

Blue Tongue Virus in Dairy Cattle in the Southern Region of Minas Gerais, Brazil - Serological Survey

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ABSTRACT

Background: Blue tongue (BT) is a noncontagious viral disease transmitted by hematophagous arthropods, especially of the genus *Culicoides*. The economic impact of the disease is related not only to deaths in sheep herds but also to the possible correlation of virus infection with the development of other diseases, such as pneumonia, abortion and movement problems. The economic losses caused by Blue Tongue are linked to restrictions on the import and export of animals and their genetic material and to the reproductive disorders associated with this disease. In addition, the fact that cattle take the role of reservoir, combined with the care by other countries with outbreaks of infection and biological contamination of their products, hinders trade in Mercosul, United States and Europe. Cattle are affected by Blue Tongue Virus in endemic areas and in some epidemic areas, but the development of clinical disease is rare. The clinical signs, when evident, range from reproductive losses, such as embryonic death, abortion, fetal malformation, temporary sterility, infertility in bulls, stillbirths and the birth of weak animals. The objective of this study was to determine the epidemiological aspects of Blue Tongue Virus (BTV) infection in dairy cattle in the Lavras region, state of Minas Gerais, Brazil.

Materials, Methods & Results: A cross-sectional study was conducted to evaluate the frequency of cattle and herds seropositive for Blue Tongue in the southern region of Minas Gerais. In this study, 54 dairy farms were visited. A total of 586 serum samples were collected from cows of reproductive age. Sampling was random, and serum samples were collected from lactating cows over 24 months of age by puncture of the jugular vein and/or coccidian vein. The samples were transported and stored at the Setor de Patologia Veterinária, at the Universidade Federal de Lavras (SPV-UFLA), where they were centrifuged, and the serum aliquots were obtained, transferred to microtubes and kept at -20°C until the serological tests were performed. The samples were tested with the agarose gel immunodiffusion test (AGID) for anti-blue tongue virus antibodies. The AGID test is more practical and is the main method used to identify Blue Tongue Virus seroprevalence in different ruminant species. They are considered important tools for epidemiological surveillance of the disease. A prevalence of 83.28% was observed among animals that were seropositive for Blue Tongue Virus (488/586; IC 95% = 80.0 - 86.21). In addition, 100% (54/54; IC 95% = 93.4 - 100.0) of the farms had at least 1 positive animal, with rates ranging from 45.45% to 100% within the herds and where 22.22% of the farms had rates of 100% of the animals being positive.

Discussion: Blue Tongue is a disease known to affect domestic and wild ruminants in Brazil. However, there is a lack of more precise information about its epidemiology and occurrence in the country and of joint efforts of researchers, producers and the government to understand in detail both the biology of vectors and the viral biology of Blue Tongue Virus in Brazil. This is the first record of detection of anti-blue tongue virus antibodies in cattle in the southern region of Minas Gerais. The results suggest that Blue Tongue Virus is present in cattle in the study area.

Keywords: cows, viral disease, BTV, prevalence, AGID, anti-BTV antibodies, *Orbivirus*, *Reoviridae*.

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INTRODUCTION

Blue tongue (BT) is a notifiable disease listed by the World Organization for Animal Health (WOAH) [18]. This pathology is caused by a double-stranded RNA virus belonging to the genus *Orbivirus* of the family *Reoviridae*, whose vectors are hematophagous arthropods of the genus *Culicoides* [12]. Currently, at least 27 serotypes have been reported [5]. Infection with BTV occurs in different animal species, although significant clinical signs occur mainly in sheep. Cattle and goats are the main reservoirs of the virus.

Restrictions on the international trade of animals and their products [17], such as semen, embryos, and milk and its derivatives, can cause great economic losses to Brazil, although no precise estimate has yet been provided [11]. These cattle are affected by BTV and may develop clinical signs ranging from reproductive losses, such as embryonic death, abortion, fetal malformation, temporary sterility, infertility in bulls [14], stillbirths and birth of weak animals [10]. These animals play an important role in the epidemiology of the disease by becoming reservoirs of the virus [2,6]. Brazil has climatic conditions that favor the multiplication and maintenance of BTV vectors, enabling the disease to spread silently. However, little is known about the disease and serotypes present in the country, which hinders the implementation of safe measures for animal movement [10]. This study aimed to evaluate the seropositivity of the Blue Tongue Virus (BTV) in a dairy region situated in the southern region of the state of Minas Gerais.

