GENETIC DIVERSITY OF PESTIVIRUSES: CONSEQUENCES FOR TAXONOMY, DETECTION AND MOLECULAR EPIDEMIOLOGY

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The pestivirus genus of the family *Flaviviridae* comprises four species and a number of additional viruses. Bovine viral diarrhoea virus type 1 (BVDV-1) mainly infects cattle but also sheep, pig, roe deer, deer, alpaca and buffalo. Abortion, birth of persistently infected animals and in some special cases highly fatal mucosal disease may appear in infected cattle. BVDV type 2 (BVDV-2) infects cattle and sheep in which infection is characterised by similar clinical signs as infection with BVDV-1, but some highly virulent BVDV-2 strains may cause haemorrhagic syndrome. Classical Swine Fever Virus (CSFV) infects only pigs. Infected animals may develop a lethal haemorrhagic fever which results in significant economic losses for the pig industry. Border Disease Virus (BDV) mainly infects sheep but virus was also detected in goat, pig, cattle, reindeer, and chamois. Clinical manifestations in infected sheep are abortion, stillbirths, barren ewes and the birth of small weak lambs. In addition to these four established species, the Pestivirus genus contains a number of "unconventional" representatives, including the giraffe, Bungowannah, antelope and HoBi-like pestiviruses.

The pestivirus genome is composed of a single-strand positive oriented RNA of about 12.5 kb size. The 5' and 3'-ends of the genome flank an ORF encoding a polyprotein composed of around 4000 amino acids. This polyprotein is cleaved by viral and cellular proteases into four structural (C, E^{rns}, E1, E2) and 7-8 nonstructural (N^{pro}, p7, NS2-3, NS4A, NS4B, NS5A, NS5B) proteins (Meyers and Thiel, 1996). The most conserved part of the genome is the 5'-untranslated region (5'-UTR), the most variable one is the immunodominant E2 region.

The rapid development of molecular-genetic techniques, especially the polymerase chain reaction (PCR), sequencing of DNA and computer-assisted phylogenetic analysis significantly improved typing of pestiviruses. The 5'-UTR, N^{pro} and E2 form the basis for typing pestiviruses at the genetic level. Methodologically, DNA fragments prepared by RT-PCR from these genomic regions are sequenced, the nucleotide sequences are compared with other sequences deposited in international databases (GenBank) using computer programs, such as Clustal W. Phylogenetic trees are constructed using computer programs such as MegAlign, Phylip, Mega. A phylogenetic tree represents a graphical presentation of the evolutionary distances between nucleotide sequences analysed and shows the relationship between pestivirus strains involved in the study.

Genetic analysis improved the taxonomy of pestivirus species and revealed new pestiviruses. In the early stages of pestivirus research, three pestivirus species, namely BVDV, CSFV and BDV were recognised. When an additional pestivirus infecting cattle was detected in Canada and the USA around 1990, the original BVDV was renamed BVDV-1 to differentiate it from the new BVDV-2 strains (Ridpath et al, 1994, Pellerin et al., 1994). To date, four additional pestiviruses are referred as "atypical", without a status as species: Giraffe pestivirus (Avalos-Ramirez, et al., 2001), originally isolated from a Giraffe in Africa; Ho_Bi-like pestivirus isolates detected in some foetal calf sera used for the cultivation of cells (Schirrmeier et al., 2004) and recently also detected in naturally infected cattle in Thailand (Stahl et al., 2007) and Italy (Decaro et al., 2011); a pestivirus isolate from pronghorn antelope (Vilcek et al., 2005), and Bungowannah virus isolated in Australia from pigs suffering from myocarditis (Kirkland et al., 2007).

At present, a new nomenclature of pestiviruses is being discussed among specialists working in the pestivirus field. The main problems are the taxonomic names for atypical pestiviruses, namely for Ho_Bi-like viruses, giraffe pestivirus, Bungowannah pestivirus. For example, one suggestion is to name Ho_Bi-like pestiviruses as Brazil virus (virus for the first time found in foetal calf serum originating from Brazil) or BVDV type 3 (BVDV-3). Another suggestion is to name pestiviruses as pestivirus-1, pestivirus-2, etc. There is also suggestion to divide pestiviruses into ruminant and porcine groups and call them as ruminant or porcine pestivirus 1, pestivirus 2, etc. replacing old names as BVDV, CSFV and BDV. The final agreement is not achieved yet.

Genetic analysis of pestiviruses revealed their high genetic variability and grouping of viral isolates below the species level (Avalos-Ramirez, et al., 2001; Becher et al., 1999; Becher et al, 2003). When CSFV isolates collected from different parts of world were analyzed, they clustered into four main phylogenetic groups (1, 2, 3, 4) with several clusters (1.1, 1.2., 2.1., 2.2., 2.3. etc) (Paton et al., 2000). The BDV isolates were divided into 7-8 genotypes (De Mia et al., 2005; Oguzoglu et al., 2009).

