Prevalence of Bluetongue virus serotype 4 in cattle in the State of Sao Paulo, Brazil

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Keywords
Bluetongue virus, Competitive ELISA, Orbiviruses, Serotype 4, South America, Virus neutralization.

Summary
Bluetongue (BT) is considered endemic in several regions of Brazil. The State of Sao Paulo was divided into 7 cattle production regions (circuits) according to the different systems of breeding, operational and logistical capacity of the state veterinary service. At least 1 animal from each property (a total of 1,716 farms) was tested by competitive ELISA for the presence of antibodies against BTV. Sero-positive sera were subsequently also tested by virus neutralization tests (VNT) using serial dilutions from 1:10 (cutoff) up to 1:640 (in MEM). BTV-4 neutralizing antibodies were detected in 86% (1,483/1,716) of the animals tested. These results show that BTV-4 is endemic and widespread in the State of San Paulo and indirectly confirm that in the State there are favourable conditions for the multiplication of competent vectors. However, as no clinical signs have ever been reported in cattle in the region, BTV-4 infection is likely to occur silently in the State of Sao Paulo.

Prevalenza del sierotipo 4 del virus della Bluetongue nei bovini dello stato di San Paolo, Brasile

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Parole chiave
Bluetongue, Brasile, Virus neutralizzazione, Orbivirus, ELISA competitiva, Sierotipo 4 del virus della Bluetongue, Sud America.

Riassunto
La Bluetongue (BT) è endemica in diverse regioni del Brasile. Lo Stato di San Paolo è stato diviso in 7 regioni (circuiti) in relazione ai diversi sistemi di allevamento bovino e alle capacità logistiche e operative del servizio veterinario statale. Almeno unanimale per ogni allevamento (per un totale di 1.716 aziende) è stato esaminato con test ELISA competitivo per la presenza di anticorpi anti-BTV. Sero-positive sera sono state successivamente anche testate con test neutralizzazione virale (VNT) utilizzando diluzioni seriali da 1:10 (limite massimo) fino a 1:640 (in MEM). BT-4 neutralizing antibodies sono state riscontrate in 86% (1,483/1,716) degli animali testati. Questi risultati indicano che BT-4 è endemico e diffuso in Stato di San Paolo e indirettamente confermano che in questo stato esistono condizioni favorevoli per la riproduzione di vettori competenti. Tuttavia, data l’assenza di segni clinici, è probabile che BT-4 in Stato di Sao Paulo sia silente.
**Introduction**

Bluetongue (BT) is an infectious disease caused by the Bluetongue virus (BTV), which is transmitted by adult Culicoides biting midges (Tabachnick 2004). The Bluetongue virus is classified as the ‘type species’ of the genus Orbivirus, within the family Reoviridae. There are 27 BTV serotypes that have been recognized so far, including recently discovered serotypes: BTV-25, Toggenburg Orbivirus (TOV) isolated in Switzerland (Hofmann et al. 2008); BTV-26 isolated in Kuwait (Maan et al. 2011), and BTV-27, isolated in Corsica (Jenckel et al. 2015).

Bluetongue is classified as a ‘notifiable disease’ by the World Organization for Animal Health (OIE 2014) and has important socioeconomic impacts that affect international trade in animals and their products. The disease was first described in South Africa in the late 18th century and was called epizootic malignant catarhal fever of sheep (Hutchén 1902). In 1905 the name ‘bluetongue’ was proposed (Ronderos et al. 2003) due to the inflammation and cyanotic appearance of the tongue and oral mucosa, (MacLachlan 1994). In 1906, it was demonstrated that the disease was caused by a virus, when blood of sick sheep was injected into susceptible animals, thereby reproducing clinical disease (Lobato 1999). According to Cunha and colleagues (Cunha et al. 1987, Cunha et al. 1988), BT emerged in Brazil due to the importation of infected animals.

The disease affects ruminants of economic interest, including sheep, goats, cattle, buffalo, and deer, although clinical signs are most often observed in sheep and certain deer species. The most commonly observed clinical signs are facial oedema, erosion and ulceration of the gastrointestinal tract, coronitis lameness, and fever, with a high rate of mortality, reproductive problems (Lobato 1999).

