program, combined with constant epidemiological investigations carried out at the state and national levels, will contribute more solidly to the detection and elimination of LVPR outbreaks, improving the health of herds and stimulating the growth of the production of sheep across the country.

Conclusion

Seropositivity of sheep to MVV in the city of Porto Acre raises the possibility that the acquisition of small ruminants from other states represented a risk to sheep health and highlights the need for systematic investigations on the prevalence of LVPR and its risk factors in the state of Acre. Considering that its occurrence is inversely proportional to the consolidation of the local production chain, harmful to animal welfare and limiting market advantages, obtaining more information about the epidemiological condition of the disease promotes the development and adoption of control and prevention measures that avoid the entry of animals carrying the virus in the Acre herd.

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