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First record of antibodies to the bluetongue virus in ewe (*Ovis aries*) in the state of Amazonas, Brazil*

Primeiro registro de anticorpos para o vírus da língua azul em ovino (*Ovis aries*) no estado do Amazonas, Brasil*

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Resumo

O objetivo do trabalho foi descrever o primeiro registro de anticorpos para o Vírus da Língua Azul (VLA) em ovino, no estado do Amazonas. A ovelha, que se encontrava em gestação gemelar, pariu no dia 9 de maio de 2015, porém um cordeiro faleceu horas após o parto. Foi então solicitado serviço veterinário por parte do proprietário, onde foi observado emaciação, perda de lã, pirexia, apatia, dispneia, secreção nasal mucoide, edema facial, lingual e submandibular. Houve visita da Agência de Defesa Agropecuária do Estado do Amazonas na propriedade e coletadas amostras de sangue do animal. O sangue total e soro foram enviados ao Laboratório Nacional Agropecuário, no qual foi possível detectar a presença de anticorpos específicos para VLA, através do teste de Imunodifusão Dupla em Gel de Ágar. A ovelha foi submetida a uma nova coleta de sangue, seguindo os mesmos protocolos e as amostras foram enviadas ao Instituto Biológico de São Paulo, confirmando diagnóstico. O animal em estado clínico grave, não resistiu e veio a óbito em julho de 2015. A ocorrência de um caso alóctone, em uma área de ocorrência de insetos vetores, pode desencadear um processo de endemia na região amazônica. Com isso, o controle epidemiológico destas ocorrências, se fazem necessários, afim de se evitar a disseminação da doença no país.

Palavras-chave: alóctone, endemia, imunodifusão dupla em gel de ágar, vetores.

Abstract

The objective of this work was to describe the first record of antibodies to the Bluetongue Virus (BTV) in ewe, in the state of Amazonas. The ewe, which was in twin pregnancy, gave birth on May 9, 2015, but a lamb died hours after delivery. Veterinary service was then requested by the owner, where emaciation, loss of wool, pyrexia, apathy, dyspnea, mucoid nasal secretion, facial, lingual and submandibular edema were observed. There was a visit by the Agricultural Defense Agency of the State of Amazonas to the property and blood samples were collected from the animal. The whole blood and serum were sent to the National Agricultural Laboratory, where it was possible to detect the presence of specific antibodies to BTV, through the Agar Gel Double Immunodiffusion. The ewe was submitted to a new blood collection, following the same protocols and the samples were sent to the Biological Institute of São Paulo, confirmed diagnosis. The animal in a serious clinical condition, could not resist and died in July 2015. The occurrence of an allochthonous case, in an area where vector insects occur, can trigger an endemic process in the Amazon region. With this, the epidemiological control of these occurrences is necessary, in order to avoid the spread of the disease in the country.

Keywords: agar gel double immunodiffusion, allochthonous, endemic, vectors.

Introduction

Bluetongue Virus (BTV) is an etiological agent of the Reoviridae family, genus *Orbivirus*, which develops a non-contagious, notifiable disease affecting sheep, goats, buffaloes, cattle, deer and camels (Maan et al. 2016; OIE 2021). With a cosmopolitan distribution, BTV is transmitted mainly by insect vectors of the *Culicoides* genus, however, other forms of transmission have already been documented, such as contaminated semen and the trans-placental (Bowne and Jones 1966; Napp et al. 2011; Saegerman et al. 2011). Countries with tropical and subtropical climates have a higher occurrence of the disease due to the favoring of the multiplication of these parasites, associated with the climatic conditions of each region (Gerry and Mullens 2000).

It was reported for the first time in Brazil, in cattle and sheep, in the state of São Paulo by Silva (1978). Since then, several epidemiological surveys have been carried out in the country,

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Tomich et al. (2009) showed a prevalence of 42 % in cattle, for the region of Nhecolândia, in Mato Grosso do Sul. In the same year, Nogueira et al. (2009) 65 % in sheep, in the region of Araçatuba, state of São Paulo, Pinheiro et al. (2013) with 27.3 % in sheep, from the municipalities of Quixadá, Tauá, Jaguaribe, Várzea Alegre, Massapê, Sobral and Granja in Ceará, Sbizera et al. (2017) with 31.1 % in sheep, from the municipalities of Candoi and Pitinga, in Paraná. For the northern region Abreu et al. (1983) reported a prevalence in cattle of 15.9 % in the state of Roraima, 25.5 % in Amazonas, 32.5 % in Pará and 21.3 % in Amapá.

