The occurrence of bluetongue virus (BTV) in the blood of susceptible animals, tested in the frame of the BT national monitoring programme. The rRT-PCR assay was applied to virological examination of animals imported from BT-affected countries. On December 5, 2007, the BTV RNA was detected for the first time in blood samples of seropositive cattle from Germany. So far, the presence of the RNA was detected in 37 samples of blood collected from German cows and in one sample taken from Dutch fallow deer. The presence of viral RNA was also found in the blood taken from a 4-week-old calf born from BT positive dam imported from Germany. It was an evidence of the vertical transmission of BTV. The long persistence of BTV in blood of infected animals was demonstrated. The viral RNA was detectable as long as one month after the first collection. Taking into consideration the above results, the implemented virological monitoring tests, in parallel with the surveillance studies, should be continued to monitor the actual BT status in Poland.

Key words: cattle, bluetongue virus, virological analysis, rRT-PCR.
susceptible animals, tested in the frame of the BT national monitoring programme.

**Material and Methods**

**Blood samples.** A total of 10,925 EDTA treated blood samples collected from susceptible animals imported to Poland from BT-affected countries and from Polish cattle kept together with the imported BT positive animals were delivered by the District Veterinary Inspectorates to our Laboratory from December 15, 2007 to November 4, 2008. The blood samples from six BTV-positive cows imported from Germany were taken two times: on August 12 and on September 11, 2008 (Table 2). The blood samples were kept at 4°C until the use. Blood samples from uninfected and experimentally infected sheep (collected 5 d after infection) provided by CRL BTV, Pirbright, UK, were used as a negative (K-) and positive (K+) controls, respectively.

**RNA extraction and rRT-PCR assay.** RNA was extracted from the blood samples by the use of the QIAamp Viral RNA Mini Kit, according to the method recommended by the manufacturer. The rRT-PCR was carried out as described previously (11).

**Results**

Out of 10,925 blood samples, 39 (0.35%) were found to be positive in the applied rRT-PCR (Table 1). Most of BTV positive animals were detected in Podlaskie and Wielkopolskie Voivodeships - 14 (0.13%), and 9 (0.08%) respectively. Six (0.05%) positive cattle specimens were found in Kujawsko-Pomorskie and five (0.04%) in Lubelskie and Mazowieckie Voivodeships. All of 37 BTV positive specimens were from cattle imported to Poland from Germany, whereas one positive fallow deer out of nine tested was of Dutch origin. Besides, one 4-week-old calf born in Poland from a seropositive German dam was found to be positive.

An example of rRT-PCR results is presented in Fig. 1. Out of 24 seropositive cattle imported in August 2008 to Grudziądz District (Kujawsko-Pomorskie Voivodeship), six animals (samples No. 9, 11, 13, 14, 21, and 22) were recognised as a positive (CT<42.0).

* calf born in Poland from German origin BT positive dam

<table>
<thead>
<tr>
<th>Voivodeship</th>
<th>Animal species</th>
<th>Country of origin</th>
<th>Date of blood collection</th>
<th>Number (%) of BTV positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubelskie</td>
<td>cattle</td>
<td>Germany</td>
<td>January 2008</td>
<td>5 (0.04)</td>
</tr>
<tr>
<td>Mazowieckie</td>
<td>cattle</td>
<td>Germany, Poland*</td>
<td>January 2008</td>
<td>5 (0.04)</td>
</tr>
<tr>
<td>Podlaskie</td>
<td>cattle</td>
<td>Germany</td>
<td>December 2007 – April 2008</td>
<td>14 (0.13)</td>
</tr>
<tr>
<td>Wielkopolskie</td>
<td>cattle, fallow deer</td>
<td>Germany, the Netherlands</td>
<td>April–March 2008</td>
<td>9 (0.08)</td>
</tr>
<tr>
<td>Kujawsko-pomorskie</td>
<td>cattle</td>
<td>Germany</td>
<td>August 2008</td>
<td>6 (0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39 (0.35)</td>
</tr>
</tbody>
</table>

