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Outbreak of Bluetongue virus serotype 4 in dairy sheep in Rio de Janeiro, Brazil

Mario Felipe Alvarez Balaro,1 Michele dos Santos Lima, Claudia Del Fava, Glenda Ribeiro de Oliveira, Edviges Maristela Pituco, Felipe Zandonadi Brandão

Abstract. In late January 2013, 10 nonpregnant Lacaune dairy ewes raised under extensive husbandry management on a farm in Rio de Janeiro, Brazil, presented with the general clinical signs of lethargy, hyporexia, edema of the face, hyperemia of the exposed parts of the skin, mouth lesions, pyrexia, and lameness. Additionally, 2 pregnant ewes died suddenly after the onset of respiratory signs. The complete blood counts and biochemistry analyses showed neutrophilic leukocytosis with monocytosis and reactive lymphocytes, normocytic normochromic anemia and increased aspartate aminotransferase levels. Postmortem examination revealed erosions on the lingual mucosa, bilateral submandibular ganglia infarctions, yellow foamy fluid accumulation in the trachea and bronchial bifurcation, pulmonary congestion, and edema associated with hemorrhagic lesions on the pulmonary artery and heart. The clinical and pathological findings were suggestive of bluetongue. For a molecular and virological diagnosis, tissue samples were analyzed by Bluetongue virus—specific real-time reverse transcription polymerase chain reaction (qRT-PCR), and viral isolation was performed in embryonated chicken eggs. For viral typing, positive tissue and egg-isolated samples were analyzed by qRT-PCR using primers and probes specific for the structural VP2 gene in genome segment 2 of all 26 serotypes. There are still no contingency plans for responding to an outbreak of bluetongue disease in Brazil, and this episode emphasizes the need for continuing serological and entomological surveillance programs. Additionally, this report describes the isolation of Bluetongue virus serotype 4 in sheep in the Americas.

Key words: Bluetongue virus; Orbivirus; small ruminants; South America.

Bluetongue is a viral disease of ruminants and camels whose vector is the hematophagous Diptera order of the genus Culicoides. Bluetongue virus (BTV; family Reoviridae, subfamily Sedoreovirinae, genus Orbivirus) is characterized as a nonenveloped double-stranded RNA virus with icosahedral symmetry. There are at least 26 serotypes of BTV worldwide.9 In South America, Central America, and the Caribbean, serotypes 1, 3, 4, 6, 8, 11, 12, 14, and 17 have been detected by serotype-specific antibodies, and serotypes 1, 3, 4, 6, 8, 12, and 17 have been detected by virus isolation.6 In the United States, serotypes 1–3, 5, 6, 9–14, 17, 19, 22, and 24 have been identified.11 Furthermore, the clinical presentation of bluetongue is a very rarely reported condition. In Brazil, serotype 12 has been isolated in asymptomatic cattle and symptomatic sheep and goats.1,2 Serotype 4 has been isolated in asymptomatic cattle in Brazil and Argentina.3,4 However, to the authors’ knowledge, serotype 4 had never been isolated in sheep in the Americas.

Bluetongue outbreak in sheep in Rio de Janeiro, Brazil

In late January 2013, 10 nonpregnant Lacaune dairy ewes raised under extensive husbandry management on a farm in Rio de Janeiro, Brazil, presented a history of apathy, inappetence, edema of the face (Fig. 1), arching of the back, and lameness. Additionally, 2 pregnant ewes in the last trimester of gestation died suddenly after the onset of respiratory signs.
The clinical findings and laboratory test results for the 10 ewes are summarized in Table 1. For symptomatic treatment, streptomycin (4 mg/kg) with penicillin (12,000 IU/kg) was administered intramuscularly every 24 hr for 3 days. After 1 week, only 1 ewe continued to exhibit prostration and hyporexia.

A week later, 1 ewe aborted twins in the last trimester of gestation. The following clinical signs were observed for this female: hyporexia, prostration, head and neck edema, halitosis, ulcers inside the oral cavity, anorexia, and death. At necropsy, there were erosions on the lingual mucosa, bilateral submandibular ganglia infarctions, yellow foamy fluid accumulation in the trachea and bronchial bifurcation, pulmonary congestion, and edema associated with hemorrhagic lesions on the pulmonary artery and heart. Representative sections from different organs were fixed by immersion in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm, routinely processed, and stained with hematoxylin and eosin. Histologic examination indicated severe and diffuse acute pulmonary edema, multifocal hemorrhages in the myocardium and cardiac papillary muscles, vasculitis and multifocal intra-mural hemorrhages of the pulmonary artery (Fig. 2), discrete focal ulcerative stomatitis, discrete nonpurulent rumenitis and reticulitis, nephrosis, and purulent ulcerative pododermatitis.