Infection in cattle is inapparent in most cases (Riet-Correa et al. 1996), but with greater susceptibility in sheep and goats (MacLachlan 2004). In small ruminants, the main clinical signs are: pyrexia of approximately 41 °C, swelling of the tongue, sometimes cyanotic, which may be on the outside of the mouth, facial swelling, neck swelling, pulmonary edema, dyspnea, mucoid nasal discharge, excessive salivation with foam, hyperemia in the oral and nasal mucosa, diarrhea and foot injuries (Erasmus 1975; Backx et al. 2007).

Standard serological tests for the detection of specific antibodies, such as Agar Gel Immunodiffusion, are recommended by the World Organization for Animal Health (OIE) for the certification of animals for the international transit of ruminants since 1982 (Lobão et al. 2014). However, the lack of specificity is the main disadvantage of this test, as it only determines positive and negative animals, not identifying the serotypes (Biihrer et al. 2020).

Vaccination is performed in some endemic regions of Africa, the United States and Europe, but in Brazil there are no licensed vaccines available on the market. The lack of epidemiological data on circulating serotypes in herds in each region, associated with the absence of inactivated vaccine for all serotypes and the possibility of disease development with the use of vaccines with live attenuated virus in some susceptible sheep breeds, make its application as a sanitary prophylactic measure (Campos 2020).

BTV infection not only affects the agricultural sector causing abortion, reduced milk production and restriction of animal transit, but is also related to the conservation of wild species, such as the deer, which acts as a host for the disease (Vosdingh et al. 1968; Tomich et al. 2009). In the Amazon region, sheep are presented as an alternative for the production of meat, milk and leather, as they need smaller areas for their creation when compared to cattle and have a reduced production cycle, integrating agroecological systems (Pereira 1999).

According to data from the Instituto de Desenvolvimento Agropecuário e Florestal Sustentável do Estado do Amazonas, in 2012, 864 raisers were assisted, with a herd of more than 23 thousand animals, including goats and sheep, with a production of 180 tons of meat/year, with highlighting the municipalities of Boca do Acre, Rio Preto da Eva and Autazes, which concentrated the largest herds in the state (IDAM, 2012). In 2018, the state already had a herd of 63.000 goats and sheep, occupying the third and fifth position in the ranking of the number of animals in the states of the northern region, respectively (IDAM, 2020).

The implementation of sanitary barriers to prevent the spread of the virus in the region and the use of technology to add value to the herd, in more sustainable breeding systems, are essential measures to develop the sheep and goat farming sector in the region. Therefore, the objective of the present study was to describe the first record of antibodies to the BTV, in the municipality of Lábrea, state of Amazonas, in a dorper sheep, from the municipality of Hidrolândia, state of Goiás.

Case description

In December 2014, a Dorper ewe, approximately 24 months old, was acquired by a property in the municipality of Lábrea (7°15'36''S 64°47'57''W), state of Amazonas, from in the municipality of Hidrolândia (16°58'5''S 49°13'54''W), state of Goiás. The ewe that was in twin pregnancy, gave birth on May 9, 2015, but a lamb died less than 24 hours after delivery.

A few days later, an autonomous veterinary medical service was requested by the owner, where weight loss, loss of wool, pyrexia of 40.5 °C, apathy, dyspnea, mucoid nasal secretion, facial, lingual and submandibular edema were observed (Figure 1). Given this, the therapeutic protocol instituted was the administration of Benzylpenicillin Procaine, Benzylpenicillin Benzathine and Flunixin Meglumine. There was a small clinical improvement, but with the worsening of the condition whenever the animal was exposed to the sun.

Figure 1: (A) Physical restraint for clinical examination and blood sample collection. (B) Tongue swelling. (C) Facial swelling. (D) Submandibular swelling

On June 8, 2015, the Agricultural and Forestry Defense Agency of the State of Amazonas (ADAF - portguese acronym), carried out a technical visit to the property, noting during anamnesis that the animal had already presented the aforementioned clinical signs for a week. On that occasion, blood samples were collected by puncture of the external jugular vein, using a sterile needle and vacuum test tube tube with ethylenediamine tetraacetic acid (EDTA) for molecular analysis and a vacuum test tube without anticoagulant for serological analysis, after antisepsis of the site with cotton and 70 % ethyl alcohol.

The sample for molecular biology was placed in an isothermal box and centrifuged at 4000g in the laboratory for 4 minutes to remove the plasma. Three washes were performed with sterile 0.9 % sodium chloride solution and centrifuged at 4000g for 4 minutes, in order to obtain the red blood cell concentrate. Finally, the sample was stored at -80 °C.

