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A SURVEY FOR BVDV ANTIBODIES IN CATTLE FARMS IN SLOVAKIA AND GENETIC TYPING OF BVDV ISOLATES FROM IMPORTED ANIMALS

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A serological survey for bovine viral diarrhoea virus (BVDV) antibodies on a collection of 1295 serum samples obtained from 6–12 months old cattle originating from 45 farms in Slovakia was carried out. On 13 farms more than 90% of the examined animals were seropositive, on 14 farms 71–90% seroprevalence was observed, on 13 farms only 50–70% animals were found to be positive for BVDV antibodies, while the remaining 5 farms showed fewer than 50% seropositive animals. The average incidence of BVDV antibodies (around 70%) was similar as determined 30 years ago. Of 84 serum samples from seronegative animals originating from 14 farms in which 70–98% seropositivity was observed, six were positive in Ag-BVDV ELISA indicating persistently infected (PI) cattle. On a farm to which animals were imported from abroad, a BVD outbreak was observed. Of 110 animals tested, four were positive in Ag-ELISA indicating the presence of PI cattle on this farm. Genetic typing of two isolates from imported animals performed by RT-PCR (324/326 primers from 5´-UTR), sequencing of PCR products and computer-assisted phylogenetic analysis revealed that they belong to BVDV-1h group.

Key words: Bovine viral diarrhoea virus, BVDV, antibody, genetic typing

Bovine viral diarrhoea virus (BVDV), the causative agent of bovine viral diarrhoea (BVD) and mucosal disease (MD), together with classical swine fever virus and border disease virus belong to the genus *Pestivirus* of the family *Flaviviridae*. Two genotypes of BVDV were recognised so far. BVDV genotype 1 (BVDV-1) is spread worldwide. BVDV genotype 2 (BVDV-2) has recently been discovered in Canada and the USA (Pellerin et al., 1994; Ridpath et al., 1994) and sporadically also in several European countries (Wolfmeyer et al., 1997; Letellier et al., 1999; Vilček et al., 2001), including Slovakia (Vilček et al., 2002).

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BVDV-1 infections may occur in subclinical form or manifest themselves in respiratory, reproductive or enteric signs. Pregnant animals infected during the first trimester of gestation give birth to persistently infected (PI) offspring. PI animals do not develop BVDV specific antibodies and shed noncytopathic virus throughout their lifetime, infecting naive animals (Nettleton and Entrican, 1995). Superinfection of PI cattle with antigenically related cytopathic BVDV strain may develop to highly fatal MD (Brownlie et al., 1987). The BVDV-2 infections of cattle result in similar clinical signs as observed for BVDV-1 infections, except for an acute infection with highly virulent strain, when thrombocytopenia and haemorrhagic syndrome may develop (Carman et al., 1998).

Serological surveys for BVDV antibodies suggested that cattle are very often infected with BVDV. Over 70% seroprevalence was observed in cattle in some countries (Niskanen, 1993), but in other countries or geographic regions lower values were also found (Niskanen, 1993; Rossmanith and Deinhofer, 1998). PI animals were detected in a range of 1–2% (Houe, 1999).

Screening for PI animals in a herd is based on the detection of specific BVDV antigen in serologically negative animals of the age of 6 months (Alenius et al., 1997; Lindberg and Alenius, 1999). As PI animals represent a source of virus, BVDV infection spreads easily in herds when those animals are not separated from the herd and the trade with livestock is not controlled properly. All the eradication programs against BVD/MD are based on the elimination of PI animals from cattle farms (Alenius et al., 1997; Lindberg and Alenius, 1999; Rossmanith et al., 2001).

Molecular genetic techniques, especially the polymerase chain reaction (PCR) and reverse transcription-PCR (RT-PCR) significantly improve laboratory diagnosis of viral infections (Belák and Ballagi-Pordány, 1993). Several RT-PCR assays have been developed for the detection of BVDV (Belák and Ballagi-Pordány, 1991; Ridpath et al., 1993; Vilček et al., 1994). Due to high variability of BVDV strains, PCR primers are often selected from the evolutionary conserved 5′-noncoding region (5′-UTR) (Ridpath et al., 1993; Vilček at al., 1994).

PCR coupled with direct sequencing and phylogenetic analysis is the best approach for the typing of pestiviruses into genotypes or subgenotypes (Hofmannn et al., 1994). Molecular epidemiology provides important information on the origin of infection and the spread of infection among farms (Haas, 1997; Vilček et al., 1999).

The aim of our work was to perform a survey for specific BVDV antibodies on cattle farms in Slovakia and for the identification of PI animals. In addition, classical and molecular genetic techniques were applied to detect and type BVDV in imported cattle.

Materials and methods

Serum samples. A collection of 1295 serum samples obtained from 6–12 month old cattle originating from 45 farms in Slovakia in 2000 was analysed (Fig. 1). In an average, 20–30 (rarely around 50) animals were checked from each farm. Animals were not vaccinated against BVD/MD. The serum samples were collected by standard procedure randomly without the evaluation of animal health status.

