Prevalence of bovine herpes virus type 1 in small herds of young beef cattle in south-eastern Poland – a preliminary study

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Received: September 15, 2015 Accepted: December 03, 2015

Abstract

The study was performed on nasal swabs, tracheal samples, and sera obtained from young beef heifers aged between 6 and 12 months, from farms in eastern and south-eastern Poland. The samples were evaluated using bovine herpesvirus 1 (BHV-1) ELISA kits (ELISA BHV1 antibody and ELISA BHV1 antigen) and PCR. Among all the animals examined, 37 (32.2%) were positive in the ELISA BHV1 antigen test. The presence of BHV-1 was confirmed by PCR in 42 (36.5%) animals. In the ELISA BHV1 antibody test, 39 (33.9%) seropositive animals were identified. The presence of BHV-1 positive samples was observed in all the examined breeds of young cattle. There were no significant differences (P ≤ 0.05) in BHV-1 positive samples. The results indicate that the incidence of BHV-1 infections in feedlot cattle herds studied was 32.2%–36.5%, which suggests that preventive measures should be implemented in order to limit transmission of the virus.

Keywords: beef cattle, bovine herpes virus 1, virus infection surveillance, Poland.

Introduction

Infectious bovine rhinotracheitis (IBR) is a highly contagious infectious disease caused by bovine herpesvirus 1 (BHV-1) in cattle of all ages and breeds. Analysis of the properties of the virus demonstrated that it is a member of the Alphaherpesvirinae subfamily, 150–200 nm in size, and contains a double-stranded DNA genome of approx. 140,000 base pairs. During acute primary infection, the virus is excreted over a period of 10 to 17 d, with the highest virus titre estimated between 4 and 6 d after infection. The virus can affect young calves and adult animals with the same intensity (14, 20). Besides respiratory disease, the virus causes other clinical syndromes, such as infectious pustular vulvovaginitis or balanoposthitis, conjunctivitis, abortion, encephalitis, and generalised systemic infections.

BHV-1 infection causes immunosuppression which increases susceptibility to secondary bacterial infections (9, 16). The particularly high costs associated with the infection are estimated at 3 billion USD annually in U.S. feedlots and about 600 million Euro in European countries (2, 19). Reducing infections caused by BHV-1 in cattle significantly improves health and weight gain, which is especially important in beef cattle. The most important problem, however, involves passage of the virus in a latent state following the acute phase of the disease. Research has shown that in many cases of infection no clinical signs are observed, and these animals are clinically healthy, ‘latently infected’ animals. In stress conditions such as shipment, transport or mixing of calves, the latent virus can be reactivated and the animals can spread the virus (5, 7).
Animals with the subclinical form of the disease pose the main threat to other individuals in the herd and contribute to uncontrolled transmission of the virus to other herds, e.g. as a result of mating or purchasing animals for stock replacement. Confirmation of the presence of BHV-1 infection in cattle herds leads to reductions in export of cattle for stock replacement from countries and herds that are not considered to be free of BHV-1. Thus control and elimination of the infections can have direct economic benefits both for farmers and for the cattle industry of the entire country. Numerous studies have also shown that the best means of eradicating BHV-1 is to slaughter rather than vaccinate seropositive animals (1). However, it has been shown (8) that although BHV-1 infections have been eradicated in many European Union countries, the virus still causes economic losses to dairy and beef production in Europe and the United States.

The problems described above indicate the necessity of conducting diagnostic tests for infections induced by BHV-1. Many European countries, including Austria, Denmark, Norway, Sweden, Finland, Switzerland, and Germany (not all states), have been declared to be free of the virus (1) thanks to implementation of programmes for the control and elimination of BHV-1 infections.

Given the lack of relevant information regarding the prevalence of BHV-1 infections in beef cattle in Poland, the aim of the study was to estimate the prevalence of BHV-1 in small herds of beef cattle of 12 months of age at farms located in eastern and south-eastern Poland.

