

Is Bluetongue Virus a Risk Factor for Reproductive Failure in Tropical Hair Sheep in Brazil?

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ABSTRACT

Background: Bluetongue is a vector-borne viral disease transmitted by midges from the genus *Culicoides*. The disease can infect most of the ruminant and camelid species, but the severe disease is most often seen in European wool and mutton sheep breeds. In this sense, there is a gap in the knowledge on BTV infection in hair sheep breeds from tropical zones. Thus, this study aimed at establishing whether exposure to BTV is a risk factor for reproductive failure in Santa Inês ewes, a hair sheep breed, reared under tropical conditions in Brazil.

Materials, Methods & Results: A retrospective cross-sectional study was carried out in sheep farms in São Paulo state, Brazil, after the rainy season. Serum samples from 110 Santa Inês ewes with a history of reproductive disorders, in the last 6 months, which were included: abortion, premature birth, stillbirth, retention of placenta, infertility, estrus repetition, fetal malformation, weak lamb birth and neonatal death were collected. The presence of antibodies against BTV was assessed by agar gel immunodiffusion method (AGID). Serology to the infectious agents *Brucella ovis*, *Lepstospira* spp., *Toxoplasma gondii*, *Neospora caninum* and *Campylobacter* sp. were also assessed. Bivariate associations between the outcome and individual explanatory variables were assessed using the Fisher's exact test. Abortion was the most common reproductive disorder (53%; 74/139) observed, followed by estrus repetition (12%; 17/139) and infertility (11%; 15/139). Other disorders related to the conceptus totaled nearly one fourth of the reported disorders. A total of 20% (22/110) of the ewes were seropositive to BTV. A higher frequency of BTV seropositive than BTV seronegative ewes with a history of abortion was found. Also, abortion with seroreactivity to BTV was tested for prevalence ratio that showed 1.38 [95% CI 1.10-1.74; $P = 0.030$]. With regards to the abortion involvement of other infectious diseases associated with the seropositive ewes to BTV, more than a half of ewes (53%; 10/19) were solely seropositive for BTV.

Discussion: In the current study, it was detected 20% (22/110) of seropositive ewes to BTV. These findings demonstrated that even though the BTV has been considered endemic in tropical countries such as Brazil, there are regions or microclimates in which the virus cannot be present or in varied prevalence. The history of abortion was identified as the potential factor associated with BTV seropositivity in Santa Inês ewes. Equally, the differential diagnosis for other infectious agents related to abortion demonstrated the unique presence of antibodies against BTV in more than half of all cases. Other studies with native sheep flocks in Iran and Nepal also demonstrated a strong positive correlation between abortion history and seropositivity for BTV. Thus, it is possible that in other continents of the world, under tropical conditions, the virus does not behave the same asymptomatic infection such as have been reported for native sheep breeds in Africa. In conclusion, it was demonstrated that one-fifth animals were positive for antibodies against BTV clearly implying the viral spreading in the local hair sheep flocks. These findings highlight the importance of surveillance related to BTV in endemic areas. Therefore, it is recommended to strengthen the surveillance system for BTV within Brazil and to educate farmers about the management and control of this disease.

Keywords: abortion, infertility, estrus repetition, Santa Inês breed, *Orbivirus*.

Descritores: abortamento, infertilidade, repetição de estro, raça Santa Inês, *Orbivirus*.

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INTRODUCTION

Bluetongue is a vector-borne viral disease transmitted by midges from the genus *Culicoides* that affects ruminants and camelid species. The disease has been considered endemic in most areas of South America, Central America and the Caribbean because of the appropriate climate conditions for vector maintenance, however, the clinical presentation of the disease has rarely been reported [12].

The infection of pregnant ewes may lead to transplacental infection and it might result in early embryonic death, fetal mummification, abortion, stillbirth and birth of weak lambs. Moreover, it has also been described the birth of non-viable offspring with severe central nervous system deformities as porencephaly, hydrocephalus, hydranencephaly and brain cysts [1,23,25,26].

