



Pestivirus Species Potential Adventitious Contaminants of Biological Products

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Abstract

Bovine Viral Diarrhea virus species of the genus Pestivirus, important pathogens affecting zootechnics worldwide, have been reported as adventitious contaminants of biological products for veterinary and human use. The *Bovine Viral Diarrhea virus 1* species showed potential for an emerging zoonosis. According to World Organization for Animal Health (Office International des Épizooties: OIE), Bovine Viral Diarrhea is a notifiable disease of importance to international trade. Recently, *Bovine Viral Diarrhea virus 3* tentative species have been isolated from Brazil and Thailand. The virus has been diffused from South America to other countries probably through the commercialization of contaminated fetal bovine serum.

Keywords: Contamination; Biological products; Bovine viral diarrhea virus; Pestivirus

Bovine viral diarrhea virus 1 (BVDV-1) and *Bovine viral diarrhea virus 2* (BVDV-2) are established species of the genus Pestivirus of the family *Flaviviridae* [1], responsible for cosmopolitan disease affecting cattle and other ruminants, presenting a wide range of clinical manifestations, with relevant impact on zootechnic production. According to World Organization for Animal Health (Office International des Épizooties: OIE), Bovine Viral Diarrhea is a notifiable disease of importance to international trade [2]. BVDV-1 and BVDV-2 are genetically related to a third recently proposed tentative species, *Bovine viral diarrhea virus 3* (BVDV-3), also known as Hobi group [3]. The new putative Pestivirus species was first identified in Europe in Fetal Bovine Serum (FBS) imported from Brazil [3]. Isolates were further reported from naturally infected domestic animals [4,5]. In particular, only 3 strains of BVDV-3, D32/00_Hobi [3], Hobi/Brazil/200/2002 [4] and Th/04_KhonKaen [5], have been initially reported from Brazil and Thailand. New isolates from domestic animals and adventitious contaminants of biological products were further reported from different countries [6-10]. Hobi-like strains have been detected recently also in Italian cattle herds with respiratory distress and reproductive failures [11,12].

A relevant and common aspect related with pestiviruses is their potential as contaminants of biological products. The contamination by animal pestiviruses may occur in biological products for veterinary and human use and the problem seems to be worldwide. The occurrence of contamination of biologicals, cell cultures, primary cell cultures and cell lines, human included, have been ascribed to non-cytopathic (NCP) strains of BVDV-1 [13-19]. Different reports indicated BVDV contamination in vaccines for veterinary use [20-23] but could not specify the involved BVDV species. BVDV-1 contamination of vaccines for veterinary use was reported by Harasawa [24,25]. Also BVDV-2 was reported as a contaminant of vaccines for veterinary use, and responsible of an outbreak of BVDV in Dutch cattle farms subsequent to the use of contaminated vaccines [26]. The BVDV-1 contamination of live virus vaccines for human use was also reported to occur in Japan [27,28], USA [29] and Europe [30]. During an experimental study in Japan, pestiviral RNA was also detected in live virus vaccines for human use [27,28]. The vaccines were produced by different pharmaceutical companies, regularly authorized and commercialized: two monovalents against mumps and rubella and two polyvalents against measles, mumps and rubella. Comparative analysis of the nucleotide sequence at the 5'-UTR identified BVDV RNA as the contaminant. Similarly,

interferon for human use was found contaminated by BVDV-1 RNA [31]. Further studies demonstrated the occurrence of Pestivirus contamination in live virus vaccine for human use also in Europe [30]. The characterization, based on primary nucleotide sequence homology and secondary palindromic sequence structure in the 5'-UTR, revealed positive samples contaminated with the BVDV-1 genotype Ib. The most probable source of contamination is suggested to be non-cytopathic BVDV infected bovine fetal serum commonly used as medium supplement for the cell cultures [32-34].

