GENETIC PROFILES OF BOVINE HERPESVIRUS 1 STRAINS ISOLATED FROM CATTLE IN POLAND

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Abstract

The purpose of the study was to determine subtypes of BHV1 strains isolated from cattle in Poland. In total five different strains of BHV1 were isolated during the last years. All isolates as well as four archival and two reference BHV1 strains were analysed by PCR and restriction enzyme analysis. Specificity of the strains was confirmed by PCR with primers complementary to the nucleotide sequence of gD gene. Subsequently, genomic DNA of the tested strains was digested with endonucleases Hind III and Hpa I. Restriction enzyme analysis revealed that all recently isolated BHV1 strains belonged to the BHV1.1 subtype. Among archival viruses, two strains had restriction pattern similar to the subtype BHV1.1 and two others to the subtype BHV1.2a. The presented study showed that currently subtype BHV1.1 is dominant in cattle population in Poland.

Key words: cattle, bovine herpesvirus 1, genetic profile, restriction enzyme analysis.

Bovine herpesvirus type 1 (BHV1) belongs to the Herpesviridae family, Alphaherpesvirinae subfamily (14, 18). Initially BHV1 strains were divided into two types, the genotype BHV1.1 associated with infectious bovine rhinotracheitis (IBR) and BHV1.2 associated with infectious pustular vulvovaginitis (IPV). Finally, on the basis of the restriction enzyme analysis of viral DNA three subtypes of BHV1 have been identified: subtypes BHV1.1 and BHV1.2a responsible for IBR outbreaks and abortions and subtype BHV1.2b associated with infectious pustular vulvovaginitis IPV or infectious pustular balanoposthitis (IPB) (11). Strains of the subtype 1 are prevalent in Europe, North America, and South America. Strains of the subtype 2b are less pathogenic than subtype 1 and are frequently isolated in Australia and Europe. Viruses isolated from buffaloes and goats and previously identified as BHV1 by serological tests have different restriction enzyme patterns to subtypes of BHV1 and now are regarded as separate viruses and have been classified as BHV2 and caprine herpesvirus, respectively. The bovine herpesvirus causing meningoencephalitis previously identified as BHV1.3 subtype has been classified as BHV5. Independently of differences in the structure of the BHV1 genome there is only one serotype of the virus.

BHV1 can cause different clinical syndromes in cattle such as IBR, IPV or IPB, conjunctivitis, abortions, and generalised systemic infections in young calves (7, 9, 12, 13, 22, 23). The most frequent form of BHV1 infection is IBR. The disease is most frequently observed in cattle managed under intensive conditions like feedlots. The clinical signs and pathological changes of BHV1 infection of cattle are not characteristic and laboratory confirmation is therefore essential. The incubation period ranges from 2 to 7 d. The disease is characterised by high fever, up to 41°C, lasting for 4-5 d, reduced appetite, and apathy. Dairy cows show a significant drop in milk production (25). The initial serous nasal discharge often becomes mucopurulent within few days. The mucosa of the nares becomes reddened and erosions may be present. Some animals develop unilateral or bilateral conjunctivitis and have a clear ocular discharge, which may later become mucopurulent. In feedlot cattle, a severe necrotising laryngotracheitis and pneumonia, complicated by secondary bacterial infections, can be observed (8). A consequence of a respiratory BHV1 infection of seronegative pregnant cows is abortion. Since abortions can occur as long as 90 d after infection, it might be difficult to relate the abortions to BHV1 infection, especially if the infection is mild or subclinical. Naturally occurring abortions are usually observed after the 5th month of pregnancy. Clinical symptoms preceding the abortion often are not observed.

Cattle of all ages and breeds are susceptible to infection with BHV1, but the disease is most common in animals over 6 months of age. The virus is usually introduced into a herd with acutely infected animals (26). Such animals shed large amounts of the virus with nasal and ocular secretions and infect susceptible individuals. The virus is spread within a herd mainly by horizontal transmission such as close contact between animals. Semen of infected bulls may contain BHV1 and
the virus can be transmitted to cows by natural mating or artificial insemination (10, 17, 24). Following a primary infection with BHV1, a latent infection in the trigeminal or sacral ganglia, depending on the route of infection, can be established (2, 3, 16). That is why infected animals become lifelong carriers of the virus. Due to stressful conditions or corticosteroid treatment, latent BHV1 can be reactivated and re-excreted at irregular intervals. Since most animals do not show clinical signs following reactivation it is impossible to predict when an outbreak might occur or which animal is responsible for the outbreak. Therefore, cattle latently infected with BHV1 can also be a source of infection. The virus is relatively stable and can persist in the environment for several days. Due to its stability, BHV1 can also be transmitted mechanically by nose tongs or direct contact with secretions and excretions in feed bunks.

