Incidence and isolation of bluetongue virus infection in cattle of the Santo Tomé Department, Corrientes Province, Argentina

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Summary

Sentinel herds were monitored for the detection of bluetongue (BT)-specific antibodies and virus over two periods, namely: June 1999 to August 2000 and September 2000 to April 2001. Herds were located in Santo Tomé (Herds 1 and 2) where BTV activity was known to occur. From June 1999 to August 2000, the cumulative incidence (CI) of bluetongue virus (BTV) infection was 0% and 35% in Herds 1 and 2, respectively. In the second period, the CI of BTV infection was 10% and 97% in Herds 1 and 2, respectively. The virus was isolated from red blood cells of animals that seroconverted and was identified as serotype 4. Averages of the monthly maximal temperatures were always above 19°C. However, averages of the monthly median temperatures were below 19°C and averages of the monthly minimal temperatures were below 15°C from May 2000 to August 2000. There was no viral activity detected at that time. *Culicoides insignis* was identified as the predominant potential vector species (99%) trapped near sentinel herds. Although clinical disease has never been reported in Argentina, viral activity was detected and the virus has been isolated in sentinel herds.

Keywords

Argentina – Bluetongue virus – *Culicoides* – Infection – Disease – Serotypes.

Introduction and objectives

Bluetongue (BT) virus is present worldwide in the tropics and subtropics and can cause disease in domestic and wild ruminants. The virus is transmitted by some species of haematophagous *Culicoides* midges (2). In South America almost all the countries have serological evidence of BT virus (BTV) infection (1) but only four outbreaks of the disease have been reported. The importance of BT resides in economic losses due to the restrictions to international movement of ruminant livestock and germplasm. Although 24 serotypes of BTV have been recognised in the world, BTV distribution seems to follow ecological factors (4).

The objective of this study was to investigate the pattern of transmission of BTV in cattle in Argentina and to isolate the virus.

Materials and methods

Sentinel herds were monitored serologically from June 1999 to August 2000 and from September 2000 to April 2001. Herds were located in Santo Tomé (Herds 1 and 2) in the Corrientes Province, areas where BTV activity was known to occur. In the first period, 30 BTV competitive ELISA (c-ELISA)-negative, 6-9-month-old female cattle were selected from each herd (Herds 1 and 2). The c-ELISA kits were supplied by Veterinary Diagnostic Technology, Wheat Ridge in Colorado (USA). Blood samples were collected monthly except for January 1999 and July 2000. In the second period, 40 seronegative 6-9-month-old female cattle were selected from each herd (Herds 1 and 2). Samples were collected in September 2000, December 2000, March 2001 and April 2001. Light traps were located close to the sentinel animals to collect potential vectors of BTV

from Herd 1. Red blood cells from those animals that seroconverted were processed for virus isolation by inoculation into embryonated chicken eggs and cell cultures (5).

Results

From June 1999 to August 2000, the cumulative incidences (CI) of BTV infection were 0% (0/34) and 35% (11/31) in Herds 1 and 2, respectively (Figs 1 and 2).