Large-scale epidemiological investigations on Pestivirus BVDV species strains (BVDV-1 n 306; BVDV-2 n 88; BVDV-3 n 30) evaluated according to the palindromic nucleotide substitution (PNS) at the 5' untranslated region of RNA [35] revealed that the 24.53% (104 out 424) were adventitious contaminants of biological products. Among BVDV-1 strains, allocated in genotypes BVDV-1a, BVDV-1b, BVDV-1c, BVDV-1d, BVDV-1f and BVDV-1i, 66 (21.56% within the species) were detected as contaminants from FBS, cell lines, vaccines for veterinary and human use and interferon samples for human use. The strains were reported from investigations performed in Europe (Austria, Belgium, Denmark, France, Germany, Ireland, Italy, Slovakia, Spain, Sweden, Switzerland, UK), but also from America (Argentina, Brazil, Canada, Colombia, Dominican Republic, Mexico, USA), Asia (China, India, Japan), South Africa, Australia and New Zealand. Twenty BVDV-2 strains (22.72% within the species), detected as contaminants, were allocated mainly in the geno group BVDV-2A from North America (USA, Canada), Europe (Belgium, Italy, Netherland and UK) and Japan. Only 3 contaminant strains were allocated in the geno group BVDV-2B from Argentina, Brazil and Mexico.

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In addition to BVDV-1 and BVDV-2, also the BVDV-3 (Hobi group) strains have been isolated from contaminated batches of fetal bovine serum [7-9]. Eighteen strains (60% within the species) originated from Australia, Brazil, Canada, China, Italy, Mexico, Thailand and USA. Among BVDV-3 strains, only those isolated from Thailand and China showed a certain divergence, and they were clustered in separate genomic variants. The other BVDV-3 strains isolated from other countries resulted homogeneous. This suggested a common origin for the spreading of the pathogen from South America, chronologically first reported originating from Brazil [3], and circulating in this country at least since 2002 [4]. The isolation of BVDV-3 in cattle in a country, for example in Italy [11,12] or Australia [9], does not necessarily represent the endemic status of the virus in the area, but more probably simply indicates the presence of the pathogen introduced from another country, unless supported by evidence of genomic characteristics related to geographical origin. In Australia, the genus Pestivirus is represented by the Bungowanah tentative species [36], showing nucleotide sequences that express the evolutionary history of the virus specific to the territory. The BVDV-3 strains isolated in Australia did not show particular sequence characteristics, and they were very similar to the prototype strains isolated in South America. Taking into account the genetic and antigenic difference between BVDV-3 and the other two BVDV species, the related lower performance of tests designed for the screening of BVDV-1 and BVDV-2 [3,37], and the subsequent laboratory diagnostic difficulties to detect BVDV-3, more probably, the pathogen could have been disseminated undetected in biological products such as FBS, despite stringent control measures.

The contamination of biological products acquires particular importance when considering that the Pestivirus can be potential for emerging zoonotic infections. The role of pestiviruses in human pathology remains unknown, and data on Pestivirus epidemiology in humans are still fragmentary. During a seroepidemiological survey undertaken in northern Italy [38], the Personnage Turner syndrome [39], a rare form of post viral mononevritis of unknown etiology, was diagnosed in a patient involved with an outbreak of BVDV which had a fatal issue on all the infected animals (nine adult cows, rare occurrence for BVDV and expression of a particular virulence of the implied strain). The patient developed anti-BVDV antibody titers which remained very high during the four following monitoring years [40]. Furthermore, seropositivity to BVDV was found in human immunodeficiency virus positive patients, in a study undertaken in Zambia and Europe [41,42]. A non cytopathic strain, classified in the BVDV-1c genotype, was isolated from a human buffy coat sample from a 31 days viremic patient originated from Belgium [43]. In a study conducted in USA, in mothers with microcephalic infants, two sera were positive for antibodies against the NADL strain of BVDV [44]. Pestivirus antigen was detected in 23.6% fecal samples from children, under two years, from Arizona, USA, affected by gastroenteritis often associated with respiratory illness, where no pathogen could be identified [45]. Similar preliminary studies in children from Baltimore and Houston, USA, and Dhaka, Bangladesh, showed Pestivirus associated gastroenteritis. In the USA, specific anti-BVDV antibodies were reported in the 40% of tested sera from identical twins discordant for schizophrenia [46]. This aspect was particularly interesting considering Pestivirus tropism for nervous cells in animal pathology. Other studies suggested a link between Pestivirus and neurological disorders in humans. Infection during pregnancy and a possible association with some forms of cerebral White Matter Damage (WMD) in preterm neonates have been investigated [47-51].

