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Risk factors associated with the occurrence of antibodies against bluetongue virus in sheep in the state of Paraná, Brazil

Abstract

Bluetongue is an arbovirus that affects different animal species, being most relevant in sheep because of the possible disease severity. This study aimed to evaluate the occurrence of antibodies against bluetongue virus (BTV) in sheep in the central-western region of Paraná, Brazil, and the risk factors for infection in the species. This is the first study to determine these factors in the southern region of Brazil. A serological survey using agar gel immunodiffusion was positive in 28.1% (108/350) of the animals tested, and 91.66% (11/12) of the farms analyzed had at least one seropositive animal. Furthermore, contact between sheep and cattle was determined as the main risk factor for BTV infection in sheep, followed by flooding, a history of abortion, and contact with rivers and riparian forests. In conclusion, the occurrence of anti-BTV antibodies in flocks in Paraná was moderate however, the virus is still circulating in these animals, so greater epidemiological surveillance and implementation of programs to control the disease are necessary.

Keywords: bovine, bluetongue, serology, southern Brazil, vector

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Abbreviations: BTV, bluetongue virus; AGID, gel immunodiffusion; OR, odds ratio; CI, confidence interval; cELISA, competitive enzyme-linked immunoassay

Introduction

Bluetongue is a notifiable arboviral disease whose etiological agent is the bluetongue virus (BTV), an enveloped virus of the genus Orbivirus, family Reoviridae. It is an infectious disease affecting different animal species, especially domestic and wild ruminants, causing significant economic losses.[1,2]

The enveloped virus that causes this infection is transmitted mainly by mosquitoes of the genus *Culicoides* sp.; however, other less common forms of transmission have been reported, such as intrauterine transmission,[3,4] through the colostrum or fetal appendages,[5,6] through contaminated semen,[7] and through iatrogenic transmission.[8] In addition, other insect species were also described as possible vectors of the disease.[9]

Its clinical presentation varies greatly with the animal species infected, with sheep being the most vulnerable species.[2] These animals tend to present more severe clinical signs such as fever, apathy, loss of appetite; lesions in the digits and ungual apparatus, difficult locomotion; crusts and erosions in the oral and nasal cavities;[10] reproductive disorders such as infertility, abortion, and fetal malformation; respiratory complications, such as dyspnea due to pulmonary edema, which in super-acute cases results in sudden death.[11]

Other ruminants, such as cattle, which are usually asymptomatic, are important virus reservoirs. In this species, the viremia, the phase in which *Culicoides* sp. transmits the infectious agent to the vector, mainly susceptible animals, lasts up to 120 days.[12]

In Brazil, many studies have focused on the seroprevalence and risk factors for BTV infection in cattle,[12,13,14]

While few studies analyze risk factors for infection in sheep.[15]

Knowing about the bluetongue disease is essential to controlling and mitigating it, considering the relevance of this disease, also called catarrhal fever, and the variety of elements that can increase its occurrence in sheep. [16] Therefore, given the above, this study aimed to determine the occurrence of anti-BTV antibodies in sheep in Paraná, Brazil, outlining the risk factors for BTV infection in sheep in that region.

Material and methods samples

A total of 358 blood samples were obtained from male and female sheep aged over 12 months, raised in 12 different farms located in the central western region of Paraná, Brazil. The sampling process was conducted in a farm-wise distribution. (Table 1) The collected samples were aliquoted into 1.5 mL plastic microtubes and sent for diagnostic testing.

At the time of collection, a questionnaire was administered to the farmers to evaluate possible risk factors further. Information such as the presence of cattle on the farm, the presence of flooded areas, a history of abortion, the presence of rivers, and a history of sudden death were evaluated.

Diagnostic test

An agar gel immunodiffusion (AGID) commercial kit (VMRD®, USA) was used to evaluate the presence of anti-BTV antibodies, according to the manufacturer's recommendations.

Statistical analysis

The data were analyzed using the Fisher's exact test and the Pearson's chi-square test, and the odds ratio (OR) was calculated at a 95% confidence interval (CI). A p-value < 0.05 was defined as statistically significant.