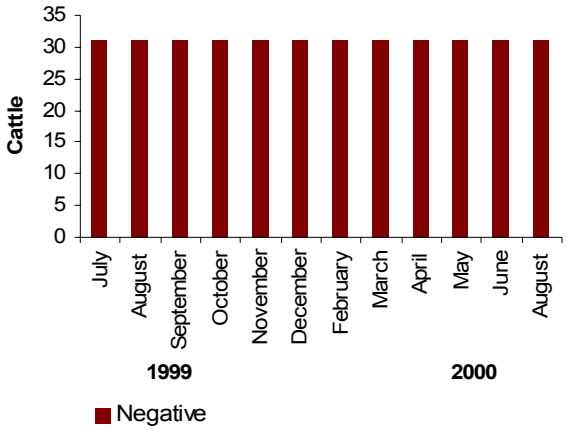

Figure 1
Serological monitoring of cattle in Argentina (Herd 1), 1999-2000

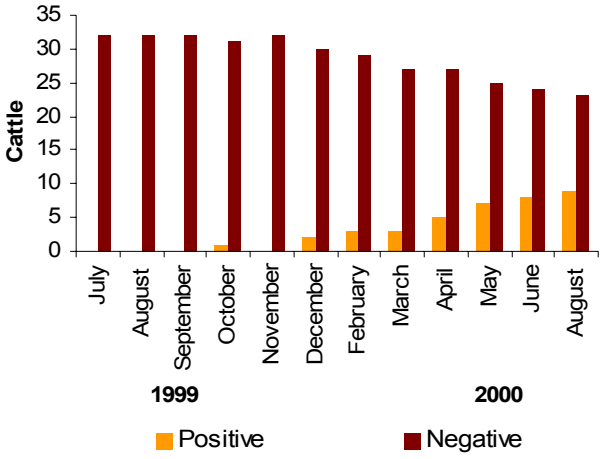

Figure 2
Serological monitoring of cattle in Argentina (Herd 2), 1999-2000

Cattle were seropositive from October to May in Herd 2. The highest monthly CI were recorded from March to April in Herd 2. No seroconversion was detected later than May. Seroconversions were identified between March and April 2001 in Herd 1 (Fig. 3) and between December and March 2000-2001 in Herd 2 (Fig. 4) The CI for these herds were 10% (5/50) and 97% (39/40), respectively (Figs 3 and 4).

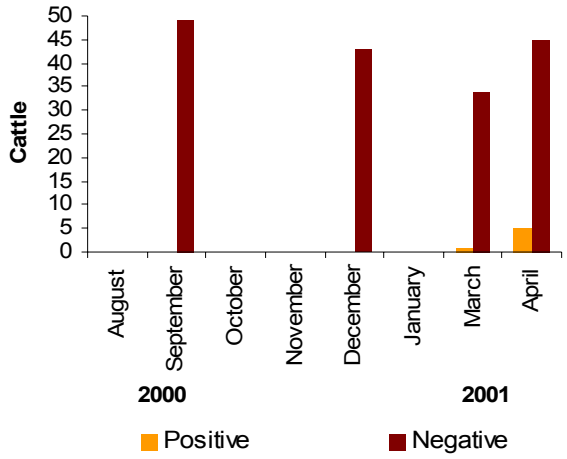

Figure 3
Serological monitoring of cattle in Argentina (Herd 1), 2000-2001

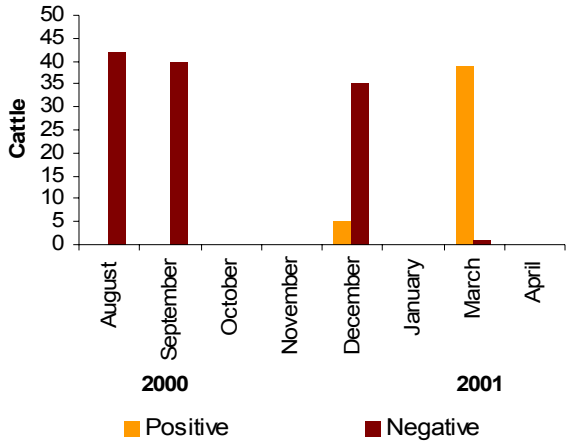

Figure 4
Serological monitoring of cattle in Argentina (Herd 2), 2000-2001

Averages of the monthly maximal temperatures were always above 19ºC. However, averages of the monthly median temperatures were below 19ºC and averages of the monthly minimal temperatures were below 15ºC from May 2000 to August 2000 (Fig. 5). No viral activity was detected during this period.

Culicoides insignis was identified as the predominant potential vector species (99% of 3 082 midges trapped close to the sentinel herds) (6).

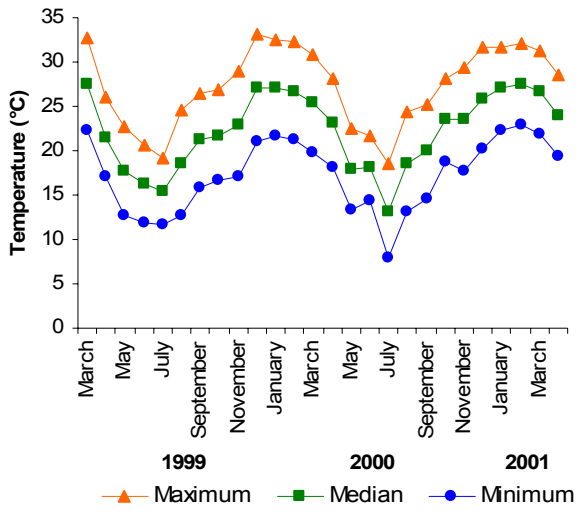

Figure 5
Monthly temperatures in Ituzaingó-Santo Tomé, Argentina, March 1999-April 2001

