GENETIC DIVERSITY OF RUMINANT PESTIVIRUSES COLLECTED IN SARDINIA ALONG 5 YEARS (2005-2009)

Silvia Dei Giudici, Annalisa Oggiano, Giantonella Puggioni, Maria Paola Madrau, Angela Maria Rocchigiani, Anna Pina Murtino, Pier Paolo Angioi, Cristiana Patta, Maria Nicoletta Ponti

Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

Introduction: The genus Pestivirus belongs to the family Flaviviridae and includes four main genotypes: bovine viral diarrhoea virus types 1 and 2 (BVDV 1 and BVDV 2), border disease virus (BDV) of sheep, and classical swine fever virus (CSFV). Pestiviruses are distributed worldwide and are responsible of economically important syndromes in cattle and sheep. The aim of our work was to study the genetic variability of the Pestivirus circulated in Sardinia in the years 2005-2009.

Material and methods: A total of 1609 samples (placenta, brain and spleen of foetuses) collected from 135 cattle and 371 sheep-goat farms with a clinical disease report compatible with a pestivirus infection, were analyzed to detect Pestivirus, using different diagnostic methods: DIF, ag-elisa, RT-PCR. 16 positives samples, 13 from sheep, 2 cattle, 1 goat, were phylogenetically analyzed. RNA was extracted using Trizol (Invitrogen) according with the manufacturer's instruction. A fragment of 288 bp from the 5’NCR of the genome was amplified by one-tube RT-PCR using primers 324 and 326 (Vilcek et al. 1994). Purified amplicons were sequenced and the sequences were compared with reference strains using ClustalW program. The phylogenetic analysis was performed using the Neighbor-joining method and the MEGA program. Statistical analysis of phylogenetic tree was determined by bootstrap analysis carried out on 1000 replicates.

Results: All the viruses clustered into two genotypes: BVDV1 and BDV, no BVDV2 strains were found. Bootstrap analysis confirmed an high confidence (over 85%) in the groupings. The bovine and goat samples were all BVDV1 subgroup 1b; three sheep samples were BDV and ten were BVDV1, they were further subdivided into subgroups 1a, 1b, 1e, 1k.

Conclusions: In this work we investigate the genetic diversity of pestiviruses collected in Sardinia, the Italian region that have the major number of sheep (3,274,518). Our data show that in the island spread different subtype of BVDV1: 1a, 1b, 1e, 1k (first report in Italy). All the isolated of BDV clustered in the same group found by De Mia et al (2005), giving strength to their hypothesis on a new cluster of BDV. The high number of BVDV found in the sheep samples suggests that BVDV could be the major cause of Pestivirus infection in sheep. Although the single cluster are strongly supported by bootstrap values, it is under way the analysis of other genomic regions (N-pro, E2) to confirm our results.

Keywords: Pestivirus, phylogenesis