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Detection of a Hobi-like virus in archival samples suggests circulation of this emerging pestivirus species in Europe prior to 2007

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ABSTRACT

The first reported incidence of Hobi-like viruses in Europe dates to a 2010 outbreak of respiratory disease in cattle in Italy. In this study, a Hobi-like virus was detected in archival samples, collected in 2007 in Italy from a cattle herd displaying respiratory disease, during the validation of a nested PCR protocol for rapid characterization of bovine pestiviruses. Phylogeny conducted with full-length pestivirus genomes and three informative genomic sequences, placed the strain detected in the samples, Italy-129/07, into the Hobi-like virus branch. Italy-129/07, similar to other Hobi-like viruses isolated in Italy, was more closely related to viruses of South American origin, than Hobi-like viruses of Southeast Asian origin. This suggests a possible introduction of this emerging group of pestiviruses into Italy as a consequence of using contaminated biological products such as fetal bovine serum originating in South America.

This report of a Hobi-like virus associated with respiratory disease along with the full-genomic characterization of the virus detected provides new data that contributes to the body of knowledge regarding the epidemiology, pathobiology and genetic diversity of this emerging group of pestiviruses. Importantly, it dates the circulation of Hobi-like viruses in Italy to 2007, at least three years before previous reports.

1. Introduction

Hobi-like viruses, also known as bovine viral diarrhea virus (BVDV) type 3, are a novel group of viruses that has been tentatively proposed as a new species within the genus Pestivirus (Bauermann et al., 2013). Frequently detected in commercial batches of fetal bovine serum (FBS), mostly of South American origin, or contaminated cell cultures (Schirrmeier et al., 2004; Stalder et al., 2005; Liu et al., 2009b; Ståhl et al., 2010; Xia et al., 2011, 2013; Peletto et al., 2012; Mao et al., 2012), Hobi-like strains were only sporadically associated with clinical disease in cattle and buffaloes. Clinical presentation was similar to that induced by extant BVDV-1/BVDV-2 (Stalder et al., 2005; Cortez et al., 2006; Ståhl et al., 2007; Decaro et al., 2011, 2012a, 2012c). More recently, the ability of this group of viruses to generate cytopathogenic strains (Decaro et al., 2012c) and to induce persistent infections (Decaro et al., 2013a) was reported. Although their natural hosts range is unknown, under experimental conditions Hobi-like viruses infect cattle, sheep and pigs, causing mild disease and viral shedding (Decaro et al., 2012b; Larska et al., 2012; Ridpath et al., 2013). Noteworthy, several independent studies indicated that Hobi viruses display a poor cross-antigenicity against extant BVDVs (Bauermann et al., 2012; Larska et al., 2012; Decaro et al., 2013b).

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The first European reported outbreak of Hobi-like virus was in Italy in 2010. The present report describes the detection, and the full genome analysis, of a Hobi-like virus in archival samples from an Italian diagnostic laboratory. The archival samples were collected from clinical outbreak of respiratory disease in cattle occurring in 2007. This predates the previous isolation of Hobi-like virus in Italy by three years. Phylogenetic analysis indicates that the 2007 virus is similar to the 2010 Italian Hobi-like isolate and other Hobi-like strains isolated from Brazil. While the route of introduction of Hobi-like virus into Europe is unknown, the similarity to Brazilian origin isolates suggests one possibility would be introduction from South America possibly from the use of contaminated fetal bovine serum originating in Brazil.

2. Materials and methods

2.1. Sample origin and clinical outbreak

The Hobi-like strain (Italy-129/07) was serendipitously detected in a nasal swab during the screening of archival samples, collected during years 2005–2011 from Italian cattle herds with clinical forms suggestive of BVDV infection, with the aim to validate a novel nested PCR assay for rapid detection and discrimination of bovine pestiviruses (Decaro et al., 2012d). It previously had been identified as a BVDV-2 strain based on a published nested PCR protocol (Sullivan and Akkina, 1995). Subsequently, it was found that this protocol mischaracterizes Hobi-like strains as BVDV-2 (Decaro et al., 2012d). A PCR protocol designed to detect Hobi-like virus (Decaro et al., 2012d) and sequence analysis of the generated amplicon characterized the strain as true Hobi-like pestivirus (Decaro et al., 2012d).