Results

Anti-BTV antibodies were detected in 108 of 350 animals tested, reaching a positivity of 28.1%. As for the 12 farms evaluated in the study, the percentage of serologically positive animals ranged from 81.57% (31/38), in farm number four, to 0% (0/7), in farm number eight. (Table 1)

Table 1 Total samples tested for the presence of anti-BTV antibodies in sheep by farm evaluated in the central- western region of the state of Paraná, Brazil

Farm no.	No. Tested samples	No. Positive samples (%)
1	14	2 (1.42%)
2	17	13 (76.47%)
3	20	7 (35%)
4	38	31 (81.57%)
5	11	5 (45.45%)
6	22	5 (22.72%)
7	20	3 (15%)
8	7	0 (0%)
9	14	1 (7.14%)
10	42	12 (28.57%)
11	15	4 (26.66%)
12	138	18 (13.04%)
TOTAL	**358**	**101 (28.1%)**

Table 2 Analysis of risk factors associated with BTV infection in sheep flocks in the region of Guarapuava, Paraná, Brazil

Variable	OR	CI95%	*P*
Contact with cattle	3.8	[1.56–9.10]	0.002*
Flooded areas (lakes/ponds/dams)	1.5	[0.83–2.56]	0.188
Abortion history	1.2	[0.66–2.10]	0.586
Contact with rivers/riverine forests	1.6	[0.98–2.72]	0.057
History of sudden death	0.8	[0.48–1.23]	0.279

*Statistically significant: p < 0.05.

Of the elements evaluated as possible risk factors for BTV infection in sheep, the only contact with cattle showed statistical significance (p = 0.02, OR = 3.8, CI 95% = 1.56–9.10) (Table 2). In addition to this element, although not statistically significant, the presence of flooded areas (OR = 1.5, CI95% = 0.83–2.56), contact with rivers and riparian forests (OR = 1.6, CI95% 0.98–2.72), and a history of abortion (OR = 1.2, CI95% = 0.66– 2.10) were also determined as risk factors, while sudden death (OR = 0.8, CI95% = 0.48–1.23) was considered a protective factor.

The sheep had contact with cattle in eight of the nine farms, with the percentage of seropositive animals > 10%. Only farm number seven showed a percentage of infection above 10% despite denied contact between sheep and cattle; however, this farm had flooded areas and a history of abortion. There was also no contact in the three farms with the lowest percentages of infected animals (in descending order, farms nine, one, and eight, with farms nine and eight denying the occurrence of all questioned elements).

Discussion

The high rate of anti-BTV antibodies in the flocks evaluated in this study, with 91.66% of the farms having at least one seropositive animal (11/12 farms), indicates that the BTV is widespread in sheep flocks in Paraná, corroborating Scolari *et al*.[17] who stated that the virus is endemic in almost the entire country, but the serological rates for anti-BTV antibodies markedly vary between the regions studied.

This study corroborates the results presented by Sbizera *et al*.[18] who found seropositive sheep in 100% of the farms tested in Paraná. However, those authors detected a higher percentage of positive animals, 64.81%, compared to 28.1% (101/358) in this study. This discrepancy can be justified by the population evaluated or evenby the diagnostic method used, the competitive enzyme-linked immunoassay (cELISA) in the study by Sbizera*et al*.[18] and the AGID in this study, which can present false-negative results (lower sensitivity) and cross–reactions with other arboviruses.[2]

As for the agents considered as possible risk factors for the occurrence of anti-BTV antibodies in sheep in the present analysis, only the connection between sheep and cattle was statistically significant (p = 0.002, OR = 3.8, CI_95% 1.56–9.10). This result indicates that sheep managed near cattle are 3.8-fold more likely to come intocontact with BTV, which is justified by the fact that cattle are a viral reservoir.[19]

This ruminant species has a long viremia period, which can last up to four months, while in sheep, the viremia usually lasts less than 15 days. This extremely extensive phase in cattle increases the possibility of the infected animal being targeted by the mosquito vectors, enabling transmission.[12] Moreover, mosquitoes of this genus have a predilection for cattle, which further emphasizes the role of these ruminants as amplifiers of the dissemination of this arbovirus, commonly associated with an asymptomatic infection, which goes unnoticed, contributing to virus maintenance in the herd.[20]

Therefore, the easy contact these vectors have with cattle in the BTV viremia phase, subsequently infecting the sheep due to the proximity between these species when managed together, increases the chances of BTV infection in the most vulnerable species.