Differential diagnosis of BT is of great importance, due to similar clinical signs with foot and mouth disease, malignant catarrhal fever, contagious pustular dermatitis, poxvirus infections, border disease, and ‘foot root’ Actinobacillosis.

As BT is transmitted by insects of the genus Culicoides, knowledge of the competent vector species in different ecosystems is important, in order to understand the epidemiology of the disease. The ability of adult Culicoides to transmit BTV varies with the species and is markedly influenced by climate, temperature, air humidity, and the rainfall (Mellor 1996, Mellor 2000).

Two BTV outbreaks have been reported in Brazil. The first occurred in 1980, when the virus was isolated from 4 cattle exported to the USA (Groocock and Campbell 1982). The second one was reported in 2002, when BTV was isolated in 12 sheep during an outbreak in the State of Paraná (Clavijo et al. 2002).

Bluetongue is considered endemic in several regions of Brazil, due to the climate and temperature favourable to Culicoides. Several serological surveys show a high prevalence, ranging from 10% to 100%, but without clinical disease in cattle, indicating that BTV can spread around the country ‘silently’. To date, little is known about the existing BTV serotypes and about the species of Culicoides involved in disease transmission.

**Materials and methods**

In Brazil, the number of cattle in commercial management in 2012 was 211,279,082, of which 10,757,383 belong to Sao Paulo1. This number represents approximately 5.1% of the national herd. According to the Köppen-Geiger climate classification, the State of San Paulo has 7 climate types, the one covering the entire central part of the state is the dominant one, and it is characterized by a highland tropical climate, with rain in Summer dry Winters, with an average temperature above 22°C in the warmer months.

The State of San Paulo was divided into 7 cattle production regions (Figure 1), according to the different systems of breedings, operational, and logistical capacity of the state veterinary service. For detection of BTV-specific antibodies, a screening test was performed by competitive ELISA solid phase (ELISA CFS) supplied by Panaftosa (Rio de Janeiro). One positive animal from each property (1,716 samples) was selected to evaluate the presence of antibodies against BTV serotype 4 (BTV-4) by virus neutralization test (VNT). A volume of 50 µl of each serial serum dilution (from 1:10 to 1:640 in MEM) was added to

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Table I. Number and percentage of cows not reactors and reactors according to antibodies titers by virus neutralization assay to BTV-4 in the 7 Circuits of State of São Paulo, in 2011.

<table>
<thead>
<tr>
<th>Titers express in arithmetic numbers</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>N* %</td>
<td>N* %</td>
<td>N* %</td>
<td>N* %</td>
<td>N* %</td>
<td>N* %</td>
<td>N* %</td>
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<td></td>
<td>19</td>
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<td>30</td>
<td>28</td>
<td>10.9</td>
<td>33</td>
<td>14.2</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>9.5</td>
<td>66</td>
<td>25.1</td>
<td>59</td>
<td>22.9</td>
<td>72</td>
<td>31</td>
</tr>
<tr>
<td>20</td>
<td>27</td>
<td>11.1</td>
<td>57</td>
<td>21.7</td>
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<td>19.4</td>
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<tr>
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<td>55</td>
<td>22.6</td>
<td>48</td>
<td>18.3</td>
<td>67</td>
<td>26</td>
<td>36</td>
<td>15.5</td>
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<tr>
<td>80</td>
<td>46</td>
<td>18.9</td>
<td>9</td>
<td>3.4</td>
<td>36</td>
<td>14</td>
<td>16</td>
<td>6.9</td>
</tr>
<tr>
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<td>42</td>
<td>17.3</td>
<td>1</td>
<td>0.4</td>
<td>13</td>
<td>5</td>
<td>6</td>
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<tr>
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<td>8.2</td>
<td>3</td>
<td>1.1</td>
<td>4</td>
<td>1.6</td>
<td>2</td>
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</tr>
<tr>
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<td>11</td>
<td>4.5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.4</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Total of animals analyzed</td>
<td>243</td>
<td>263</td>
<td>258</td>
<td>232</td>
<td>246</td>
<td>227</td>
<td>247</td>
<td>1,716</td>
</tr>
</tbody>
</table>