The importance of the bluetongue in wild ruminants and naturalized sheep breeds is still unclear [7]. The most of indigenous African sheep breeds do not show clinical signs of infection by BTV, so the disease has not been considered with a major significance in many sheep rearing rural communities in Africa [9]. Clinical disease is well established in some wild ruminant species in North America [14], domestic sheep in Asia [26] and native sheep breeds in the Middle East where it has been demonstrated economic losses by BTV infection [17,18]. Thus, this study aimed at establishing whether exposure to BTV is a risk factor for reproductive failure in Santa Inês ewes, a Brazilian hair breed, kept under tropical conditions in Brazil.

MATERIALS AND METHODS

Animals and study design

A retrospective cross-sectional study was performed in 17 meat sheep farms located in São Paulo state (average latitude 23° 32' 51" S) from April to November, after the rainy season. The animals were raised under semi-intensive or intensive husbandry management. A questionnaire was administered to farmers to collect data and serum samples from animals with previous history of reproductive disorders, in the last 6 months, which were included: abortion, premature birth, stillbirth, retention of placenta, infertility, estrus repetition, fetal malformation, weak lamb birth and neonatal death. By this screening method, serum samples from 110 Santa Inês ewes, a local hair sheep

breed, were collected. Each farm was sampled by one to 30 serum samples. It is also important to highlight that one ewe could present more than one reproductive problem and it was considered for epidemiological analyses.

Blood collection and laboratory tests

Blood samples were collected by jugular venepuncture into vacuum tubes (BD Vacutainer®)¹ containing gel clotting activator to obtain serum. In sequence, blood samples were centrifuged (2,600 x g), aliquoted into plastic microtubes², identified and stored in a freezer at -20°C for serologic analyses. Serum samples were tested for antibodies against BTV using the agar gel immunodiffusion (AGID) [31]. The antigen used was produced in the Research Laboratory in Animal Virology at Veterinary School (UFMG) [4].

Other local infectious agents related to reproductive disorders in sheep were sought as a differential diagnostic for BTV. All BTV seropositive ewes in association with history of abortion were serologically tested against *Leptospira* spp., *Brucella ovis*, *Toxoplasma gondii*, *Neospora caninum* and *Campylobacter* sp. The microscopic agglutination test (MAT), was performed against the following 24 leptospira serovars: Australis, Bratislava, Autumnalis Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Sentot, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Copenhagen, Javanica, Panama, Pomona, Pyrogenes, Patoc, Hardjo (Hardjoprajitno), Hardjo (Hardjobovis), Wolffi, Shermani, and Tarassovi [6]. Seroreactivity against *B. ovis* was analysed by complement fixation [21], and against *T. gondii* and *N. caninum* was by indirect immunofluorescence assay (IFA), it was considered a cutoff point of 1:50 for *T. gondii* [5] and 1:64 for *N. caninum* [3]. The bacterial isolation of *Campylobacter* sp. was carried out from ewes faecal samples [27].

Statistical analysis

Data were compiled in Microsoft Excel³ spreadsheets and checked for possible inconsistencies. All analyses were performed using the BioEstat[®] 5.0 software⁴. Bivariate associations between the outcome and individual explanatory variables were assessed using the Fisher's exact test. Liberal cut-off of 5% was used for variable selection to be included in the multivariable analysis. Due to the prevalence obtained (20%), the risk calculation for the occurrence of reproductive disorders by serology status was performed by

the prevalence ratio [2]. *P*-values less than 0.05 were considered significant.

RESULTS

A total of 139 reproductive problems were found. Abortion was the most frequent (53%; 74/139) declared, it was followed by estrus repetition (12%; 17/139) and infertility (11%; 15/139). Birth of weak lambs (14), fetal malformation, neonatal death (11), stillbirth, and premature birth (1) totalled, together, one fourth (23%; 32/139) of the findings. Retention of placenta (1%; 2/139) was considered a minor reproductive problem (Figure 1).