MATERIALS AND METHODS

Experimental design

The blood samples were transported and stored at the Setor de Patologia Veterinária (SPV), at Universidade Federal de Lavras (UFLA), where they were centrifuged for 3 min at 136 g, and the serum aliquots obtained were transferred to microtubes and stored at -20°C until testing.

The sampling was randomized per convenience, from locations within a radius of up to 120 km from the city of Lavras; all locations had specialized technical assistance where serum samples were collected by puncture from lactating cows older than 24 months of age from the jugular vein and/or coccidian vein. The intra-herd sampling was completed after obtaining a sensitivity and specificity of the herd above

90% for the disease tested. Based on these calculations, 11 animals were randomly sampled at each farm visited, totaling 586 samples for this study. Animals in the gestational transition period (animals 30 days before birth and 30 days after birth were not collected) because there is a change in the immune profile at this stage of life [1].

At the time of collection, a questionnaire was applied to each property, with questions covering the collection of data associated with the production characteristics and sanitary practices of the sampled properties.

Serological analysis

For the detection of precipitating antibodies against BTV, the agarose gel immunodiffusion test (AGID) technique was used. This test was performed at the Veterinary School of Universidade Federal de Minas Gerais (UFMG). A 0.85% sodium chloride solution (NaCl) and agarose at a final concentration of 0.9% in deionized distilled water were used, to be deposited on Petri dishes. The antigen was placed in the center, and the positive control serum was placed in three other wells at a volume of 25 µL in each well, with the samples to be tested being placed in the remaining wells. The Petri dishes were kept in a humid chamber with 0.5% copper sulfate for 48 h at room temperature.

The AGID reading was performed through indirect light and dark background, observing the precipitation lines formed between the antigen and the antibody. The identity of the line formed with the positive control serum was the basis for reading the test. The antigen was produced by Veterinary School of UFMG using BTV 4 and the positive control serum from positive bovine serum previously tested and titrated by Veterinary School of UFMG.

Data analysis

Descriptive analyses of the properties and frequency distribution between properties were performed with a confidence interval of 95% of the interview variables. R software¹ was used, with the use of the included packages Dplyr, knitr, kableExtra e binGroup.

The coordinates of the farms were marked to obtain the georeferencing of each of the properties by the Global Positioning System (GPS), according Figure 1. For mapping and identification of spatial points, the data were entered into the GPS software² and image editing software³.

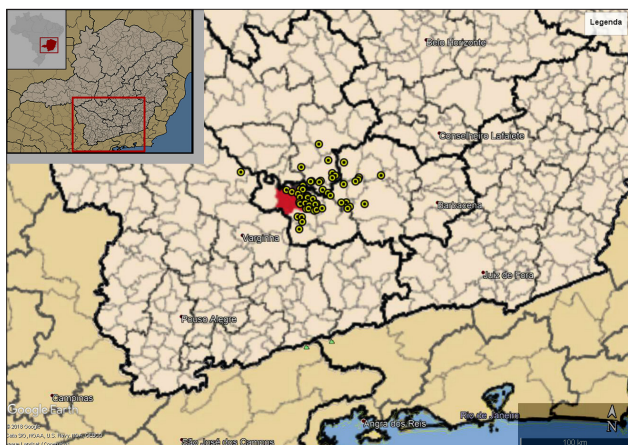


Figure 1. Geographical distribution of dairy farms surveyed for the occurrence of Blue Tongue Virus in southern Minas Gerais, Brazil during the year of 2018.

RESULTS

Through the questionnaire applied to the farmers, the characterization of the farms was made by observing the system of rearing, reproductive and sanitary management, presence of other animal species on the property, movement of animals and the occurrence of disease on the property.