Results

Since all sampled cows were older than 24 months, all tested positive to the ELISA assay. The samples were subsequently submitted to virus neutralization tests against BTV-4. In this assay 86% (1,483/1,716) of the tested animals were positive, with titers ranging from 10 to ≥ 640 (95% confidence interval (CI) lower and upper limit 84.7 to 88.0). Table I shows the number and percentage of cows not reactors and reactors according to neutralising antibodies titers to BTV-4 in the 7 Circuits of State of São Paulo. Figure 2 shows the spatial distribution of farms with BTV-4 positive cattle (red in different intensity) according to neutralising titers and BTV-4 negative cattle (green) in State of São Paulo in 2011.

Discussion

The results revealed a high prevalence of antibodies against BTV-4 in cows. Although no clinical signs of BTV were observed during the study, the presence of antibodies indicates that there was viral circulation in the study area.

The high BT sero-prevalence is consistent with other reports of national herds (Cunha et al. 1987, Castro et al. 1992, Melo et al. 2000, Bernardes 2011), which present similar results in regions with favourable climate conditions to complete the BTV transmission and infection cycle. In 2009, Teomichi and colleagues (Teomichi et al. 2009) found 42% (92/219) of seropositivity in bovines in Pantanal Sul mato-grossense. In the same year, in Corumbá, Brazil, it was found 51.3% (181/353) of seropositivity using the agar gel immune-diffusion (AGID) technique. In the State of Rio Grande do Sul, Costa and colleagues (Costa et al. 2006) verified a low sero-prevalence of BTV in cattle (0.6%). Although there were also some infected Culicoides, the climate conditions were unfavourable for vector multiplication. In regions with arid and semi-arid climate topology, as in the Sertao in the State of Paraíba (Alves et al. 2008), the prevalence of BTV in sheep was 8.4%. In the micro-region of Juazeiro in the State of Bahia, Souza and colleagues reported 0.43% of animals with antibodies against BTV (Souza et al. 2010). This low sero-prevalence may be due to temperature and humidity conditions in the region, which make vector multiplication more difficult.

One of the risk factors for BTV infection may be the intensive indoor farming of livestock, which may increase the probability of bites by vector insects. According to Cunha and colleagues (Cunha et al. 1988), these animals are most susceptible to vector bites, perhaps due to the higher concentration of animals or because of the characteristics of the facilities, such as high humidity, standing water, and organic material, which favour the appearance and multiplication the vector midges. In this study the high prevalence of the infection may also be partially explained by the sampling of 2 year old
females, which have a higher probability of having suffered a previous/multiple infections.

The results confirmed that BTV infection occurs silently in cattle in the 7 circuits of the State of San Paulo. Although the clinical signs of BTV infection are usually less severe in cattle than in sheep, the lack of apparent clinical signs may reflect circulation of a low virulence strain or adaptation of the animals to the strain(s) that are circulating in Brazil.

The results also indicate that the temperature and humidity conditions in the State of San Paulo are favourable for the multiplication and maintenance of competent vector midge species, supporting endemic circulation of BTV. These data agree with those provided by Groocock and Campbell (Groocock and Campbell 1982) concerning the circulation of BTV-4 in Brazil.

The diagnosis and confirmation of BT disease lead to the imposition of restrictions of animals movement and trade in their products, affecting both animal health and agribusiness. However, the lack of accurate data concerning the distribution of BTV-4 in the State of San Paulo has more difficult to make decisions concerning prevention and infection-control strategies.

In conclusion, a high prevalence of BTV-4 was detected in cattle in all circuits of the State of San Paulo, demonstrating virus circulation in many regions. Notably, in region 2 (Figure 2), on the border with the state of Minas Gerais, there were animals positive to ELISA and negative to BTV-4 VNT (possible cross-reaction), suggesting the presence of another serotype of BTV in that region.
References