For serology, the blood was kept at rest at room temperature until complete retraction of the clot for separation of the serum fraction and subsequent packaging in an isothermal box. In the laboratory, centrifugation was performed at 900g for 5 minutes, and the serum was transferred to a properly identified 1.5 ml microtube and stored in a freezer at -20 °C.

Whole blood and serum were sent to the National Agricultural Laboratory, following the protocols recommended by the Ministry of Agriculture, Livestock and Supply (MAPA - portguese acronym), subjected to tests for Foot-and-Mouth Disease, Sheeppox, Vesicular Stomatitis, Contagious Ecthyma and Bluetongue (BT). In which it was possible to detect the presence of specific antibodies of the BTV, through the test of Agar Gel Double Immunodiffusion (AGID), however it was not detected in the RT-PCR.

In order to rule out the possibility of RNA degradation during the first process, the animal was submitted to a new blood collection, following the same protocols described above, and the samples were sent to the Biological Institute of São Paulo. Again, the result was negative in RT-PCR and positive for detection of specific antibodies to BTV in the AGID test.

The animal, which was in a serious clinical condition, could not resist and died in the first half of July 2015.

Discussion

The positive result for the BTV, through the AGID test, associated with the clinical signs of the disease, prove the animal's contact with the virus, generating the registration of the disease in sheep in the zoosanitary indicators of the MAPA for the state of Amazonas. The first record of detection of antibodies to BTV in sheep in the state describes an allochthonous case of the disease, since the animal comes from the municipality of Hidrolândia, state of Goiás. However, for the maintenance of the virus in nature, the presence of vector insects is necessary, associated with the climatic conditions of the region, such as humidity and temperature, favoring its multiplication, characterizing the endemic disease (Gerry and Mullens 2000).

However, Abreu (1983) describes that the prevalence of the disease in cattle in the state of Amazonas is 32.5%, according to a survey carried out by MAPA, with 40.705 female cattle over 30 months of age, in 3.814 properties distributed in three microregions homogeneous in the state.

Sheep belonging to European lines of fine wool are more susceptible to BTV infection than sheep from tropical and subtropical regions where the virus is endemic (MacLaclan 2004). However, in the present study, the occurrence of the disease in the state was recorded in a dorper sheep, a breed originally from South Africa, released with aptitude for beef production (Souza 2015).

The event of the death of one of the lambs can be associated with the clinical signs of the disease. Saegerman et al. (2011) reported that in a herd of sheep in Belgium, where an outbreak of BTV infection occurred, between November 2007 and May 2008, 26 aborted fetuses were observed, in a total of 300 sheep. Trans-placental infection can occur with serotype 8, resulting in hydranencephaly and abortion, causing fetal death (Vercauteren et al. 2008).

Antoniassi et al. (2010) described that some of the clinical signs observed in an outbreak in the state of Rio Grande do Sul in sheep were edema, pyrexia, apathy, dyspnea and excessive nasal secretion, signs similar to those found in the sheep in this study. The mortality rate is influenced by factors such as the pathogenicity of the viral strain and the worsening of the clinical picture by exposure to sunlight (Erasmus 1975).

RT-PCR has high sensitivity and specificity for all serotypes and strains of BT, however, the ideal is that the collection be performed during the viremia period of the disease, which in sheep can vary from 14 to 28 days (Aradaib et al. 1994). In the present study, the animal was in an area of difficult access and for logistical reasons it was not possible to quickly store the sample for later shipment to the laboratory, a fact that possibly caused degradation of the samples, making the positive molecular diagnosis unfeasible.

Neutralizing and non-neutralizing antibodies develop around 7 to 14 days after infection, therefore, after viremia, the AGID test shows whether the animal came into contact with the infectious agent, through the presence of antibodies to the BTV, as shown. In the present study. However, the disadvantage is the lack of specificity and the possibility of cross-reacting with other Orbiviruses, which can be retested using a specific assay for serogroup LA (OIE 2021).

Conclusion

The occurrence of an allochthonous case of ovine BT, in an area with the presence of competent vectors, can trigger a process of endemic introduction in the Amazon region. Thus, the epidemiological control of these occurrences, entomological investigations, control in the transport of imported animals, as well as the correct diagnosis, are of fundamental importance on the part of field veterinarians and are necessary by the inspection bodies of each state, in order to to prevent the spread of the disease in the country.

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