Fig. 1. Logarithmic fluorescence plots versus cycle number resulting from the determination of BTV RNA in blood samples (9, 11, 13, 14, 21, and 22) collected from imported animals.
Table 2
CT values of blood samples obtained by rRT-PCR assay

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>1st collection</th>
<th>2nd collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>21.02</td>
<td>27.49</td>
</tr>
<tr>
<td>11</td>
<td>22.72</td>
<td>28.41</td>
</tr>
<tr>
<td>22</td>
<td>22.04</td>
<td>28.03</td>
</tr>
<tr>
<td>13</td>
<td>27.16</td>
<td>30.80</td>
</tr>
<tr>
<td>14</td>
<td>25.76</td>
<td>29.78</td>
</tr>
<tr>
<td>21</td>
<td>27.8</td>
<td>31.42</td>
</tr>
</tbody>
</table>

Three out of six BTV positive blood samples (9, 22, and 11) had CT values of 21.02, 22.72, and 22.04, respectively, whereas samples No. 13, 14, and 21 had higher CT values (Table 2). The blood samples collected from the same animals one-month later (2nd collection) were still BTV positive; however, the CT values of these samples were much lower, in the range 27.49-31.42 (Table 2).

Discussion

Recently observed animal migrations due to international trade and import of BTV susceptible animals was the reason for the decision of BT monitoring studies in the population of animals imported to Poland from BT-affected countries. That is why, since October 2006, we started examining the seroprevalence of BTV-specific antibodies in serum samples collected from susceptible animals imported to Poland from EU countries after June 15, 2006 (12). The serological studies were carried out in 2007 (13) and were continued in 2008. Apart from this, at the end of 2007, we performed the first BT virological assays by the rRT-PCR. On December 5, 2007, the viral RNA was detected for the first time in blood samples of seropositive cattle from Germany (11). So far, the presence of BTV RNA was detected in 37 samples of blood collected from German cows and one sample taken from a Dutch fallow deer (Table 1). The most of the BTV positive animals were found at the beginning of 2008, when the BT epidemiological situation in North-Western Europe was still disadvantageous. The animals imported from BT-affected premises were the source of BTV. It was surprising that the presence of viral RNA was found in one sample of blood taken from a 4-week-old calf born from a BT positive dam. On the basis of the above result, the vertical transmission of BTV was presumed. Recently, the evidence for the transplacental transmission of the field strain of BTV in cattle was confirmed by Menzies et al. (10). The authors found the presence of BTV RNA in three calves born from BTV-seropositive but RT-PCR negative dams imported from the Netherlands into Northern Ireland. The possibility of horizontal transmission of BTV in the absence of any detectable adult vector insects, by spread of the virus by a direct route, most probably by the ingestion of the placenta infected with the virus was also suggested (10).

The pathogenesis of BTV infection is similar in sheep and cattle, and most probably, in all species of ruminants (5). The BTV is associated with the red blood cells. It may persist in these even after the development of a high humoral antibody response (2). It was shown that the BTV RNA could be detected in the whole blood of infected sheep for at least 30 d post infection (d p.i.) and in the blood of infected cattle even up to 90 d p.i. (1, 6). It is difficult to estimate the probable time of infection for BTV positive cattle detected in our laboratory, but the long persistence of BTV in blood of these animals was confirmed. The viral RNA was detectable in blood samples one month after the first collecting, although the CT values were much lower (Table 2).

In conclusion, it may be assumed that the detected BTV positive animals could be the potential source of BTV in our country, after culicoides midges started activity in the environment. The undertaking of appropriate measures (e.g. the elimination of BTV positive animals) was the only means to prevent the further transmission of BTV into the environment. Therefore, the implemented BTV virological tests in parallel with the surveillance investigations should be continued to monitor the actual BT status in Poland.

References