BHK cells inoculated with four samples developed CPE and were positive by direct and indirect immunofluorescence with BTV-specific reagents. Those samples examined by electron microscopy showed virus particles with BTV morphological characteristics. Blood samples and tissue culture supernatants were positive with the RT-PCR technique with primers corresponding to segment 3 of the BTV genome. Using microseroneutralisation and RT-PCR, with primers corresponding to the segment 2 of the BTV genome, the four samples were identified as serotype 4 (Fig. 6).

Three out of four Argentinian virus samples tested (20, 99 and 102) gave positive results in RT-PCR assays with BTV-4 specific primers, generating a cDNA band of the expected size (2.2 kB) which was detected after agarose gel electrophoresis (Fig. 6). The cDNA from virus sample 20 was subsequently used as template for sequencing reactions and analysis (accession number AJ585169). A comparison of 687 nucleotides near to the 5′ end of genome segment 2 was made with sequence, that was also derived from a South African reference strain of BTV-4 (accession number AJ585125) and a 9.4% nucleotide sequence difference was detected. According to previous phylogenetic analyses of genome segment 2 from different BTV isolates (3), this level of homology indicates that the two strains are from the same serotype, although not closely related. Data concerning these virus isolates is available at: iah.bbsrc.ac.uk/dsRNA_virus_proteins/ReoID/btv-4.htm.

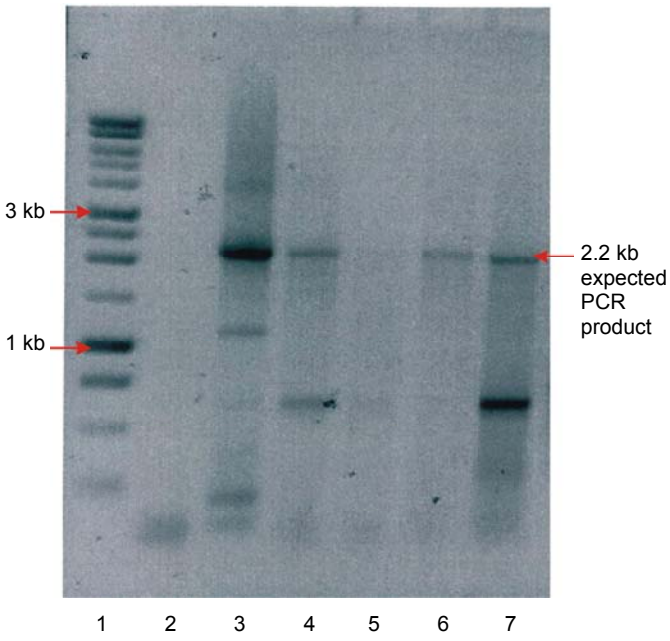

Figure 6
Agarose gel electrophoresis of cDNAs generated by RT-PCR, using BTV-4 specific primers and RNA templates extracted from infected BHK cells
Lane 1 1 kb marker
Lane 2 negative control
Lane 3 BTV-4 South Africa
Lane 4 BTV-4 Argentina No. 20
Lane 5 BTV-4 Argentina No. 82
Lane 6 BTV-4 Argentina No. 99
Lane 7 BTV-4 Argentina No. 102

Discussion

Although clinical disease has never been reported in Argentina, viral activity was detected and the virus has been isolated from sentinel herds. This first isolation of BTV in Argentina was identified as serotype 4. There was a marked variability in the CI of BTV infection among herds and between years. Absence of BTV activity from May to September suggests that low temperatures resulted in low or no vector activity. *Culicoides insignis* seems to be the most likely vector of BTV in this area.

Acknowledgement

This project was supported by SECYT, PICT/97 08-00000-01071.

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