A serological study of BoHV-1 distribution was conducted in Lithuania from 2005 to 2009. Antibody level was measured using a commercial ELISA. For serological examination, 15,368 random blood samples from cattle of different age, gender, and size of herd, which was unvaccinated against IBR, were collected in 37 districts. It was registered that 11.97% of BoHV-1 were seropositive samples. It was also shown that BOHV-1 is most widespread in cattle herds with population >200 individuals (14.79%). Comparison of different sex groups of cattle revealed that the highest number of infected animals was identified in cows (34.64%) and the lowest in bulls (2.01%). In heifers the number of infected animals was 10.01% and in calves – 4.14%. It was shown that seroprevalence of BoHV-1 infection in Lithuania increased with age of animals. The highest prevalence of BoHV-1 (53.98%) was registered in cattle aged more than 7 years.

**Key words:** cattle, bovine herpesvirus, antibodies, Lithuania.

Bovine herpesvirus type 1 (BoHV-1), responsible for infectious bovine rhinotracheitis, is widespread worldwide (23). It belongs to the genus Varicellovirus, family Herpesviridae, sub-family Alphaherpesvirinae. According to DNA analysis, this virus can be classified into sub-types: 1.1 – respiratory infections; 1.2 – respiratory and genital infections; 1.3 – neurological infections; sub-types 2a and 2b. The virus of sub-type 2 is less virulent than the virus of sub-type 1 (2, 7, 9, 14, 23). Rhinitis, fever, salivation, necrosis of mucous membrane of nose, injury of genital mucosa, and sometimes death are the symptoms associated with BoHV-1 infection. Other possible symptoms are purulent necrotic inflammation of vagina or prepuce, endometritis, late abortions, or milk drop (3-5, 18). Yet, the pathology of genitals and reproductive dysfunction are the most widespread among the mature animals subclinical forms of the infection (18, 20). The immunity acquired after the infection is presumed to persist for life. Maternal antibodies have a biological half-life of about three weeks. Occasionally antibodies are detected in animals aged up to 9 months and rarely in older individuals (8).

At present, IBR is identified by serological methods: virus neutralisation (VN) and ELISA. These methods are usually applied for the identification of BoHV-1 antibodies in serum (6). In addition, gE ELISA is used for detection of antibodies to BoHV-1 in cattle vaccinated with marker vaccines (19, 21).

Preliminary investigations in Lithuania have shown that IBR is a rather relevant problem (15). Further investigations have revealed an increasing trend of BoHV-1 prevalence (16). Thus it was necessary to analyse the IBR prevalence in cattle population in Lithuania and to define ways and forms of BoHV-1 transmission. The obtained results would contribute to development and practical application of effective control and prevention measures.

The main objective of the present study was to analyse the IBR epidemiological situation in cattle in Lithuania in 2005–2009, and to estimate the association of BoHV-1 prevalence with cattle age, and the type and size of a herd.
Material and Methods

Materials. Serological study of BoHV-1 in cattle in Lithuania was carried out during a five year period from 2005 to 2009. The blood samples for serological examination were collected from different cattle farms in 37 districts. The samples were collected randomly from animals of different age and gender, unvaccinated against IBR, which were bred in herds of different size. The total of 15,368 blood serum samples was examined. The animals were grouped according to their age, gender, and herd size (Table 1; Figs 2 and 3).

Serological tests. The examination of serum samples was performed at the Departments of Virology of the National Food and Veterinary Risk Assessment Institute and Lithuanian Veterinary Academy. Commercial standardised ELISA kits (POURQUIER® ELISA IBR-IPV gB Serum, Blocking ELISA/Screening, Institute Pourquier, France) were used for the detection of antibodies to BHV 1 gB glycoprotein. All testing was completed according to the manufacturer’s instruction.

Statistical analysis. The confidence interval (CI) of BoHV-1 seroprevalence and statistical significance of the differences between prevalence percentages were calculated at 95% probability following the standard methods.

Results

BoHV-1 Antibodies were detected in 1,839 (11.97%) of examined cattle serum samples. The highest number of seropositive animals was identified in 2009 - 17.88% (95% CI 15.07%–20.69%). The number of seropositive animals over the 5 year period decreased from 12.13% (95% CI 11.14%–13.12%) in 2005 to 9.54% (95% CI 8.35%–10.73%) in 2007. However, from 2008, the number of seropositive animals started to increase and 17.88% (95% CI 15.07%–20.69%, P<0.05; Fig. 1) of cattle were positive to BoHV-1 in 2009.

It was shown that BOHV-1 is most widespread in cattle herds with population higher than 200 animals (14.79%). In smaller herds, the number of seropositive animals was statistically significantly lower (from 0.79% to 6.59 %, P<0.05; Table 1).