Material and Methods

Animals and samples. Nasal swabs (n = 115) and sera (n = 115) were obtained from young beef heifers aged from 6 to 12 months and suspended in 1 mL of Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich, Germany). The material included also tracheal samples (n = 15) obtained from dead calves with symptoms of respiratory syndrome. The material was collected from the animals with clinical respiratory disease (cough, exudates from respiratory tract, and fever) or animals without disease symptoms that were kept in the same stalls. It should be emphasised that the samples were taken during a routine veterinary examination conducted in order to develop a treatment and prevention scheme following introduction of the animals to new environmental conditions. Newly purchased calves were kept in a livestock system which put them in direct contact with older beef cattle, aged over 12 months.

All samples were stored at -80°C until analysis. The calves were of Simmental (n = 56), Charolais (n = 35), Belgian Blue (n = 18), and Limousin (n = 6) breeds and came from 35 small beef farms (no more than 20 calves) located in eastern and south-eastern Poland. The number of samples obtained from each farm represented a minimum of 10% of the herd. The calves had not been vaccinated against bovine respiratory syncytial virus (BRSV), bovine viral diarrhoea virus (BVDV), parainfluenza virus-3 (PI-3), or BHV-1. The samples were evaluated using ELISA kits and PCR.

Due to the age of the calves, maternal antibodies could not be detected, as in most cases maternal antibodies are undetectable by six months of age according to Cho et al. (3).

ELISA procedure. A BIO K 189 Pulmotest BHV-1 ELISA kit for antigen (ELISA BHV1 Ag) detection in nasal swabs was obtained from Bio-X Diagnostics (Belgium). Sample preparation, the test procedure, and calculation of the results were performed according to the manufacturer’s instructions. The limit of positive results for the antigen was 0.15. Any sample that yielded a difference in optical density greater than or equal to 0.15 was considered positive. Conversely, any sample that yielded a difference in optical density less than 0.15 was considered negative.

BHV1 antibodies were detected in serum using an indirect ELISA kit (ELISA BHV1 Ab, Cypress Diagnostics, Belgium) according to the manufacturer’s recommendations. Absorbance was measured using a Bio-Rad (USA) 680 ELISA reader at a wavelength of 450 nm.

DNA extraction and PCR reaction procedures. DNA was extracted from supernatants containing secretions from the nasal cavity or trachea with a Qiagen DNA Mini kit 50 (Qiagen, the Netherlands). The PCR mix consisted of 2.5 μL of 10 × DNA polymerase buffer, 1 μL of 10 mM dNTP mix (Fermentas, Lithuania), 1 μL of 5 mM solution of each primer (gDp1, gDp2), 1 μL of thermo-stable RED TaqTM DNA polymerase (Fermentas, Lithuania), and 16.5 μL of DNA-RNA free water (Sigma-Aldrich, Germany). The mixture was supplemented with sterile water to a final volume of 25 μL. The primers used to detect the gene encoding the protein were gD BHV-1 p1 5’- GCT GTG GGA AGC GGT ACT - 3’ and gD BHV-1 p2 5’- GTC GAC TAT GGC CTT GTG TGC - 3’, yielding products of 468 bp (16). For the PCR, denaturation was conducted for 5 min at 94°C and 35 cycles were performed under the following conditions: denaturation for 1 min at 94°C, hybridisation for 45 s at 62°C, and elongation for 1 min at 72°C. The last cycle was performed in total and the elongation step after the last cycle was prolonged to 8 min. PCR products were evaluated in 1.5% agarose gel stained with ethidium bromide. The products obtained were analysed using Quantity One software (Bio-Rad, USA).

Statistical analysis. Statistical analysis of the differences between the results obtained in the ELISA BHV1 Ag and PCR and between the results obtained in
the ELISA BHV1 Ab and ELISA BHV1 Ag tests as continuous variables was performed by the chi-square ($\chi^2$) test for independence using the contingency $2 \times 2$ in Statistica 10.0 software (StatSoft, USA).