Notwithstanding the high production and control standards applied

especially in western countries, there arises the problem about safety of the actual production methods of biological products. Therefore, laboratory testing is a key element and requires particular attention. Tests have to be performed taking into account the heterogeneity of pestiviruses. The analysis antigen substrates have to be selected to detect the broadest spectrum of genomic variants. Furthermore, standard procedures (e.g. immune-fluorescence and serum neutralization tests), when applied without prior concentration of high volumes, might not detect low virus titres. Bolin et al. [14] reported that BVDV RNA detection by PCR from experimentally infected monkey cells was unsuccessful unless sequential passages on turbinate bovine cells were made. In addition, inactivation procedures, as irradiation, might be inefficient. However, the major concern is represented by the potential of BVDV-3 as contaminants of biological products, especially taking into account that the current BVDV diagnostic tests may fail to detect HoBi-like viruses or to differentiate between BVDV and HoBi-like viruses [3,37]. Furthermore, available commercial serological tests for BVDV do not reliably detect HoBi-like virus exposure, and cross protection against HoBi-like viruses conferred by current BVDV vaccines is likely limited [37].

It is important to enhance the laboratory capacity to increase analytical sensitivity and specificity. Characterization procedures should be applied also to increase our understanding of epidemiological dynamics and provide elements to ensure traceability. The PNS procedure [52] demonstrated the potential for its application to epidemiological investigations on pestiviruses, including traceability purposes, through the identification of genomic characters linked to the geographical origin, animal host and virulence of strains. Genetic variation could be related with diffusion of the BVDV-2 species variants in different geographic areas [53]. Chronologically, the species emerged in North America in 1978, spreading in UK and Japan, continental Europe, South America and New Zealand. Correlation between clinical features related with isolation of BVDV-2 strains and genetic variation indicated specific genomic variants related with hemorrhagic syndrome [53]. The PNS procedure was useful also for the evaluation of the probable common origin of the BVDV-3 species spreading via commercialization of FBS batches from South America [35].

Furthermore, contamination of biological products by BVDV species implies the risk of iatrogenic infection by potentially hypervirulent strains capable to determine severe clinical courses. From both BVDV-1 and BVDV-2 species have been reported strains responsible of hemorrhagic syndromes. BVDV-2 species includes the strains isolated from outbreaks hemorrhagic syndrome characterised by thrombocytopenia and high mortality in USA and Canada [54-56]. Hyper-virulent strains 890 and CD-87 have been previously included in BVDV species in a separate cluster [55,57]. Correlation between clinical features related with isolation of BVDV-2 strains with genetic variation indicated that genotype 2a; subgenotype 1, variants 4 and 5 were related with hemorrhagic syndrome [53]. The hemorrhagic syndrome has been reported also in European cattle [24,36,58-60]. The European strains of the Culi series and strains L256 and Marloie showed high virulence causing hemorrhagic syndrome, characteristic also with BVDV-2 strains. Among BVDV-1 species, bovine strains associated with clinical forms of hemorrhagic syndrome belonged to the genotype BVDV-1b (Culi4 and Culi6 in BVDV-1b1 group and Marloie in BVDV-1b2) [61].

Other particularly important aspects related to the potential of iatrogenic infections are the possible contamination of modified live vaccines and the capacity of pestiviruses to cross placental barrier and determine vertical transmission [30]. Therefore, a definitive risk during pregnancy has to be taken into account.

In conclusion, giving the importance of safety of biological products, accurate and most sensitive monitoring for adventitious Pestivirus should be recommended to manufacturers, and applied in the framework of inspection controls on biological products, in respect with the general rules on the safety of pharmaceutical products, which clearly excludes any kind of contamination. Controls should be particularly accurate on products such as FBS imported from tropical countries which have generally reduced laboratory capabilities, and obtained from indigenous herds potential carriers of Pestivirus species. Furthermore, taking into account the zoonotic potential of BVDV-1, particular attention should be focused to prevent contamination of biological products for human use.

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