The collections were performed in dairy farms in southern Minas Gerais, Brazil, (5.56%) and had mixed dairy and beef production. The predominant type of animal was European milk cows (61.11%), followed by crossbred animals (35.19%) and finally Zebu cattle (3.70%). The most practiced farming system in the region was semi confined (75.93%), followed by extensive (18.52%) and confined (free-stall; 5.56%). All farms performed milking twice a day, and the most commonly used milking system was the milking parlor (66.67%), followed by foot milking (31.48%), and only 1 milking facility had manual milking. The median number of lactating cows was 33.5, and the median daily production on the farms was 485 L of milk.

The health problems reported by the owners during the visits were mastitis (85.19%), abortions (83.33%), ectoparasites (83.33%), diarrhea (66.67%), retained placenta (50%), pneumonia (33.33%), pododermatitis (27.78%) and endoparasites (22.22%).

The farms had flooded areas (46.30%), areas of concentration of the animals where the animals remained together, and the time of milking (72.22%).

The data referring to other animal species present in the properties were available on Table 2.

Questions were asked regarding the health of the herd in terms of the vaccinations administered to the animals, with the following results: brucellosis = 53/54 (98.15%); leptospirosis = 25/54 (46.30%); and Bovine Viral

Diarrhea (BVD) and Infectious Bovine Rhinotracheitis (IBR) = 25/54 (46.30%). Regarding the diagnostic tests performed on the animals against the main reproductive diseases, the producers responded that tests were performed in relation to brucellosis (30/54 = 55.56%), leptospirosis (2/54 = 3.70%), and IBR/BVD (1/54 = 1.85%), but none of the farms performed tests for Neospora and blue tongue.

The data regarding the characteristics of 54 dairy farms with seropositive cows for Blue Tongue Virus could be observed in Table 1. In relationship of the movement of animals in 39 farms (72.22%), producers had purchased the cows from: 11.11% (6/54) agricultural fairs; 1.85% (1/54) traders or 59.26% (32/54) other farms.

In the farms visited in relation to reproductive management, natural mount was present (48.15%) at the farms, followed by natural mount and artificial insemination (38.89%) and only artificial insemination (12.96%).

The data describing the presence of all animal species living in the 54 properties is available in Table 2.

The research for antibodies against the blue tongue virus showed that 83.28% (488/586; IC95% = 80.0 - 86.21) of the animals were seropositive in all the farms assessed, and in 100% (54/54; IC95% = 93.4 - 100.0) of farms, at least 1 animal was positive in the tests. The prevalence of positivity ranged from 45.45% to 100% within the herds (Figure 2), where at 12/54 (22.22%) of the farms, all animals examined were positive for BTv.

Table 1. Characteristics of 54 dairy farms with seropositive cows for Blue Tongue Virus in the region of southern Minas Gerais, Brazil during the year of 2018.

Characteristics	Positive	
	N	%
Type of farm		
Milk	51/54	94.44
Mixed	3/54	5.56
Breeding system		
Confined	3/54	5.56
Semiconfined	41/54	75.93
Extensive	10/54	18.52
Animal breeds		
Zebu	2/54	3.70
European milk	33/54	61.11
Crossbred	19/54	35.19
Purchase of Animals		
Yes	39/54	72.22
At fairs	6/54	11.11
From traders	1/54	1.85
From other farms	32/54	59.26

Table 2. Animal species living at the same habitat with seropositive cows for Blue Tongue Virus in 54 dairy farms situated in the region of southern Minas Gerais, Brazil during the year of 2018.

