PREVALENCE OF THE BLUETONGUE VIRUS ANTIBODIES IN RUMINANTS IMPORTED TO POLAND IN 2008

WIEŚŁAW NIEĐBALSKI

Department of Foot and Mouth Disease, National Veterinary Research Institute, 98-220 Zduńska Wola, Poland
wieslaw.niedbalski@piwzp.pl

Received for publication November 27, 2008

Abstract

The aim of this study was to evaluate the occurrence of antibodies to the bluetongue virus (BTV) in animals imported to Poland in 2008, the calves born to bluetongue positive cows and Polish-origin animals kept together with imported cattle. From January 1 to December 15, 2008, a total of 25,495 samples of sera was tested using the c-ELISA and direct ELISA. Out of the tested sera, 1,511 (5.92 %) were found to be positive for BTV. The majority of seropositive cattle were imported to Poland from Germany (987; 65.3%) and the Netherlands (290; 19.2%). Maternal antibodies were detected in 129 (8.5%) samples of sera taken from calves born to seropositive dams of German and Dutch origin. The high number of seroreagents was the result of bluetongue vaccination implemented in BTV-infected EU member States in 2008. In conclusion, it can be stated that surveillance studies should be continued to monitor the actual bluetongue status of Poland. However, an ELISA for the differentiation of infected and vaccinated animals should be introduced to laboratory practice to determine the number of BTV post-infected seropositive animals in the population of imported animals.

Key words: imported ruminants, bluetongue virus, antibodies, Poland.

Bluetongue (BT) is an economically-important infectious but non-contagious, arthropod-borne viral disease. It affects camels and all domestic and wild ruminant species, including sheep, cattle, goats, deer, antelopes, and elk (7). It is caused by the bluetongue virus (BTV), the species of the genus Orbivirus within the family Reoviridae and 24 serotypes (BTV-1 to BTV-24) having been identified so far over the world (2). BTV is a small (about 70 nm in diameter)icosahedral virus with a ten-segmented, double-stranded RNA (dsRNA) genome, which encode for seven structural proteins (VP1-VP7) and four non-structural proteins (NS1-NS3 and NS3A) (15). The most common symptoms of BT include fever, catarrhal stomatitis, rhinitis, enteritis, and lameness. The mortality rate can vary from 0% to 30%, but may reach 75% (8).

The distribution of BT is determined by the geographical distribution of the arthropod vector and includes Africa, southern Asia, Australia, the Middle East, and the Americas (17). Historically Europe has experienced only sporadic incursions of BT, involving a single-virus serotype on each occasion (9). However, since 1998, BT outbreaks have occurred annually, involving strains from six distinct BTV serotypes – BTV-1, 2, 4, 8, 9, and 16 (10). Since August 2006, for the first time, the BTV has crossed latitude 50°N and BT outbreaks occurred in the northern part of Europe: the Netherlands, Belgium, Germany, France, and Luxembourg (16, 18). In 2007-2008, BTV-8 spread to the other regions of Europe, where disease had never been observed before: the United Kingdom, Denmark, Switzerland, Czech Republic, Sweden, Spain, Hungary, and Austria (Fig. 1).

In total, 2,358 outbreaks caused by BTV serotype 8 and 1,772 outbreaks caused by BTV serotype 1 were reported by EU member States from May 1, 2008 to December 2, 2008 (3). On October 24, 2008, the Netherlands reported the occurrence of BTV-6 on its territory. This serotype had not been reported in the EU before and was not known to be present in Europe or surrounding areas. The origin of the serotype is still unknown. Recently, the disease outbreaks caused by BTV serotype 6 were also observed in Germany, close to the Dutch border (Fig. 1). At present, all EU member states infected by BTV use vaccination as the most effective tool to control the disease. Voluntary vaccination of cattle and sheep is implemented in the United Kingdom and the Netherlands, while mandatory vaccination is performed in Belgium, Denmark, France, Germany, Italy, Luxembourg, Portugal, and Spain.