Fig. 1. The location of farms in Slovakia from which serum samples were collected. Squares: Western Slovakia, circles: Central Slovakia, stars: Eastern Slovakia, X: location of farm 'X'

Serological and antigenic analysis. The BVDV-specific serum antibodies were determined using indirect ELISA Ab kit (Svanova Biotech, Sweden and Test Line, Brno, Czech Republic). Specific p80/125 BVDV antigen was detected in blood using SERELISA assay (Rhone Merieux, France).

Characterization of farm 'X' with BVD outbreak. More attention was focused on a farm with dairy cattle (farm 'X') in which BVD outbreak appeared. All the 102 pregnant cows were imported to this farm from abroad in 1999. An outbreak of BVD was reported in the herds while the animals were in quarantine. Most animals suffered from fever (40.5–41 °C), anorexia, cough, diarrhoea, nasal and eye discharge, stillbirth, and malformation of newborn calves. Many abortions and death in the first days after calf birth were also observed.

Analysis of samples from animals on farm 'X'. Serological analysis was carried out on 102 cows and 8 calves in farm 'X'. Seronegative animals were checked by Ag-ELISA. Four antigen-positive blood samples were also analysed by molecular genetic techniques. The total RNA was isolated from sera by Trizol (Gibco

BRL) according to the manufacturer's instruction. In addition, a total RNA was also separated from bulk tank milk collected in farm 'X' according to the method described by Drew et al. (1999). The BVDV nucleic acid was detected by RT-PCR using the 324/326 primers, which were selected from the 5´-untranslated region of the pestivirus genome (Vilček et al., 1994). Details of the synthesis of cDNA and PCR are given elsewhere (Vilček et al., 1994). Two PCR products obtained from blood and bulk tank milk samples were sequenced in both directions by PCR primers using commercial sequencing kit employing fluorescent labelled dideoxynucleotide terminators (Perkin Elmer, USA). The nucleotide sequences were analysed on an ABI PRISM sequencing device and they were proof-read by SeqMan II program from the Dnastar software package (Dnastar, Inc., WI, USA). The phylogenetic tree was constructed using MegAlign program from Dnastar. Some representative BVDV strains from our previous study (Vilček et al., 2001) and reference BVDV strains were also included in the phylogenetic study.

Results

Of 1295 serum samples originating from individual animals housed on 45 cattle farms, the specific BVDV antibodies were detected in 894 animals (69%). However, the occurrence of antibodies significantly varied among the herds. Seropositivity higher than 90% was detected on 13 farms, 71–90% seroprevalence was observed on 14 farms, while lower values of seropositivity between 50–70% were found on 13 farms. Cattle from 5 farms showed less than 50% seropositivity. Comparing three Slovakian geographic regions, the distribution of BVDV antibodies was not equal. In Western Slovakia two farms showed more than 90% seroprevalence, 7 farms showed 71–90% and 7 farms indicated 50–70% BVDV seropositivity. The corresponding numbers of farms from Central and Eastern Slovakia were 4/2/2 and 7/5/4 farms, respectively.

Eighty-four serum samples of seronegative animals originating from 13 farms with the highest seropositivity (70–98%) were used for the detection of specific BVDV antigen in order to identify PI animals. Six samples of 462 animals belonging to the 13 mentioned farms were found to be positive for BVDV antigen, which represent 1.3% PI animals.

On the farm 'X', where imported animals became affected by BVDV infection with clinical signs representing BVD outbreak, 92 of 110 animals tested (83.6%) were positive for BVDV antibodies. Two cows and two seronegative calves were identified by Ag-ELISA as PI animals, which was also confirmed by RT-PCR. Sufficient amount of PCR product was obtained for sequencing from the blood sample of a young PI calf with malformation of jawbone. Further PCR product for sequencing was obtained from a bulk tank milk sample collected in the same farm.

A 98% similarity between two nucleotide sequences of PCR products was observed. Genetic typing using phylogenetic approach suggested that both viral samples belong to relatively rare BVDV-1h genetic group. Two other BVDV isolates originating from a country from which cattle were imported to farm 'X' were also clustered in BVDV-1h genetic group (Fig. 2).