In the beginning of genetic analysis of BVDV-1 isolates two subgroups (BVDV-1a and BVDV-1b) were identified only. When more isolates were analysed, 12 phylogenetic subgroups (subgenotypes, subtypes) were recognised, namely BVDV-1a to BVDV-1k and l (Jackova et al., 2008; Vilcek et al., 2001) and additional subgroups were described, now to BVDV-1p. Some of BVDV-1 isolates were found in many countries around the world (BVDV-1a, BVDV-1b) others are predominant or unique to some countries (BVDV-1i for UK, BVDV-1k for Switzerland). The BVDV-2 isolates which mainly circulate in cattle farms in USA and Canada but also in South America and Asia, and occasionally found in Europe (for example in Germany, France, Austria, Belgium, Slovakia) were grouped into 2-4 subgroups (Flores et al., 2002). Any of BVDV-1 or BVDV-2 subgroups can be specifically recognised by antigenic analysis. There is no clear evidence that the isolates belonging to the particular phylogenetic subgroup develop special clinical signs in infected cattle.

High genetic variability of BVDV isolates complicates the development of specific molecular-genetic assays based on RT-PCR or real-time RT-PCR for diagnostic laboratories. The primers for PCR assays were usually selected from the evolutionary highly conserved 5r'-untranslated region of the pestivirus genome (Hoffmann et al., 2006; Letellier et al., 2003; Vilcek et al., 1994).

Rapid sequencing of PCR prepared DNA fragments and phylogenetic analysis of nucleotide sequences is useful for the establishment of molecular epidemiology. In combination with classical epidemiological investigation, molecular epidemiology provides useful information for the identification of sources of infection and to study the spread of infection between farms. Molecular epidemiology was successfully used in the BVDV eradication program in Sweden (Stahl et al., 2005) and Austria (Hornberg et al., 2009). In Switzerland, where a BVDV eradication program is being implemented, large collection of pestivirus isolates detected in infected cattle are analysed at the genetic level.

There is no doubt that the classical (epidemiological investigation on farms, detection of BVDV antibodies and antigen) and molecular-genetic approach (detection of BVDV by RT-PCR, real-time RT-PCR, sequencing of PCR products and phylogenetic analysis) which started in Ukraine with the introduction of common Swiss-Ukrainian-Slovak SCOPES project can help to fight BVD and to improve the economic performance of cattle farming.

