

Summary

Bovine viral diarrhoea (BVD) caused by a pestivirus of cattle is one of the most important diseases of cattle which can be manifested by clinical signs including gastrointestinal disorders, respiratory and reproductive problems resulting in significant economic losses in modern cattle production. Ability of BVDV to cross the placenta during early pregnancy (40-120 days) can result in a birth of persistently infected (PI) calves. PI animals shed large quantities of virus in all their secretions and excretions throughout life and are the primary reservoirs for BVDV and the major source of infection in a herd. Eradication of infection is based on the identification and removal of PI animals from a herd. Herd prevalence of PI animals is low and ranges from 0.5% to 2%, therefore the detection of these animals requires testing the of whole herd. Furthermore, high genetic and antigenic diversity of the virus makes it difficult to identify all infected animals. Genetic diversity of BVDV in Poland has not been studied before. Therefore, the aim of the study was genetic characterization and phylogenetic analysis of BVDV isolates circulating in Poland.

Phylogenetic analysis was based on two regions of BVDV genome 5'UTR and N^{pro}. As my study has shown all BVDV isolates belonged to BVDV-1 genotype, subtypes 1b, 1d, 1e, 1f and 1g and to BVDV-2 genotype, subtype 2a. Genotypes BVDV-1a (dominant in vaccines) and atypical bovine pestivirus, BVDV-3 were not identified. In herds where more than one PI animal was identified all shedders were infected with the same subtype of BVDV, due to high nucleotide sequence homology among isolates. Only in one herd two different BVDV subtypes, 1b and 1f, were identified. Genotype 2 of BVDV which caused significant economic losses in the cattle herd vaccinated against BVDV-1 was isolated from apparently healthy animals. There was no correlation between genotype of the virus and its geographical occurrence. On the basis of virological and phylogenetic studies it was concluded that BVDV is widespread in Poland and the percentage of PIs at the herd level remained at the normal level below 3%.

The second stage of the study was to develop the method of genotyping the isolates without sequencing and to determine the status of BVDV infection, based on the results of phylogenetic analysis. Testing by real-time RT-PCR with TaqMan probes allowed for rapid, qualitative and quantitative evaluation for the presence of BVDV in herd. This method could be used to test animals regardless of their age, immunological status or the subtype of BVDV which caused the infection. Distinction between PI and TI (transiently infected) animals was possible using the cut-off values in a single test. High sensitivity of the developed method

exceeding the sensitivity of ELISA Ag allowed the testing of pooled samples reducing the cost of analysis. The method is characterized by high reproducibility, sensitivity and specificity.

Implementation of the method of restriction fragment length polymorphism (RFLP) analysis of the amplification products of standard RT-PCR (based on the sequences of the field isolates) allowed the effective distinction of BVDV-1 subtypes 1a, 1b, 1d, 1e, 1f, 1g and BVDV-2a. Using the selected enzymes, seven restriction patterns were obtained, each specific for only one subtype of the virus. With RFLP analysis it was possible to distinguish a new pattern for a new subtype i.e. BVDV-1e, which has never been detected in Poland before. This typing result was confirmed by sequencing. The study allowed to conclude that the restriction analysis is a fast genotyping method with the accuracy similar to sequencing.

The development of the method of cross priming amplification (CPA) with the use of two sets of primers allowed rapid and at low cost identification of BVDV-1 and BVDV-2 infections. CPA assay does not require sophisticated and expensive laboratory equipment and it can be performed in a water bath under isothermal conditions. The sensitivity of the CPA was assessed with a serially diluted standard RNA and it was sufficient to detect persistently infected animals. This is a qualitative method that does not allow to differentiation of virus genotypes. Using this methods we were able to detect all BVDV genotypes which have been identified in Poland so far. Both sets of primers used for the detection of BVDV-1 and BVDV-2 were not able to detect atypical pestiviruses. There were no false-positive results neither in negative samples nor in the negative control. Obtained results were confirmed by real-time RT-PCR and standard RT-PCR. Developed method is the first in the world, where the CPA technique was used to detect BVDV infection.