The serological screening to BTV revealed 20% (22/110) of seropositive ewes. Besides, most of these had a previous history of abortion (86.4%; 19/22). Only a few ewes had a history of neonatal deaths (15%;

3/22) and birth of weak lambs, premature birth, and stillbirth (4.5%; 1/22) [Table 1]. It was found a higher frequency of BTV seropositive than BTV seronegative ewes with a history of abortion. Moreover, a higher frequency of BTV seronegative ewes with estrus repetition and infertility was also observed. Abortion with seroreactivity to BTV was tested for prevalence ratio that showed 1.38 [95% CI 1.10-1.74; *P* = 0.030] (Table 1).

With regards to the abortion involvement of other infectious diseases associated with the 19 BTV seropositive ewes, it was found a positive serology in 32% (6/19) of these for *T. gondii*, 10% (2/19) for *N. caninum* and 5% (1/19) for *Leptospira* serovar Autumnalis. All BTV seropositive ewes were negative to *Campylobacter* sp. and *B. ovis*. In short, more than a half of ewes (53%; 10/19) were solely seropositive for BTV.

Table 1. Outcomes of bivariate analysis of association between bluetongue seroprevalence in Santa Ines ewes and the individual explanatory variables on reproductive failures.

Variable	Negative n (%)	Positive n (%)	<i>P</i> value ¹	PR (CI 95%)	<i>P</i> value ²	CI
Abortion	55 (62.5)	19 (86.4)	0.026	1.38	0.0301	1.10-1.74
Estrus repetition	17 (19.3)	-	0.015	-	-	-
Infertility	15 (17.1)	-	0.027	-	-	-
Weak lamb	13 (14.8)	1 (4.5)	0.178	-	-	-
Neonatal death	8 (9.1)	3 (15.0)	0.381	-	-	-
Foetal malformation	2 (2.3)	-	0.638	-	-	-
Retained Placenta	2 (2.3)	-	0.638	-	-	-
Premature birth	-	1 (4.5)	0.200	-	-	-

¹Associations evaluated by Fisher's exact test. ²Association evaluated by prevalence ratio. PR: Prevalence ratio CI: Confidence interval.

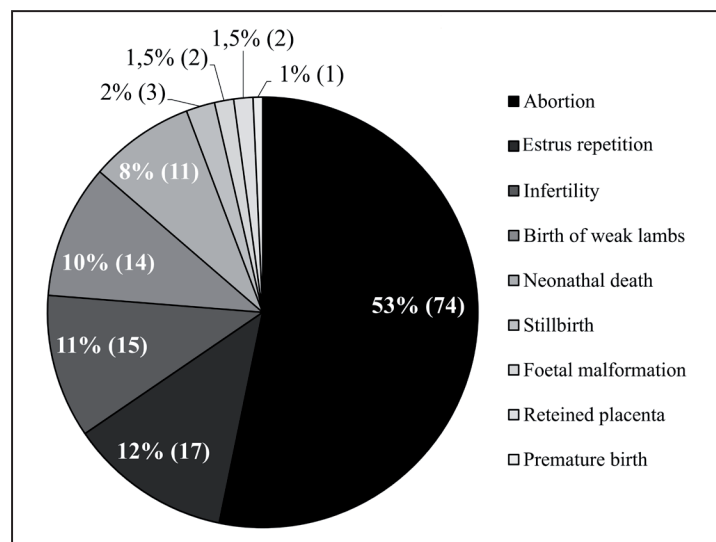


Figure 1. Frequency distribution for reproductive failures (n = 139) found in Santa Inês ewes (n = 110) in São Paulo State, Brazil.

