SURVIVAL OF BOVINE ENTEROVIRUS STRAIN LCR-4 IN WATER, SLURRY, AND SOIL

HALINA OLSZEWSKA, ZBIGNIEW PALUSZAK1, AND ZDZISŁAW JARZĄBEK2

Department of Animal Hygiene and Microbiology, Faculty of Animal Breeding and Biology, University of Technology and Life Sciences, 85-084 Bydgoszcz, Poland
1Department of Microbiology, Faculty of Agriculture, University of Technology and Life Sciences 85-029 Bydgoszcz, Poland
2Department of Virology, National Institute of Hygiene, 00-791 Warsaw, Poland
haol@wp.pl

Received for publication December 21, 2007

Abstract

The study of the bovine enterovirus survival in water, slurry, and two layers of black soil, was carried out at 4°C and 20°C under laboratory conditions. Samples of soil, water, and slurry were infected with a suspension of the enterovirus. The titre of the virus at 4°C was determined on the day of inoculation (day 0) and on 7, 21, 42, 70, and 135 d after inoculation. At 20°C, the titre was determined at day 0 and after 7, 21, 35, and 49 d. The study showed that bovine enterovirus, present particularly in the samples of water and slurry, retains its ability to cause cytopathic effects. The temperature of 20°C significantly shortened the survival of the virus in water, soil, and slurry. The process of virus inactivation in the soil proceeded faster than in slurry and water, which indicates that viruses in the soil are subjected to the action of different factors.

Key words: bovine enterovirus, survival, water, slurry, soil.

Agricultural use of slurry is an essential yield-forming factor due to its manurial constituents. Insufficiently hygienised slurry can pose a sanitary hazard connected with the risk of soil, groundwater, and plant contamination (17). Epidemic and epizootic hazards result from the fact that slurry can be contaminated with numerous pathogenic microorganisms, including the bacteria of the genera Salmonella, Brucella, Mycobacterium, and Leptospira (8, 9, 14). Moreover, it can contain numerous invasive forms of parasites such as Eimeria, Trichuris, Fasciola, and Ascaris (1). For epidemic and epizootic reasons, foot-and-mouth disease virus, transmissible gastroenteritis virus, rotaviruses, Aujeszky’s disease virus, and other viruses present in slurry seem to be of a particular importance (11, 23). Enteroviruses are particularly often isolated from slurry, and their infective titre can range from $10^3$ to $10^6$ TCID50/mL (22). The virus’s ability to retain infectivity in the environment is strongly differentiated and dependent on thermal and humidity conditions, sunlight, presence of organic matter, microflora, and many other factors (7).

The aim of the presented experiment was to estimate the survival of bovine enterovirus in water, slurry, and two layers of soil under laboratory conditions.

Material and Methods

Strain LCR-4 of bovine enterovirus (cytopathogenic bovine orphan virus) was obtained from the collection of the Animal Hygiene Institute of the Hohenheim University (Germany). The virus was propagated on cell line MDBK (Madin and Darby Bovine Kidney) using Eagle medium without bovine foetal serum, with an addition of antibiotics, as a maintaining fluid. A pool of the virus with the titre of $10^{7.0}$ TCID50/mL was used for the investigation.

Black soil, formed from humus layer and mother rock, was dried and sieved through the 1 mm sieve. The bovine slurry used in the experiment was previously examined to exclude the presence of bovine enterovirus.

The virus was diluted with bidistilled water and slurry at a 1:2 ratio. Then, 1.2 ml of the suspension was introduced into tightly closed plastic containers that contained 5 g of soil samples. In this way, 60% water saturation capacity of the soil was obtained. The dose of the virus per gram of the soil was about $1.2 \times 10^6$ TCID50. At the same time, the control samples were prepared as follows: 0.6 ml of bovine enterovirus suspension was placed in plastic containers and filled with bidistilled water or slurry up to 5 ml.

Soil and control samples were stored at 4°C and 20°C. The titre of the enterovirus was determined in samples stored at 4°C on the day of inoculation (day 0), and then on days 7, 21, 42, 70, and 135 after inoculation. In samples kept at 20°C, the titre was determined at day 0 and then after 7, 21, 35, and 49 d.
The virus was eluted from the soil by 10% bovine foetal serum at pH 10.5 (5). The eluate was used to determine the virus titre on the MDBK cell line on microplates. The titres were calculated according to the Karber method (24) and presented as TCID<sub>50</sub>/mL of water and slurry or gram of soil.

The results of bovine enterovirus survival in particular carriers were subjected to statistic analysis and regression lines were calculated with the programme Statistica Windows Start Soft 5.1.

**Results**

The dynamics of changes of enterovirus titre in water, slurry, and soil was presented in Tables 1 and 2. Virus inactivation rate illustrated by regression lines was shown in Figs 1-3. Initial titre of bovine enterovirus in water at 20°C ranged from 5.3 to 5.55 log TCID<sub>50</sub>/mL (average 5.42), while on day 49 of the study it decreased significantly and amounted to 3.55 log TCID<sub>50</sub>/mL (Table 1).