The occurrence of BT in the border countries suggests that Poland is now at high risk of BTV epizootic infection. According to the decision of the General Veterinary Inspectorate of the Ministry of Agriculture and Rural Development in Warsaw of March 26, 2008, all BTV-susceptible animals imported to Poland from BT-affected countries should be tested for the presence of BTV-specific antibodies.
Any offspring of BT-positive animals and Polish-origin animals kept together with imported animals should be also tested. That is why, since October 2006, we have started serological surveillance of all susceptible animals imported to Poland from EU countries after June 15, 2006 (11). This monitoring was continued in 2007 and about 11,000 serum samples were tested by c-ELISA (12).

The aim of this study was to determine the BT serological status of BTV-susceptible animals imported to Poland in 2008, the calves born to BT positive cows and Polish cattle kept together with the imported animals.

**Material and Methods**

**Sera.** From January 1, 2008 to December 15, 2008, a total of 25,495 samples of sera collected from cattle, fallow deer and goats imported to Poland from BTV infected EU member countries was examined. Besides, the serum samples collected from the offspring of BT-positive cows and Polish-origin cattle held on farms together with imported animals were analysed. Out of the total number of the tested serum samples, 25,485 (99.96%) samples were taken from cattle and only eight (0.03%) and two (0.01%) from fallow deer and goats, respectively. The sera were supplied by the District Veterinary Inspectorates from 14 voivodeships (Table 1), and kept frozen at -20°C until used.

**Testing.** The sera were screened using a commercially-available c-ELISA kit (VMRD Inc., Pullman, USA) according to the procedure described previously (11). Since November 20, 2008, c-ELISA has been replaced by the INGEZIM BTV DR kit (Ingenasa) based on a new direct ELISA called double-recognition ELISA. This test is based on the detection of antibodies specific to the VP7 protein of BTV, and is designed to detect antibodies during infection by any type of BTV and/or post-vaccination antibodies induced by any vaccine presenting the VP7 antigen. According to the manufacturer’s specifications, a sample is considered to be positive if its OD value at 450 nm is higher than the cut-off (15% of positive control). All positive results were confirmed by the AGID assay using a commercially-available BTV antigen and positive BTV reference antisera (Bluetongue Virus Antibody Test Kit, VMRD Inc., Pullman, USA). The test was read after 24 h of incubation at room temperature and reread after 48 h to confirm the results.

**Results**

Out of 25,495 sera tested, 1,511 (5.92 %) were found to be positive for BTV antibodies (Table 1). Most of the seroreagents were detected in Wielkopolskie, Warmińsko-Mazurskie, Podlaskie, Mazowieckie, and Zachodniopomorskie Voivodeships - 534 (2.10%), 261 (1.02%), 170 (0.67%), 160 (0.63%), and 134 (0.52%), respectively. The majority of seropositive animals were imported from Germany (987, 65.3%) and the Netherlands (290, 19.2%). Seventy nine (5.2%) animals were of Czech origin and 26 (1.72%) were from France. The rest of the positive sera (129, 8.5%) were collected from calves born to BT-seropositive dams imported from the member countries. No cattle specimens of Polish origin kept together with animals imported from the BT-affected countries were found positive for BTV antibodies.
Table 1
Presence of BTV antibody positive animals tested in 2008