Results

The results obtained in the PCR showed the presence of a product of 468 bp in 42 (36.5%) of the nasal swabs (Fig. 1). In the case of the ELISA BHV1 Ag, the total number of positive samples was 37 (32.2%) (Table 1). Samples positive for BHV-1 were observed in all breeds of calves examined. Despite the differences in absolute values in the results obtained, there were no statistically significant differences ($P \leq 0.05$) between these methods in the $\chi^2$ test (Table 2).

The highest detection of BHV-1 was observed in nasal swab samples, i.e. 42 (36.5%) positive samples in comparison to 39 (33.9%) positive sera detected in the ELISA BHV1 antibody test. In the case of samples obtained from the trachea, 12 out of 15 were positive for BHV1, representing 80% of the samples tested. However, due to the small number of samples, the results obtained cannot be compared with the results obtained in the tested sera and nasal swabs (Table 3).

The lowest number (37, 32.2%) of BHV-1-positive animals was detected in the ELISA BHV1 antigen test (Table 4). The chi-square test showed no statistically significant differences ($P \leq 0.05$) between the nasal swabs and sera results obtained in the two ELISA BHV1 tests (Table 5).

![Fig. 1. Detection of BHV-1 by PCR assay. Line 1 – pUC mix marker 8 (67–1118bp); lines 2, 6–8, and 12–15 – negative samples; lines 3–5 and 16–20 – positive samples; lines 9–10 and 21–22 – reference strain of BHV-1, IPV468](image)

Table 1. BHV-1 detection by ELISA BHV1 Ag and PCR in calves of different breeds

<table>
<thead>
<tr>
<th>Breed</th>
<th>ELISA positive</th>
<th>PCR positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simmental (n = 56)</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Charolais (n = 35)</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Belgian Blue (n = 18)</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Limousin (n = 6)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>42</td>
</tr>
</tbody>
</table>

Table 2. Statistical analysis of the ELISA BHV1 Ag and PCR results by $\chi^2$ test

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>37</td>
<td>78</td>
<td>115</td>
</tr>
<tr>
<td>PCR</td>
<td>42</td>
<td>73</td>
<td>115</td>
</tr>
<tr>
<td>Chi-square (df = 1)</td>
<td>0.48</td>
<td>P = 0.487</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. PCR results obtained in nasal swabs and tracheal fluid samples

<table>
<thead>
<tr>
<th>Material</th>
<th>PCR positive</th>
<th>PCR negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal swabs (n = 115)</td>
<td>42 (36.5%)</td>
<td>73 (63.4%)</td>
</tr>
<tr>
<td>Tracheal fluid samples (n = 15)</td>
<td>12 (80%)</td>
<td>3 (20%)</td>
</tr>
</tbody>
</table>
Table 4. Results obtained in ELISA BHV1 Ag (nasal swabs) and Ab (sera) test kits

<table>
<thead>
<tr>
<th>Material</th>
<th>ELISA BHV-1 antigen positive</th>
<th>ELISA BHV-1 antigen negative</th>
<th>ELISA BHV-1 antibody positive</th>
<th>ELISA BHV-1 antibody negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal swabs (n = 115)</td>
<td>37 (32.2%)</td>
<td>78 (67.8%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sera (n = 115)</td>
<td>-</td>
<td>-</td>
<td>39 (33.9%)</td>
<td>76 (66.1%)</td>
</tr>
</tbody>
</table>

Table 5. The statistical analysis (χ²) test between the results obtained in nasal swabs and sera in both ELISAs

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal swabs</td>
<td>37</td>
<td>78</td>
<td>115</td>
</tr>
<tr>
<td>Sera</td>
<td>39</td>
<td>75</td>
<td>114</td>
</tr>
<tr>
<td>Chi-square (df = 1)</td>
<td>0.18</td>
<td></td>
<td>P = 0.673</td>
</tr>
</tbody>
</table>

Discussion

BHV-1 was found to be present in the young feedlot heifers originating from different breeding environments. The total number of positive samples accounted for over 30% of all animals tested. The presence of the virus in the similar number of samples obtained from nasal swabs and sera confirms that both types of material can be used for analysis. Despite the differences in absolute values in the results, there were no statistically significant differences (P ≤ 0.05) in the BHV-1-positive samples. These results suggest the possibility of using both nasal swabs and sera as material for detection of BHV-1.