Additionally, it was established that BoHV-1 prevalence in cows was significantly higher compared to other animal groups and reached 34.64% (95% CI 32.97%–36.31%). The BoHV-1 seroprevalence was lowest in bulls - 2.01% (95% CI 1.27%–2.75%). It was 10.01% (95% CI 9.23%–10.79%) in heifers and 4.41% (95% CI 3.82%–5.00%, Fig. 2) in calves.

Table 1
The influence of herd size on IBR prevalence

<table>
<thead>
<tr>
<th>Number of animals in a herd</th>
<th>Total number of examined animals</th>
<th>Number of seropositive animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>505</td>
<td>4</td>
</tr>
<tr>
<td>11-50</td>
<td>914</td>
<td>21</td>
</tr>
<tr>
<td>51-100</td>
<td>1,347</td>
<td>106</td>
</tr>
<tr>
<td>101-200</td>
<td>1,912</td>
<td>126</td>
</tr>
<tr>
<td>&gt;200</td>
<td>10,690</td>
<td>1,581</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>CI, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>0.79</td>
<td>0.02 – 1.56</td>
</tr>
<tr>
<td>11-50</td>
<td>2.3</td>
<td>1.33 – 3.27</td>
</tr>
<tr>
<td>51-100</td>
<td>7.87</td>
<td>6.43 – 9.31</td>
</tr>
<tr>
<td>101-200</td>
<td>6.59</td>
<td>5.48 – 7.7</td>
</tr>
<tr>
<td>&gt;200</td>
<td>14.79</td>
<td>14.12 – 15.48</td>
</tr>
</tbody>
</table>

Fig. 1. Dynamics of the distribution of BoHV-1 seropositive animals in cattle population in Lithuania.
It was established that the numbers of seropositive animals in age groups: 0–4 months and 1–2 years were comparable and varied from 4.26% (95% CI 3.7%–4.84%) to 6.69% (95% CI 5.95%–7.43%). The number of seropositive to BoHV-1 animals significantly increased with age. In group >2–3 years, 18.26% (95% CI 16.56%–19.96%) of seropositive animals to BoHV-1 were registered. However, in group >7 years, the number of seropositive animals reached 53.98% (95% CI 49.03%–58.93%; P<0.05; Fig. 3).

**Discussion**

The investigation performed in Lithuania in 2005–2009, showed that 11.97% of the examined cattle were seropositive to BoHV-1. Antibodies to BoHV-1 were determined in all investigated cattle farms and in all age groups. These results extend our previous investigations, showing that the subclinical infection with BoHV-1 is common and widely distributed disease among Lithuanian cattle (16). The results from this study indicate that the seroprevalence of BoHV-1 has tendency to increase since 2008. These data suggest that IBR infection in Lithuania has not reached an equilibrium and seropositivity has tendency to fluctuate. The reason for the apparent increase in seroprevalence in 2008-2009 of BoHV-1 is unknown, but it is possible that the import and movement of cattle is stressful, and the infected cattle can infect susceptible cattle in the newly formed herds.

Since 1996, IBR has been identified in all European countries, in which it was studied. However, the prevalence of BoHV-1 infection between different countries is variable (10, 12, 13, 17). In some of the European countries, BoHV-1 infection level has been
In Austria, Denmark, and Switzerland eradication programmes have been implemented in some European countries. In Austria, Denmark, and Switzerland eradication programmes were very successful and for the last several years there were no seropositivity to BoHV-1. Sanitary programmes also have been implemented in Germany, Hungary, and Italy (10, 13, 17). In Finland, IBR has been eradicated (11). Similar eradication programmes have been implemented in Poland and BOHV-1 infection was eradicated in bull semen centres (12).

Our study demonstrated that cows have higher seroprevalence of BoHV-1 infection in comparison to heifers, calves, and particularly bulls. Analysis of the animals’ age influence on IBR prevalence in the cattle herds in Lithuania showed high association between serological status and cattle age. It was demonstrated that the seroprevalence of BoHV-1 antibodies increased with age. The seroprevalence was consistently higher in adult animals, particularly in cattle over 2–3 years of age. In addition, it was shown that the one of the risk factors for the presence of BoHV-1 antibodies in cattle is a large herd size. Our results regarding the association of seropositivity with increasing cattle age and herd size are in agreement with data from other countries (1, 22).

In conclusion, the results of this 5 years period study indicated that the seroprevalence of BoHV-1 in cattle in Lithuania has tendency to increase. The seroprevalence is significantly associated with cattle age and herd size. The highest percentage of BoHV-1 positive animals was identified in cows older than 2-3 years.

References