Lately new severe outbreaks of respiratory disease caused by BHV1 were observed in Poland. It may indicate that more virulent strains of BHV1 currently circulate in cattle population in our country. Therefore, the purpose of the study was to determine subtypes of field strains of BHV1 isolated from cattle with respiratory signs and compare them with a restriction pattern of some archival strains of this virus.

Material and Methods

Specimens. Nasal swabs, and tissue samples collected from cattle showing clinical signs of respiratory infection were used for virus isolation in cell culture. The nasal swabs were dipped in Eagle’s MEM containing antibiotics, thoroughly shaken, and centrifuged at 1,000 g for 10 min at 4ºC. Tissue samples were homogenised with the addition of MEM to obtain 10% suspension. The supernatants from nasal swabs and specimen suspensions were used to inoculate cell culture. Additionally, four archival BHV1 strains, the strains Los Angeles and K-22, were used as reference viruses for restriction enzyme analysis.

Virological examination. Virus isolation test was performed in MDBK cell line grown in flat bottom 24-well plates (Constar). Two-three-day-old monolayer of MDBK cells was inoculated with earlier obtained supernatant in the volume of 200 µl/well. The plates were incubated for 1 h at 37ºC and then the inoculum was replaced with maintenance medium (Eagle’s MEM with addition of 2% foetal calf serum) and incubated at 37ºC in 5% CO2 atmosphere. Infected MDBK cells were examined microscopically for the presence of cytopathic effect on the daily basis for the next 5-7 d.

Extraction of viral DNA. Isolated viruses were grown in large bottles (150 cm2) overlayed with MDBK monolayers at 37ºC. When cytopathic effect reached 90%-100%, the culture medium was clarified by low speed centrifugation and the supernatant was centrifuged at 100,000 x g for 1.5 h. The viral pellet was re-suspended in redistilled water. Subsequently, the viral suspension was treated with 0.1 mg/mL proteinase K and 0.5% sodium dodecyl sulfate for 1-2 h at 37ºC. After digestion, the viral DNA was extracted once with an equal volume of phenol:chloroform:isoamyl alcohol mixture (25:24:1), once with chloroform:isoamyl alcohol (24:1), and precipitated with 96% ethanol overnight at -20ºC. Precipitated DNA was centrifuged at 12,000 x g for 15 min at 4ºC. The pellet was then washed with 70% ethanol, air dried, and re-suspended in 50 µl of redistilled water.

PCR analysis. The set of primers selected from the sequence of gene encoding gD glycoprotein of BHV1 was used in PCR (27). PCR mix consisted of 5 µl of 10X DNA polymerase buffer, 2 µl of 10 mM dNTP mix, 1 µl of 5 mM solution of each primer, and 2.5 U of thermo-stable RED Taq™ DNA polymerase (Sigma). The mixture was supplemented with sterile water to a final volume of 50 µl. Two microlitres of extracted DNA was used in the reaction with primers. PCR conditions for DNA amplification were identical as described in original paper. PCR products were analysed in 2% agarose gel. The electrophoresis was run at constant voltage of 100 V in TAE buffer (0.004 M Tris acetate, 0.001 M EDTA) for 1 h. The PCR result was considered positive when the product of expected size was observed.

Restriction enzyme analysis (REA). Viral DNA was cleaved with Hind III and Hpa I restriction enzymes under the conditions recommended by the manufacturer (Fermentas). The digestion products were separated by electrophoresis in 0.7% agarose gels containing 1 µg/mL of ethidium bromide. The gels were run at 35 V for 20 h in TAE buffer and photographed under UV light. The Hind III λ-phage DNA fragments served as standard size markers for determination of BHV1 fragment sizes.

Results

During this study five field strains of BHV1 were isolated. Four strains were isolated from nasal swabs collected from adult cattle and one from the lungs of 2 weeks old calf, which died with clinical signs of respiratory infection. Cytopathic effect characteristic for herpesviruses was observed usually during the first passage after inoculation of the cell culture.