The nasal swab was collected in 2007 from a cattle herd, consisting of about 1400 Holstein cows, located in Basilicata, a region of southern Italy neighboring Calabria. All Hobi-like virus outbreaks previously reported in Italy have occurred in Basilicata (Decaro et al., 2011, 2012a,c, 2013a). The farm was free from infectious bovine rhinotracheitis, enzootic bovine leukosis, tuberculosis and brucellosis, and no BVDV vaccination was routinely employed. Neither recent animal introduction nor contact with water buffaloes was reported. However, a water buffalo farm was distant only few kilometers and embryo transfer was routinely employed in the affected cattle herd. The herd was suffering from a respiratory disease outbreak characterized by severe pyrexia (up to 41.7 °C), drastic drop in milk production, cough and nasal discharge. Treatment of affected animals, mainly calves, with tilmicosin (10 mg/kg, SC, single administration) and oxytetracycline (11 mg/kg, PO, q 12 h for 7 days) did not result in improvement of clinical conditions. Nasal swabs collected from clinically ill animals (six 4-month-old calves and one 2-year-old cow at 8 months of pregnancy) were submitted for screening for respiratory pathogens of cattle, as previously described (Decaro et al., 2008, 2012c). Five nasal swabs from 4 calves and the cow tested positive for Bovine respiratory syncytial virus (BRSV) by RT-PCR (Valarcher et al., 1999) and 3 of them (including the cow) were found to contain also a pestivirus strain which was typed as BVDV-2 by the Sullivan and Akkina's protocol (Sullivan and Akkina, 1995). The samples were stored at −20 °C for more than 4 years and when retested by the novel nested PCR assay (Decaro et al., 2012d), they were confirmed to be pestivirus positive, but the strain was characterized as Hobi-like.

2.2. Real-time RT-PCR for Hobi-like pestivirus

The three Hobi-like virus positive nasal swabs were retrieved from archival samples and submitted to a TaqMan assay which had been developed for specific detection and quantification of this group of pestiviruses (Liu et al., 2008). Viral RNA was extracted using the QIAamp® Viral RNA Mini Kit (Qiagen S.p.A., Milan, Italy) and reverse transcription performed using GeneAmp® RNA PCR (Applied Biosystems, Applera Italia, Monza, Italy). Real-time PCR reactions and thermal protocols were carried out as previously described (Liu et al., 2008), with minor modifications (Decaro et al., 2012b). Hobi virus RNA copy numbers were calculated on the basis of the standard curves generated by 10-fold dilutions of a synthetic RNA obtained by in vitro transcription of a plasmid containing the 5′ UTR of the isolated strain.

2.3. Virus isolation attempts

For virus isolation, nasal swabs were vortexed in Dulbecco's minimal essential medium containing antibiotics (penicillin 5000 IU/ml, streptomycin 2500 μg/ml, amphotericin B 10 μg/ml). After centrifugation at 3000 × *g* for 15 min, the supernatants were used to inoculate confluent monolayers of Madin Darby bovine kidney (MDBK) cells supplemented with gamma-irradiated calf fetal serum (FBS US origin 500 ml, Lonza), which was free of pestivirus antibodies by ELISA and virus neutralization assays. Viral growth was monitored by an immunofluorescence (IF) assay using an anti-NS3 monoclonal antibody (kindly provided by Dr. P, Cordioli, Istituto Zooprofilattico Sperimentale di Lombardia ed Emilia Romagna, Brescia Italy) that is broadly reactive against all known pestiviruses.

2.4. PCR amplification of the Hobi-like pestivirus genome

As the viruses from the three samples had 100% nucleotide identity in the PCR amplified fragment corresponding to positions 1173–1383 of the sequence of BVDV-1 strain NADL (GenBank accession no. M31182) (Decaro et al., 2012d), the virus present in the cow sample was selected as prototype strain (Italy-129/07). A near full-length genome of this prototype strain was generated by means of RT-PCR amplifications carried out as described by Liu et al. (2009a) using the SuperScript™ One-Step RT-PCR for long templates (Life Technologies).

2.5. Sequence and phylogenetic analysis of the Hobi-like pestivirus genome

The generated PCR products were directly sequenced by BaseClear B.V. (Leiden, The Netherlands) and a consensus