A mean titre of the virus stored in water at 4°C changed slightly from 5.38 log TCID<sub>50</sub>/mL to 5.3 log TCID<sub>50</sub>/mL on day 135 (Table 2).

On the basis of regression lines, it was evaluated that titre reduction proceeded slowly and was 0.32 log/week at 20°C and 0.07 log/week at 4°C (Fig. 1). Adding the elution buffer to the suspension of the virus and water and virus and slurry did not affect significantly the survival of the virus (Tables 1 and 2).

Storage of slurry at 20°C resulted in a fast decrease in the virus titre from 5.05 log TCID<sub>50</sub>/mL on day 0 to the value below the detectable level (1.05 log TCID<sub>50</sub>/ml) on day 49 (Table 2). In the slurry stored at 4°C, at first a gradual decrease of virus titre from the initial 4.97 log TCID<sub>50</sub>/mL to 3.8 log TCID<sub>50</sub>/mL was observed on day 42 of the experiment, and then its increase to the level of 4.55 log TCID<sub>50</sub>/mL on day 135 (Table 2). On the basis of regression lines, it was shown that the elimination rate of bovine enterovirus at 20°C was 0.65 log/week, while at 4°C only 0.03 log/week (Fig.2).

The initial titre of the virus in the humus layer of the soil at 20°C ranged from 4.27 to 5.02 log TCID<sub>50</sub>/g of soil (on average 4.64 log TCID<sub>50</sub>/g of soil). However, on day 35 of the experiment, the titre decreased to the level of detectability of the used method (1.52 log TCID<sub>50</sub>/g of soil), and on day 49 the presence of virus was not detected (Table 2). The changes in the number of the tested virus in the soil at 4°C proceeded differently. A slight increase in the titre was observed on day 7 of the experiment (4.77 log TCID<sub>50</sub>/g of soil) in comparison with the initial value (4.52 log TCID<sub>50</sub>/g of soil). The reduction of the titre to a level of 2.52 log TCID<sub>50</sub>/g of soil was observed on day 135 of the experiment (Table 1).

The inactivation rate of bovine enterovirus in black soil was 0.61 log/week at 20°C and 0.11 log/week at 4°C (Fig. 2).

In the mother rock kept at 20°C, a considerable decrease in virus titre to 2.27 log TCID<sub>50</sub>/g of soil was noted as early as on day 21 of the experiment (Table 3). At 4°C, the elimination of the virus from the mother rock proceeded far more slowly. On day 135 of the experiment, the virus titre at the level of 3.27 log TCID<sub>50</sub>/g was found in the soil (Table 1). The reduction of the virus according to the regression analysis was 0.69 log/week at 20°C and 0.08 log/week at 4°C (Fig. 3).

<table>
<thead>
<tr>
<th>Environment</th>
<th>Time (days)</th>
<th>0</th>
<th>7</th>
<th>21</th>
<th>35</th>
<th>49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus + water</td>
<td></td>
<td>5.42</td>
<td>4.80</td>
<td>5.30</td>
<td>4.30</td>
<td>3.55</td>
</tr>
<tr>
<td>Virus + water + buffer</td>
<td></td>
<td>5.38</td>
<td>5.05</td>
<td>4.55</td>
<td>3.92</td>
<td>3.05</td>
</tr>
<tr>
<td>Virus + slurry</td>
<td></td>
<td>5.05</td>
<td>4.80</td>
<td>4.30</td>
<td>1.55</td>
<td>&lt;1.05</td>
</tr>
<tr>
<td>Virus + slurry + buffer</td>
<td></td>
<td>5.03</td>
<td>4.18</td>
<td>4.05</td>
<td>&lt;1.52</td>
<td>&lt;1.05</td>
</tr>
<tr>
<td>Humus layer</td>
<td></td>
<td>4.64</td>
<td>4.52</td>
<td>3.27</td>
<td>1.52</td>
<td>&lt;1.52</td>
</tr>
<tr>
<td>Mother rock</td>
<td></td>
<td>4.77</td>
<td>4.27</td>
<td>2.27</td>
<td>2.27</td>
<td>2.27</td>
</tr>
</tbody>
</table>

**Table 2**

Mean titres of bovine enterovirus stored in water, slurry (log TCID<sub>50</sub>/ml), and black soil layers (log TCID<sub>50</sub>/g) at 4°C

<table>
<thead>
<tr>
<th>Environment</th>
<th>Time (days)</th>
<th>0</th>
<th>7</th>
<th>21</th>
<th>42</th>
<th>70</th>
<th>135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus + water</td>
<td></td>
<td>5.38</td>
<td>5.05</td>
<td>5.05</td>
<td>4.55</td>
<td>4.80</td>
<td>5.30</td>
</tr>
<tr>
<td>Virus + water + buffer</td>
<td></td>
<td>5.38</td>
<td>n.e.</td>
<td>5.05</td>
<td>4.30</td>
<td>4.80</td>
<td>5.55</td>
</tr>
<tr>
<td>Virus + slurry</td>
<td></td>
<td>4.97</td>
<td>5.05</td>
<td>4.30</td>
<td>3.80</td>
<td>4.55</td>
<td>4.55</td>
</tr>
<tr>
<td>Virus + slurry + buffer</td>
<td></td>
<td>5.42</td>
<td>5.05</td>
<td>4.30</td>
<td>3.80</td>
<td>4.05</td>
<td>3.30</td>
</tr>
<tr>
<td>Humus layer</td>
<td></td>
<td>4.52</td>
<td>4.77</td>
<td>4.27</td>
<td>3.90</td>
<td>3.77</td>
<td>2.52</td>
</tr>
<tr>
<td>Mother rock</td>
<td></td>
<td>4.60</td>
<td>4.77</td>
<td>4.27</td>
<td>3.77</td>
<td>3.90</td>
<td>3.27</td>
</tr>
</tbody>
</table>