Specificity of the strains was confirmed by PCR with primers complementary for gD gene. The results of PCR are presented in Fig. 1. PCR products of expected size were found in all field strains of BHV1 (Fig. 1, lanes 1 to 5). Positive results were also obtained for vaccine strain Difivac (Fig. 1, lane 6) and the reference strain Los Angeles and K-22 (Fig. 1, lanes 8, 9), respectively. No amplification was observed in negative control.

During the next step, all field isolates were tested using restriction enzyme analysis. Four archival strains and the reference strains Los Angeles and K-22 were additionally included in the study. The REA patterns obtained after digestion of DNA of each isolate with Hind III enzyme are shown in Fig. 2. No differences were found in the patterns of the five field strains and they were identical as the pattern of the reference strain Los Angeles, representing BHV1.1 subtype (Fig. 2, lanes 5 to 9).
Fig. 1. Electrophoresis of PCR products for gD gene. Lane M: DNA molecular weight marker, pUC Mix 8, (Fermentas); lanes 1 to 5 field strains of BHV1, 6 - vaccine strain Difivac, 7 – negative control, 8 – Los Angeles strain (subtype BHV1.1), 9 – K-22 strain (subtype BHV1.2b).

Fig. 2. Restriction enzyme patterns of BHV1 strains after digestion with Hind III. Lanes M: DNA molecular weight marker, lambda DNA/Hind III, (Fermentas); lanes 1 to 4 – archival BHV1 strains, lanes 5 to 9 – field BHV1 strains, lane 10 - Los Angeles reference strain, lane 11 – K-22 reference strain.

Differences were observed among archival strains. Strains marked as 2/13 and Gorzow had restriction patterns typical for BHV1.1 subtype (Fig. 2, lanes 1, 2) whereas the patterns of two remaining strains marked Frezer and Muskat were similar to the pattern of BHV1.2a subtype (Fig. 2, lanes 3, 4).

Restriction patterns of the field isolates were also identical after digestion with Hpa I enzyme. Analysis of the patterns revealed that they were characteristic for BHV1.1 subtype. Among the archival strains other minor differences were found. Restriction patterns of the strains 2/13 and Gorzow differed between each other by fragment C. Differences were also observed in the patterns of the strains Frezer and Muskat. DNA of Frezer strain was cleaved into four visible fragments, while profile of Muskat strain contained five fragments of DNA.

Discussion

First attempts of classification of bovine herpesviruses using restriction enzyme analysis of viral DNA were conducted at the beginning of 1980s. A preliminary study performed by Engels et al. (6) indicated that there were at least two patterns among strains of BHV1. Metzler et al. (11), using this method, have found clear differences between restriction patterns of BHV1 strains isolated from the respiratory and reproductive tracts of cattle. Afterwards, authors from Switzerland have chosen four different restriction enzymes and developed a clustering system for classification of new BHV1 isolates (1). Comparison of restriction pattern of a new strain with patterns of archival strains stored in database allowed determining subtype of a strain and to find the strain, which has similar restriction pattern.

The emergence of a new genotype of BHV1 in United Kingdom was reported for the first time in Scotland. According to Nettleton (15), the appearance of more severe form of IBR was associated with imported cattle, which was infected with BHV1.1 subtype. Edwards et al. (4, 5) have tested by REA over 80 BHV1 strains isolated in England and Welsh in the period of 1960-1978 and showed that BHV1.1 subtype almost completely superseded strains of BHV1.2b subtype in cattle population. They concluded that in the 1960s, BHV1.2b strains were endemic in British cattle at a low prevalence and caused sporadic outbreaks of genital and relatively mild respiratory disease. From the mid 1970s, strains of BHV1.1 subtype have been dominant in cattle population causing severe IBR outbreaks.

In Poland, studies with the use of REA were done at the end of 1980s. Rola et al. (19, 20) have tested
strains of BHV1 isolated from semen of seropositive bulls and showed that infections were caused by both strains of BHV1.1 subtype and BHV1.2 subtype. Salwa (21) has demonstrated that six out of nine BHV1 isolates obtained from cattle with clinical signs of IPV had patterns similar to the subtype BHV1.2a. The results of the presented investigations showed that all BHV1 strains isolated during the last 5 years belonged to the BHV1.1 subtype. Among archival viruses, two strains had restriction pattern similar to the subtype BHV1.2a and two others to the subtype BHV1.2a. Based on the obtained results we can conclude that currently subtype BHV1.1 is dominant in cattle population in Poland.

References