### Table 1. Clinical signs and laboratory tests from 10 nonpregnant ewes affected by bluetongue disease.*

<table>
<thead>
<tr>
<th>Sheep no.</th>
<th>Age/weight</th>
<th>Clinical signs</th>
<th>Complete blood cell count and biochemistry tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/54 kg</td>
<td>Lethargy, hyporexia, healed lesions in pulvinus dentalis, ruminal hypomotility with increased borborygmus, crackles in lung auscultation, dry stools, arching of the back, and lameness</td>
<td>RBC: 7.5 × 10⁶ cells/mm³ (↓); PCV: 24% (↓); normocytic normochromic anemia; TPP: 70 g/l; Fb: 2 g/l; WBC: 12,100 cells/mm³ (↑) with reactive lymphocytes; hemolysate serum and plasma; AST: 1,209 U/l (↑); urea: 33.2 mmol/l (↑); GGT: 54 U/l (↑)</td>
</tr>
<tr>
<td>2</td>
<td>3/49 kg</td>
<td>Lethargy, hyporexia, healed lesions in pulvinus dentalis, ruminal hypomotility with increased borborygmus, dry stools with hematochezia, arching of the back, lameness, tense abdomen, and enlarged liver</td>
<td>RBC: 7.2 × 10⁶ cells/mm³ (↓); PCV: 23% (↓); normocytic normochromic anemia; TPP: 64 g/l; Fb: 2 g/l; WBC: 12,500 cells/mm³ (↑) with eosinophilia; platelet aggregation; AST: 125 U/l</td>
</tr>
<tr>
<td>3</td>
<td>3/48 kg</td>
<td>Lethargy, hyporexia, ruminal hypomotility with increased borborygmus, dry stools with hematochezia, lameness, and pyrexia (40.2°C)</td>
<td>RBC: 8.8 × 10⁶ cells/mm³ (↓); PCV: 28%; TPP: 80 g/l; Fb: 6 g/l (↑); WBC: 11,100 cells/mm³ (↑) with monocytosis; AST: 128 U/l; urea: 16.1 mmol/l (↑)</td>
</tr>
<tr>
<td>4</td>
<td>3/53 kg</td>
<td>Lethargy, hyporexia, enlarged liver, crakcles in lung auscultation, dry stools, and lameness</td>
<td>Hemolysate serum; AST: 774 U/l (↑); urea: 20.1 mmol/l (↑); GGT: 41 U/l</td>
</tr>
<tr>
<td>5</td>
<td>2/47 kg</td>
<td>Lethargy, hyporexia, ruminal hypomotility, dry stools, and lameness</td>
<td>Hemolysate serum; AST: 638 U/l (↑); urea: 8.9 mmol/l; GGT: 38 U/l</td>
</tr>
<tr>
<td>6</td>
<td>1/39 kg</td>
<td>Lethargy, hyporexia, healed lesions in pulvinus dentalis, intestinal hypermotility, enlarged liver, lameness, and pyrexia (40.0°C)</td>
<td>RBC: 9.1 × 10⁶ cells/mm³; PCV: 29%; TPP: 70 g/l; Fb: 2 g/l; WBC: 14,850 cells/mm³ (↑) with neutrophilic and monocytosis; AST: 193 U/l; urea: 34.9 mmol/l (↑)</td>
</tr>
<tr>
<td>7</td>
<td>1/37 kg</td>
<td>Lethargy, hyporexia, intestinal hypermotility, enlarged liver, lameness, and pyrexia (40.1°C)</td>
<td>RBC: 9.1 × 10⁶ cells/mm³; PCV: 29%; TPP: 70 g/l; Fb: 2 g/l; WBC: 14,850 cells/mm³ (↑) with neutrophilic and monocytosis; AST: 193 U/l; urea: 34.9 mmol/l (↑)</td>
</tr>
<tr>
<td>8</td>
<td>3/46 kg</td>
<td>Lethargy, hyporexia, ruminal hypomotility with increased borborygmus, and lameness</td>
<td>RBC: 9.1 × 10⁶ cells/mm³; PCV: 29%; TPP: 70 g/l; Fb: 2 g/l; WBC: 14,850 cells/mm³ (↑) with neutrophilic and monocytosis; AST: 193 U/l; urea: 34.9 mmol/l (↑)</td>
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<tr>
<td>9</td>
<td>1.5/41 kg</td>
<td>Hyporexia, edema and hyperemia of the face, conjunctival and episcleral congestion, crusts in the nasal plane with nasal discharge, halitosis, erosions in the mucocutaneous junction of the upper lip, small ulcers on the tongue and the lingual frenulum, whitish pulvinus dentalis, ruminal hypomotility, enlarged liver, arching of the back, and lameness</td>
<td>RBC: 9.1 × 10⁶ cells/mm³; PCV: 29%; TPP: 70 g/l; Fb: 2 g/l; WBC: 14,850 cells/mm³ (↑) with neutrophilic and monocytosis; AST: 193 U/l; urea: 34.9 mmol/l (↑)</td>
</tr>
<tr>
<td>10</td>
<td>2.5/45 kg</td>
<td>Hyporexia, edema and hyperemia of the face, conjunctival and episcleral congestion, crusts in the nasal plane with nasal discharge, halitosis, erosions in the mucocutaneous junction of the upper lip, small ulcers on the tongue and the lingual frenulum, whitish pulvinus dentalis, ruminal hypomotility, and crackles in lung auscultation</td>
<td>RBC: 9.1 × 10⁶ cells/mm³; PCV: 29%; TPP: 70 g/l; Fb: 2 g/l; WBC: 14,850 cells/mm³ (↑) with neutrophilic and monocytosis; AST: 193 U/l; urea: 34.9 mmol/l (↑)</td>
</tr>
</tbody>
</table>

* Complete blood cell count and biochemistry test reference values from Krammer (2000) and Kaneko, Harvey, and Bruss (2008), respectively. RBC = red blood cell count; PCV = packed cell volume; TPP = total plasma protein; Fb = fibrinogen; WBC = white blood cell count; AST = aspartate aminotransferase; GGT = gamma glutamyl transferase.
The clinical and pathological findings were suggestive of bluetongue. For molecular and virological diagnosis, whole blood samples, along with multifocal hemorrhaging in the intima and media. Hematoxylin and eosin (HE). Bar = 200 μm. Inset: sheets of erythrocytes between the tissue. HE. Bar = 50 μm.

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References