DISCUSSION

In the current study, 20% (22/110) of seropositive ewes to BTV was detected. Other studies carried out in the same region have shown antibody frequencies ranging from 13.4% (72/538) in young sheep [30] to 64.9% (651/1002) in adult sheep [19]. Equally, recent studies in nearby regions have also showed antibody frequencies ranging from 31.2% (34/109) to 80.4% (86/107) in sheep [16,28]. These findings demonstrated that even though the BTV has been considered endemic in tropical countries such as Brazil, there are regions or microclimates in which the virus cannot be present or in varied prevalence. Thus, the unhindered movement of animals among regions, states, and countries may favour viral dissipation into new areas [7,15].

Abortion was the most common problem reported by farmers with a high rate to BTV seropositive ewes who aborted (86.4%). It is important to highlight the serological survey was conducted after rainy seasons, the epidemiological period where clinical cases may occur in sheep. Similar research showed a presence of BTV antibodies in 82.2% of exotic ewes that had aborted in India [20]. Moreover, studies with native sheep flocks in Iran and Nepal also demonstrated a strong positive correlation between abortion history and seropositivity for BTV [8,17,18], even though other study did not find correlation between them in small ruminant flocks in Algeria [11]. Lastly, concerning to large ruminants, it was demonstrated that buffalo (OR= 3.95) and cattle (OR= 5.89) with history of abortion were much likely to be infected with the disease in Pakistan [29].

In the present study, history of abortion was identified as the potential factor associated with BTV seropositivity in Santa Ines ewes. The differential diagnosis for other infectious agents related to abortion demonstrated the unique presence of antibodies against BTV in more than half of all cases. Even with the knowledge that there are other non-infectious causes of abortion, these findings corroborated with other studies in tropical zones which have been demonstrated productive and economic losses due to bluetongue disease in native sheep breeds and not only in exotic wool sheep breeds and their crossbreeds [17,22,26]. Furthermore, clinical outbreaks of BTV in Santa Inês sheep were already reported, as well as the susceptibility of this breed to BTV infection [10,13]. Thus, it is possible that in other continents of the world, under

tropical conditions, the virus does not behave the same asymptomatic infection such as have been reported for native sheep breeds in Africa.

Recently, it was first demonstrated the transplacental transmission of BTV-1 in pregnant mice during early and mid-pregnancy [25]. In the early stage reduced implantation sites, early embryonic deaths, abortions, and necro-haemorrhagic lesions were observed. In the mid stage, congenital defects and neurological lesions in foetuses like haemorrhages, diffuse cerebral edema, necrotizing encephalitis and decreased bone size were noticed. Thus, this model can be very suitable for further research into mechanisms of transplacental transmission, overwintering, and vaccination strategies.

The estrus repetition and infertility were the second greatest cause of reproductive disorders reported by farmers. Nevertheless, there was not found a significant relationship between the seroprevalence rate for BTV and these disorders in the current study. Fertility rates are normally influenced by a range of factors such as season, age, body condition score, heat stress, lack of management, mating failures, nutritional issues, male factors, infectious diseases and others [24]. Furthermore, this study was unable to obtain significant correlations related to fetal disorders with BTV seropositive ewes. This result may be explained by the small sample size obtained from these disorders when it was compared with the greater number of abortion cases.

CONCLUSIONS

The present study demonstrated that one-fifth animals were positive for antibodies against BTV clearly implying the viral spreading in the local hair sheep flocks. These findings highlight the importance of surveillance related to BTV in endemic areas. Also, history of abortion was identified as the potential factor associated with BTV seropositivity in Santa Inês ewes. Therefore, it is recommended to strengthen the surveillance system for BTV within Brazil and to educate farmers about the management and control of this disease.

MANUFACTURERS

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Ethical approval. This research was approved by the Ethical Committee for Animal Use of the University of São Paulo, as reported in a statement issued by the Bioethics Committee of the Faculty of Veterinary Medicine and Animal Science, on 18/07/2007 and was also conducted under the ethical principles of the Sociedade Brasileira de Ciência em Animais

de Laboratório. All applicable international, national, and/or institutional guidelines for the care of animals were followed.

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

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