References

1. Avalos-Ramirez, R., Orlich, M., Thiel ,H.J., Becher, P.: Evidence for the presence of two novel pestivirus species. Virology 286, 456-465, 2001. **2.** Becher, P., Orlich, M., Kosmidon, A., Konig, M., Baroth, M., Thiel, H.J.: Genetic diversity of pestiviruses: identification of novel groups and implication for classification. Virology 262, 64-71, 1999. **3.** Becher, P., Avalos Ramirez, R., Orlich, M., Cedillo Rosales, S., Konig, M., Schweizer, M., Stalder, H., Schirrmeier, H., Thiel, H.J.: Genetic and antigenic characterization of novel pestivirus genotypes: implication for classification. Virology 311, 96-104, 2003. **4.** Decaro, N., Lucente, M.S., Mari, V., Cirone, F., Cordioli, P., Camero, M., Sciarretta, R., Losurdo, M., Lorusso, E., Buonavoglia, C.: Atypical pestivirus and severe respiratory disease in calves, Europe. Emerg. Infect. Dis. 17, 1549-1552, 2011. **5.** De Mia, G.M., Greiser-Wilke, I., Feliziani, F., Giammarioli, M., De Giuseppe, A.: Genetic characterization of a caprine pestivirus as the first member of a putative novel pestivirus subgroup. J. Vet. Med. B. Infect. Dis. Vet. Public Health 52, 206-210, 2005. **6.** Flores, E.F., Ridpath, J.F., Weiblen, R., Vogel, F.S., Gil, L.H.: Phylogenetic analysis of Brazilian bovine viral diarrhea virus type 2 (BVDV-2) isolates: evidence for a subgenotype within BVDV-2. Virus Res. 87, 51-60, 2002. **7.** Hoffmann, B., Depner, K., Schirrmeier, H., Beer, M.: A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. J. Virol. Methods 136, 200-209, 2006. **8.** Hornberg, A., Fernández, S.R., Vogl, C., Vilcek, S., Matt, M., Fink, M., Köfer, J., Schöpf, K.: Genetic diversity of pestivirus isolates in cattle from Western Austria. Vet. Microbiol. 135, 205-213, 2009. **9.** Jackova, A., Novackova, M., Pelletier, C., Audeval, C., Gueneau, E., Haffar, A., Petit, E., Rehby, L., Vilcek, S.: The extended genetic diversity of BVDV-1: Typing of BVDV isolates from France. Vet. Res. Commun. 32, 7-11, 2008. **10.** Kirkland, P.D., Frost, M.J., Finlaison, D.S., King, K.R., Ridpath, J.F., Gu, X.: Identification of a novel virus in pigs – Bungowannah virus: a possible new species of pestivirus. Virus Res. 129, 26-34, 2007. **11.** Letellier, C., Kerkhofs, P.: Real-time PCR for simultaneous detection and genotyping of bovine viral diarrhea virus. J. Virol. Methods 114, 21-27, 2003. Meyers, G., Thiel, H.J.: Molecular characterization of pestiviruses. Adv. Virus Res. 47, 53-118, 1996. **12.** Oguzoglu, T.C., Tan, M.T., Toplu, N., Demir, A.B., Bilge-Dagalp, S., Karaoglu, T., Ozkul, A., Alkan, F., Burgu, I., Haas, L., Greiser-Wilke, I.: Border disease virus (BDV) infections of small ruminants in Turkey: a new BDV subgroup? Vet. Microbiol. 135, 374-379, 2009. **13.** Paton, D.J., McGoldrick, A., Greiser-Wilke, I., Parchariyanon, S., Song, J.Y., Liou, P.P., Stadejek, T., Lowings, J.P., Bjorklund, H., Belak, S.: Genetic typing of classical swine fever virus. Vet. Microbiol. 73, 137-157, 2000. **14.** Pellerin, C., Van den Hurk, J., Lecomte, J., Tijssen, P.: Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortality. Virology 203, 260-268, 1994. **15.** Ridpath, J., Bolin, S.R., Dubovi, E.J.: Seggregation of bovine viral diarrhea virus into genotypes. Virology 205, 66-74, 1994. **16.** Schirrmeier, H., Strebelow, G., Depner, K., Hoffmann, B., Beer, M.: Genetic and antigenic characterization of an atypical pestivirus isolate, a putative member of a novel pestivirus species. J. Gen. Virol. 85, 3647-3652, 2004. **17.** Stahl, K., Kampa, J., Baule, C., Isaksson, M., Moreno-Lopez, J., Belak, S., Alenius, S., Lindberg, A.: Molecular epidemiology of bovine viral diarrhoea during the final phase of the Swedish BVD-eradication programme. Prev. Vet. Med., 72, 103-108, 2005. **18.** Stahl, K., Kampa, J., Alenius, S., Persson Wadman, A., Baule, C., Aiumlamai, S., Belák, S.: Natural infection of cattle with an atypical ‚HoBi'-like pestivirus-implications for BVD control and for the safety of biological products. Vet. Res. 38, 517-523, 2007. **19.** Vilcek, S., Herring, A.J., Herring, J.A., Nettleton, P.F., Lowings, J.P., Paton, D.J.: Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. Arch. Virol. 136, 309-323, 1994. **20.** Vilcek, S., Paton, D.J., Durkovic, B., Strojny, L., Ibata, G., Moussa, A., Loitsch, A., Rossmanith, W., Vega, S., Scicluna, M.T., Palfi, V.: Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. Arch. Virol. 146, 99-115, 2001. **21.** Vilcek, S., Ridpath, J.F., Van Campen, H., Cavender, J.L., Warg, J.: Characterization of a novel pestivirus originating from a pronghorn antelope. Virus Res. 108, 187-193, 2005.

ГЕНЕТИЧНЕ РІЗНОМАЇТТЯ ПЕСТІВІРУСІВ: НАСЛІДКИ ДЛЯ ТАКСОНОМІЇ, ВИЯВЛЕННЯ ТА МОЛЕКУЛЯРНОЇ ЕПІДЕМІОЛОГІЇ

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Рід пестівірусів сімейства Flaviviridae включає в себе чотири види вірусів та декілька додаткових вірусів. Бурхливий розвиток молекулярно-генетичних методів, зокрема, полімеразної ланцюгової реакції (ПЛР), секвенування ДНК і комп'ютерний філогенетичний аналіз, значно покращив можливості для типування пестивірусів. Немає сумнівів, що класичний підхід (епідеміологічне дослідження на фермах, виявлення антитіл і антигенів BVDV) і молекулярно-генетичний підхід (визначення BVDV за допомогою ПЦР в режимі реального часу RT-PCR, секвенування продуктів ПЛР та філогенетичного аналіз), що розпочалися в Україні в рамках спільного швейцарсько - українсько-словацького проекту можуть допомогти в боротьбі з вірусною діареєю ВРХ і підвищити економічну ефективності сільськогосподарської худоби.