Some studies on the occurrence of anti-BTV antibodies in sheep described several risk factors favoring this condition in some countries, including China,[16] Iran,[8] Pakistan,[21] and Bangladesh,[22] but so far never reporting contact between sheep and cattle as a risk factor.

In Brazil, contact between cattle and sheep has not been identified as a risk factor, but few studies have evaluated risk factors for BTV infection in sheep. In one of these studies, Alves *et al*.[23] found an occurrence of 8.4% BTV seropositive sheep (27/321) in the Sertão Paraibano mesoregion, in the state of Paraíba, Brazil, and determined as risk factors the sanitary conditions of the farms and the frequency of deworming, ruling out the contact between sheep and cattle and opposing the findings of the present study. This fact may be related to whatwas pointed out by the authors themselves, who considered the possibility of goats acting as virus reservoirs because goats were present in the farms with no cattle. Thus, contact with cattle is a more impacting element in Paraná, where cattle farming predominates over goat farming, and the opposite is observed in Paraíba, where goat farming predominates over cattle farming.[24]

Abortion history in the studied farms was also reported as a risk factor for BTV infection in sheep in Paraná, which shows that BTV infection can increase the chance of a female having an abortion by 1.2-fold, a finding compatible with the findings by Rizzo *et al*.[25] who

evaluated the relationship between BTV infection and reproductive disorders in Santa Inês sheep in São Paulo, Brazil, showing that females with anti-BTV antibodies were 1.38-fold more likely to have abortions. This phenomenon can be explained by the abortogenic effect of BTV, either due to its replication in reproductive tissue or due to general condition depletion.[11,26]

The only evaluated element that presented itself as a protective factor was the history of sudden death inthe herd (OR = 0.8, CI 95% = 0.48–1.23), indicating that this element reduces the chance of the animals being seropositive for BTV by 0.8-fold. This element allows us to determine that there is 20% less chance ofseropositive animals experiencing sudden death than seronegative animals. This fact can be explained by the numerous causes of sudden death in sheep.[27] However, several reports of BTV outbreaks in sheep flocks describe super-acute presentations that result in rapid death.[28] Bluetongue disease can be subclinical or clinical, with animal death rarely occurring in < 24 hours from symptom onset.[10]

In the state of Rio Grande do Sul, Brazil, Costa *et al*.[29] detected a low occurrence of seropositive sheep (0.16%, 2/1,341), a scenario attributed to climatic conditions—low temperatures during winter—unfavorable to the maintenance of the *Culicoides* vectors in the environment. However, those authors reported that sheep had contact with cattle in the only farm with seropositive animals, indicating that contact between these species increases the chance of BTV infection in sheep, corroborating the present study. Similarly, the same authors found that most farms studied had areas of flooding, a condition believed to favor vector multiplication, with seropositive animals not detected even in these farms, contrary to what was observed in this study, in which the presence of flooded areas and contact with rivers and riparian forests were risk factors that increased the chance of detecting anti-BTV antibodies by 1.5- and 1.6-fold, respectively.

The relevance of these elements as risk factors can be attributed to the fact that environments with wet areas, whether flooded expanses or rivers, favor the multiplication of *Culicoides*.[30] Similarly, environments rich in vegetation, such as forests, offer favorable conditions for the proliferation of the vector since, in both circumstances, insects are attracted due to moisture, organic matter, and shelter.[31] This condition increases the possibility of sheep having contact with vectors, increasing their risk of being exposed to BTV.

Conclusion

Given these results, the occurrence of anti-BTV antibodies in the sheep flocks in Paraná was moderate, indicating that the viral agent is present in herds in the state and that mixed cattle and sheep breeding; the occurrence of abortion; and the presence of flooded areas, rivers, or riparian forests favor contact of this species with the infectious agent. This is the first study to define risk factors for BTV infection in sheep in the southern region of Brazil.

Further studies are necessary to determine the seroprevalence and risk factors for BTV infection in sheep and to increase epidemiological surveillance and the implementation of virus control measures in different animal species.

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None.

Conflicts of interest

The authors declare no conflict of interest.

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