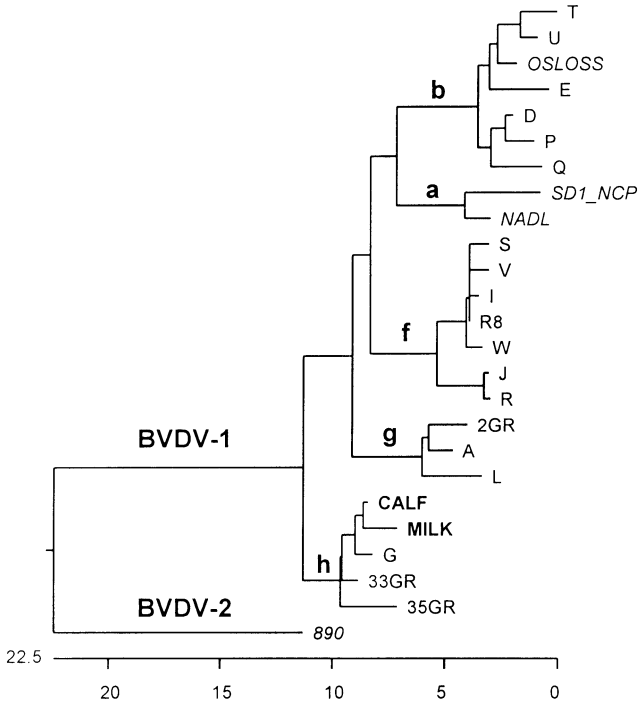

Fig. 2. Genetic typing of BVDV isolates found on farm 'X'. Two isolates from farm 'X' are labelled CALF and MILK. The phylogenetic tree was constructed using MegAlign program. The nucleotide sequences for the strains NADL, SD-1, Osloss and 890 were taken from GenBank (accession numbers M31182, M96751, M96687, U18059, respectively). The data for other isolates were taken from our previous article (Vilček et al., 2001) and our unpublished data collection. Genetic groups of BVDV-1 (a, b, f, g, h) were labelled according to Vilček et al. (2001)

Discussion

In most of the cattle herds examined in Slovakia, BVDV seropositivity ranged from 50 to 100%. In some cattle herds lower prevalence of BVDV antibodies was found. A similar prevalence of BVDV antibodies in the Slovakian cattle population was observed in an other independent but very limited study (Pistl et al., 1999). Surprisingly, our data are very close to the results of a survey for serum BVDV antibodies in cattle, which was done in Western Slovakia 30 years ago (Žuffa et al., 1972). This result indicates that BVDV has been perma-

nently present in the Slovakian cattle population as a result of underestimating the significance of BVDV infection and because of uncontrolled cattle trade.

The results on the prevalence of BVDV antibodies in Slovakia are similar to data from other European countries, where 60–85% BVDV seropositivity was detected (Houe, 1999). The low incidence of PI animals (1.3%) identified in our study also corresponded to the results found by others, who identified about 1–2% PI animals (Liess et al., 1987; Houe et al., 1999).

BVD/MD causes serious economic losses in the cattle industry (Ozsvári et al., 2001). In order to improve both health status of cattle and economic parameters in cattle herds, Scandinavian and some other European countries have already started an eradication program against BVD/MD at the regional and national level. They have demonstrated that it is possible to diminish BVDV seroprevalence significantly after the elimination of PI animals from cattle herds and the introduction of strict rules in cattle farming (Alenius et al., 1997; Linderg and Alenius, 1999; Rossmanith et al., 2001). The persistence of comparable values of BVDV seroprevalence over a period of three decades in Slovakia indicates that BVDV cannot be eliminated from cattle farms spontaneously. The vaccination of cattle against BVDV/MD was applied only in some herds where serious health problems occurred. Our data clearly indicate that the introduction of an eradication program against BVD/MD in Slovakia, and in other countries with similar epidemiological situation, is strongly needed and would be useful.

Recently, we have revealed that BVDV-1 is genetically more variable than it was previously supposed. While BVDV-1 strains were usually divided into two genetic groups, e.g. BVDV-1a ('NADL like') and BVD-1b ('Osloss like') (Pellerin et al., 1994; Ridpath et al., 1994), our data confirmed that BVDV-1 strains could be divided into at least 11 genetic groups (Vilček et al., 2001). The two very similar viral samples detected in PI young calf and bulk tank milk orginating from the cattle farm 'X', in which BVD outbreak had appeared, were genetically typed as BVDV-1h. The viruses belonging to this genetic group have been found so far in Austria, Germany and Mozambique only (Tajima et al., 2001; Vilček et al., 2001). We have to mention that one virus isolate collected in Slovakia in 1999 has also been typed as BVDV-1h (Vilček et al., 2001) but it originates from farm 'X' described in the present work. Additional six BVDV isolates found in different geographic regions of Slovakia, which were typed in our laboratory so far, do not belong to the BVDV-1h genetic group. However, no other farms in the close neighbourhood of farm 'X' where examined.

It is highly likely that two isolates belonging to BVDV-1h type were brought into Slovakia with imported animals, because they were identified in animals kept in quarantine and BVDV-1h type had not been detected previously in the country.

The present data demonstrate that BVDV widely circulates on cattle farms in Slovakia. It is highly urgent to point out a proper control of all imported cattle

for BVDV because of the high risk of importing PI animals, which represent a long-term source of infection and serious health problems in the farms. The classical and molecular genetic techniques can significantly facilitate the control of unwanted international trade of BVDV-infected cattle.

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