n.e.- not examined
**Fig. 1.** Inactivation rate of bovine enterovirus in water under laboratory conditions at 4°C and 20°C.

**Fig. 2.** Dynamics of bovine enterovirus inactivation in slurry and humus layer of black soil (II3) at 4° and 20°C.

**Fig. 3.** Dynamics of bovine enterovirus inactivation in slurry and mother rock of black soil (II4) at 4° and 20°C.
It should be noted that the elimination rate of the virus from the mother rock stored at 20°C was higher by 0.04 log/week than in slurry (Fig. 3). It is also interesting that at 20°C, both in the humus layer and mother rock, a quicker process of bovine enterovirus elimination was observed in comparison with the slurry (Figs 2 and 3).

Discussion

Enteroviruses disappear gradually from the environment, as they do not replicate beyond the living cell. Yet, their time of retaining infectivity in the environment can be long and is determined by a number of factors.

In the present studies, a small decrease in virus titre in the carriers used in the experiment (slurry, water) was noted. At 4°C, the rate of enterovirus elimination in slurry calculated on the basis of regression lines was as slow as 0.03 log/week and at 20°C, 0.08 log/week. The results obtained confirm the data reported by other authors. The remarkable effect of temperature on bovine enterovirus survival was reported by Winter et al. (25), who isolated the virus from slurry stored at 31°C up to 9 d. Studying the inactivation rate of coliphage Φ2 in slurry, Pesaro et al. (18) determined its daily elimination rate as 0.014 log in the summer and 0.05 log in the winter. Additionally, the study by Olszewska et al. (16) concerning the survival of the poliovirus in water and communal wastewater lends support to the predominant influence of temperature on the virus elimination rate from the tested samples. The long time of retaining the infectivity of bovine enterovirus in slurry in the present study, may have been affected by the process of virion absorption on stable particles of slurry. The results of the studies by Burge and Enkiri (2) as well as Lance and Gerby (13), prove a significant influence of the dry matter content and the presence of divalent ions on the processes of virus absorption.

The theoretical survival time of the tested viruses in water was also relatively long and amounted to 16.8 (20°C) and 78.0 (4°C) weeks. A high resistance of the viruses to environmental factors is reported by Winter et al. (3), who observed the time of their survival in water being 200 d. Keswik et al. (12) determined that the virus survival in drinking water was 200 d. The authors point out a favourable effect of water low biological activity on the prolongation of time needed for the elimination of the viruses from this environment. Soil environment is particularly difficult to study enterovirus infectivity. Adsorption process is a significant factor affecting the elimination rate of viruses from soil (6, 19, 20). Virus ability to adsorption depends on numerous factors, including the germ species, soil type, pH, content of silt, changeable cations, and organic matter (21). Viruses adsorbed on soil particles do not lose their infectivity and frequently survive longer than virions not connected with soil (6, 15, 26). Köhler (10) reports that bovine enteroviruses survived under diverse soil conditions from 24 to 28 weeks at 20°C, and from 52 to 60 weeks at 4°C. A shorter bovine enterovirus survival was noted in the black soil tested in the present study. The theoretical survival time calculated from regression lines was about 7 weeks at 20°C and from 39 to 52 weeks at 4°C. Surprisingly, the elimination rate of bovine enterovirus in both soil layers in the present study was quicker in comparison with both slurry and water (Figs 1, 2, and 3).

At 4°C, the elimination rate of the bovine enterovirus was slightly slower in mother rock than in the humus layer. It can be concluded that a lower biological activity positively affected the survival of virus LCR-4 in the black soil mother rock. It is supported by the study by Hurst (4), who detected viruses in fallow soils longer than in biologically active soils. The determination into the effect of particular physical and chemical soil factors on virus survival is enormously difficult due to their diversity and the complexity of biological and chemical processes that occur in the soil.

In conclusion, viruses introduced into water, slurry, and soil differed in the rate of the process of elimination they underwent. The survival of the bovine enterovirus was affected mainly by the temperature. The low temperature (4°C) resulted in the virus stability in the tested environments, while the high temperature (20°C) accelerated their inactivation. The tested enteroviruses underwent a very slow elimination in slurry and water at 4°C, and the weekly reduction rate of the virus titre was 0.03 and 0.07 log, respectively. In black soil, the process of virus elimination proceeded faster than in slurry and water, what seems to indicate that the viruses in the soil were subjected to the action of many different factors.

References