<table>
<thead>
<tr>
<th>Voivodeship</th>
<th>Country of origin</th>
<th>Number of sera tested</th>
<th>Number of sera positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolnośląskie</td>
<td>Germany, France, Austria, Czech Republic, Poland</td>
<td>736</td>
<td>24 (0.09%)</td>
</tr>
<tr>
<td>Kujawsko-Pomorskie</td>
<td>Germany, France, Italy, Poland</td>
<td>1,467</td>
<td>32 (0.12%)</td>
</tr>
<tr>
<td>Lubelskie</td>
<td>Germany, Czech Republic, Austria, Poland</td>
<td>669</td>
<td>90 (0.35%)</td>
</tr>
<tr>
<td>Lubuskie</td>
<td>Germany, Netherlands, Poland</td>
<td>556</td>
<td>51 (0.20%)</td>
</tr>
<tr>
<td>Łódzkie</td>
<td>Germany, France, Poland</td>
<td>285</td>
<td>41 (0.16%)</td>
</tr>
<tr>
<td>Mazowieckie</td>
<td>Germany, France, Czech Rep., Netherlands, Poland</td>
<td>2,916</td>
<td>160 (0.63%)</td>
</tr>
<tr>
<td>Opolskie</td>
<td>Germany, Czech Republic, Netherlands, Poland</td>
<td>1,251</td>
<td>12 (0.05%)</td>
</tr>
<tr>
<td>Podkarpackie</td>
<td>Germany, Czech Rep., France, Italy, Slovakia, Poland</td>
<td>84</td>
<td>0</td>
</tr>
<tr>
<td>Podlaskie</td>
<td>Germany, Austria, France, Czech Republic, Denmark, Poland</td>
<td>2,520</td>
<td>170 (0.67%)</td>
</tr>
<tr>
<td>Pomorskie</td>
<td>Germany, Czech Republic, Austria, Denmark, Poland</td>
<td>215</td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td>Śląskie</td>
<td>Germany</td>
<td>3</td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td>Warmińsko-Mazurskie</td>
<td>Germany, Czech Rep., France, Austria, Netherlands, Denmark, Sweden, Poland</td>
<td>1,554</td>
<td>261 (1.62%)</td>
</tr>
<tr>
<td>Wielkopolskie</td>
<td>Germany, France, Czech Rep., Netherlands, Austria, Denmark, Poland</td>
<td>10,825</td>
<td>534 (2.10%)</td>
</tr>
<tr>
<td>Zachodniopomorskie</td>
<td>Germany, Czech Republic, Netherlands, Austria, Denmark, Poland</td>
<td>2,414</td>
<td>134 (0.52%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>25,495</td>
<td>1,511 (5.92%)</td>
</tr>
</tbody>
</table>

Discussion

An effective serological surveillance is one of the essential measures for the strategy of BT control. Diagnostic tests are a major component of success in any surveillance system. A wide variety of tests are capable of detecting BTV-specific antibodies. These include agar gel immunodiffusion (AGID), complement fixation (CF), virus neutralisation (VN), and ELISA (14). The competitive ELISA (c-ELISA) is very rapid, specific, sensitive at detecting antibodies in BTV-infected animals and easy to use (1, 14), therefore was recommended for large-scale serological screening and international trade purposes (13). However, recent studies shown that this ELISA is not sensitive enough to be used for the detection of post-vaccination antibodies (4). If low levels of antibodies were present in tested serum, below the threshold of the detection of the c-ELISA, this vaccinated animal was scored as seronegative. Therefore, since November 20, 2008 we have introduced the direct ELISA (Ingenasa), recommended for the serological screening of animals vaccinated against BT (6). Our preliminary results on the population of 480 vaccinated cattle imported from Germany showed that many more samples of sera scored positive with the Ingenasa kit than with the VMRD kit (c-ELISA). We found that for some panels of sera collected from vaccinated cattle, more than 60% of c-ELISA-negative or threshold sera were scored positive by direct ELISA (data not shown). However, according to the results obtained at the Bluetongue CRL in Pirbright (UK), for animals vaccinated with inactivated BTV-8 vaccines on a single occasion, the absence of antibodies detected by the c-ELISA does not correlate with the lack of protection (5).

Due to the BT mass vaccination of all domestic ruminant species conducted in EU member States in 2008, the number of seroreagents detected in our laboratory in 2008 was much higher than before. In 2006, out of 5,757 sera, only three (0.05%) samples were found to be positive for antibodies to BTV (11). Out of 10719 samples of sera tested in 2007, 30 (0.28%) reacted positively in the c-ELISA (12). By comparison, the number of seroreagents detected in 2008 increased to 1,511 (5.92% of the total number of tested animals). Most of the seropositive animals were detected in the population of imported animals (1,382, 91.5%). Besides, maternal antibodies were detected in 129 (8.5%) samples of sera taken from calves born to seropositive dams of German and Dutch origin. At present, it is impossible to estimate the precise number of BTV post-infected seropositive animals in the population of imported animals. For this purpose, an ELISA for the differentiation of infected and vaccinated animals (DIVA test) should be applied. However, no DIVA tests are available commercially yet. During the carrying out of exercises within the BT Ring Trial 2008, only two laboratories applied with some success “in-house” DIVA assays. Therefore, the validation and commercial distribution of DIVA kits are essential for future serological monitoring, particularly for export/import purposes.

In conclusion, it can be stated that because the BT situation in EU member States is getting more complicated (new BTV serotypes detected), the
serological surveillance should be continued to monitor the actual BT status of Poland.

References