Species	N	%
Dogs	53/54	98.15
Birds	41/54	75.93
Horses	33/54	61.11
Opossums	33/54	61.11
Pigs	23/54	42.50
Cats	20/54	37.04
Capybaras	16/54	29.63
Felids	15/54	27.78
Monkeys	12/54	22.22
Deer	5/54	9.26
Sheep	2/54	3.70

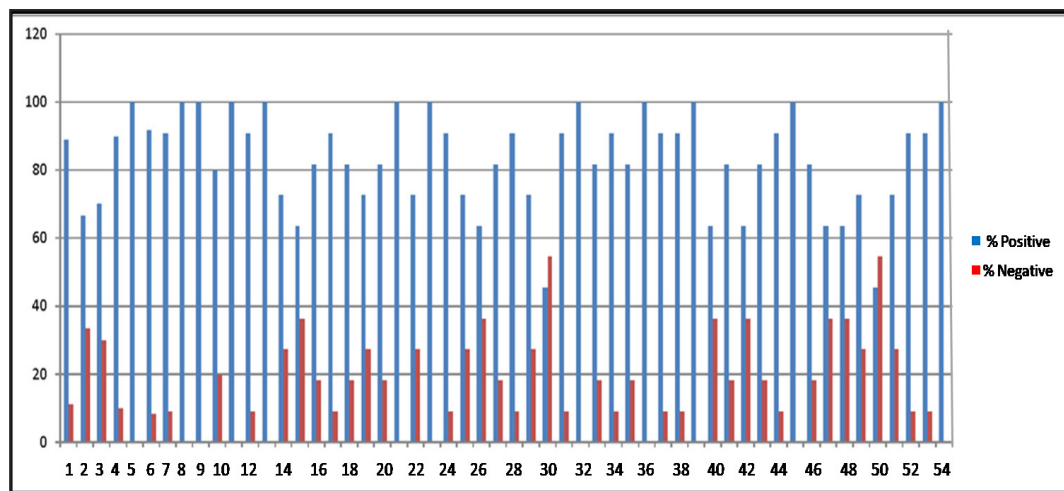


Figure 2. Percentages of seropositivity for Blue Tongue Virus in cows in the 54 farms surveyed in southern Minas Gerais, Brazil during the year of 2018.

DISCUSSION

These results confirm both the presence of the virus and the importance of dairy cattle in maintaining the virus in the region.

An important factor that may be associated with the high percentage of animals with positive serology for BTV is the fact that they are animals that live most of the time in their stable. These animals are the most susceptible to vectors, perhaps due to the high concentration of the animals [16].

The wide distribution of this virus in the state is highlighted in the literature as a characteristic of the Southeast region of Brazil. In this region, the climate and sparse rainfall throughout the year ensure

adequate humidity and temperature for the proliferation of the mosquito vector [8]. Thus, factors such as high humidity and the presence of standing water favoring the presence and multiplication of mosquitoes, which are linked to the characteristics of the properties, may also be associated with the high percentage of positives observed in the study.

The percentages found in this study differ slightly from those of other serological studies conducted in the state to verify the distribution and prevalence of BTV infection. Lower prevalence rates were observed in 59% of cattle [7] and 54.13% of sheep [4], but other factors associated with the creation and management of herds in the properties were not evaluated.

The BTV is able to infect a variety of domestic and wild ruminants, including sheep, goats, cattle, buffalo and cervids. The clinical form of the disease is most commonly observed in sheep and cervids [3,15]. However, cattle are epidemiologically important because they serve as reservoirs of the virus for prolonged periods [3,13,15].

Serological tests are important to determine the distribution of the infection and can be used to confirm the infection by the virus; however, in endemic areas, other complementary tests are needed. The control of the insect vector population is another prevention and control option and can be done through the action of insecticides. These methods can be applied to hosts and aerial and aquatic environments, in addition to the elimination of insect reproduction sites, such as swampy areas and water accumulations. However, all of these measures have been shown to be inefficient, in addition to causing environmental problems (contamination) and financial burdens [10].

CONCLUSION

The Blue Tongue is a disease known to affect domestic ruminants in Brazil. In the present study, a high prevalence of positive animals was diagnosed on the properties examined. This is the first record of the detection of anti-BTV antibodies in cattle in the southern region of Minas Gerais. However, more precise information about its epidemiology and occurrence in the rest of the country is lacking.

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Ethical approval. The execution of the present study was approved by the Ethics Committee on Animal Use (CEUA) at Universidade Federal de Lavras (UFLA) under the protocol number 017/15.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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