The lack of significant differences (P ≤ 0.05) in the positive results between the ELISA and PCR techniques suggest that both methods have high sensitivity and specificity in detection of BHV-1-positive animals. The reliability and technical superiority of the ELISA in detection of BHV-1 in cattle serum in comparison to the virus neutralisation test were also confirmed by Saravanajayam et al. (18).

Because all BHV-1-seropositive animals showed the presence of the specific viral protein gD (detected in PCR), it can be assumed that the high levels of serum antibodies were a consequence of the contact of the calves with BHV-1.

Symptoms of respiratory BHV-1 infection observed in young beef cattle may indicate a significant influence of this virus on the development of respiratory syndrome. For example, Raaperi et al. (13) found a significant correlation between high occurrence of respiratory disease in unweaned calves and the presence of BHV-1 in cows. However, these authors did not confirm a significant association between BHV-1 infection and high occurrence of respiratory disease among heifers.

The results obtained in the present study could be useful because currently there are no statistical data in Poland on the occurrence of BHV-1 in beef cattle; only the results of tests conducted on dairy cattle are available (17). In dairy cattle the percentage of cows exhibiting a positive reaction has been shown to be considerably higher than that obtained in the present study, at a level of 53% of 83 herds tested. In the case of small herds the percentage was 11.1%, compared to 73.2% in large herds.

In other European countries which did not introduce an obligatory programme to eradicate BHV-1 infections, the percentage varies. In Estonia, 22% of dairy herds are positive, and this percentage depends on herd size. There is also no systematic control programme in either dairy or beef cattle, except for bulls used for semen collection for artificial insemination (11, 12, 14).

In Slovakia, the total percentage of infected herds is 70%. In the Netherlands, at the start of the eradication programme about 25% of dairy herds and 18% of beef cattle herds were certified BHV1-free (10); the occurrence of BHV-1 was 37% in dairy cattle herds and 66% in beef cattle herds. In France, the percentage of infected dairy herds is 4.7%, while the percentage in beef herds is about 10%. The lower rates of infection in these herds are the result of vaccinations. In other European countries – Lithuania, Latvia, and Hungary – only the occurrence of BHV-1 infections was confirmed, but comprehensive epidemiological testing is lacking. In Ireland, the seroprevalence of BHV-1 in dairy cattle herds was about 74.9%, but in England these results ranged from 40% to 42.3% (4, 21). Literature data indicate that bovine herpesvirus type 1 infections were diagnosed in most countries of Europe and in the world even after implementation of eradication programmes.

At present, countries such as Germany, the Netherlands, Belgium, France, Hungary, the Czech Republic, and Slovakia have national eradication programmes in place. Ireland banned the use of non-marker IBR vaccines in 2005, although currently no eradication programme is in place there (7). In Germany and in the Italian province of Trento, an EU-approved compulsory BHV-1 eradication programme has been implemented. Canada and the United States have also control programmes in place (4).

Other EU countries, such as Austria, Denmark, Finland, and Sweden, as well as the Italian province of
Bolzano-Bozen and some German states, have declared virus-free status as a result of the implementation of programmes to control and eradicate the infection (1). Switzerland and Norway have also eradicated IBR.

In Poland, according to current legislation (15) BHV-1 is monitored. Furthermore, since 2005 many biosecurity tools have been applied, such as the introduction of only seronegative new animals onto farms, restrictions in animal movement, or specific immunoprophylaxis against IBR (6). Such measures can help to reduce the spread of BHV-1 in Poland, particularly in dairy herds.

Conflict of Interests Statement: The authors declare that they have no financial or non-financial conflict of interests regarding the publication of this article.

References