Persistent Infection Caused by Hobi-Like Pestivirus

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A calf persistently infected by Hobi-like pestivirus was monitored for about 6 months, displaying clinical signs typical of bovine viral diarrhea virus persistent infection and shedding the virus through all body secretions, with maximal titers detected in urine. This report provides new insights into the pathogenesis of the emerging pestivirus.

Hobi-like virus is an emerging pestivirus that was first recovered from contaminated batches of fetal bovine serum (FBS) (1) and subsequently isolated from naturally infected cattle and buffaloes (2–4). The virus is phylogenetically distant from extant bovine viral diarrhea virus (BVDV) types 1 and 2, so it has been proposed as a tentative species of the genus Pestivirus (5). Although Hobi-like pestivirus has been known for nearly 10 years, outbreaks of overt disease in cattle were reported only recently (6–8). The pathogenic potential of the virus was confirmed by experimental studies in vivo (9), and a cytopathogenic (CP)/non-cytopathogenic (NCP) Hobi-like virus pair was also isolated from a dead heifer (7). So far, the ability of Hobi-like pestivirus to induce immunotolerance in cattle has not been demonstrated. In the present article, we report the first case of persistent infection in a calf caused by this emerging pestivirus.

The calf, a male born in October 2011, belonged to the same herd of southern Italy where repeated outbreaks of Hobi-like-induced disease had been observed in the previous months (6–8). In comparison with animals of the same age, the calf displayed a lower birth weight and a decreased growth rate, which was suggestive of a pestivirus persistently infected (PI) animal. In February 2012, the farmer requested a clinical evaluation at which the calf exhibited ruffled hair; respiratory distress with cough and nasal discharge; and alopecic areas on the head, neck, and right shoulder and stifle consistent with a cutaneous mycosis. This was confirmed by culture of scale samples on Sabouraud’s dextrose agar, and Trichophyton verrucosum was isolated. EDTA-blood samples were collected for subsequent testing for pestivirus antibodies and nucleic acid by using the bovine viral diarrhea virus (BVDV-Ab) SVANOVIR enzyme-linked immunosorbent assay (ELISA) (Svanova Biotech AB, Uppsala, Sweden) and reverse transcription (RT)-PCR (10), respectively. Laboratory investigations revealed that the calf was pestivirus antibody negative and virus positive, with the strain being characterized as Hobi-like pestivirus by means of nested PCR (10) and real-time RT-PCR (11). To overcome the observed lack of sensitivity of the ELISA (9, 12), the calf seronegativity for Hobi-like pestivirus was also confirmed by a virus neutralization (VN) assay using the prototype isolate Italy-1/10-1 (12). The Hobi-like strain causing persistent infection was isolated on Madin-Darby bovine kidney cells, where no cytopathogenic effect was observed even after serial passage. No insertion was detected in the NS2-3 region by means of RT-PCR analysis (7). By sequence analysis of the full-length of the E2 gene (6), the Hobi-like strain was strictly related to other NCP viruses previously isolated from the same herd (6, 7), with nucleotide and amino acid identities of 99.2 to 99.6% and 98.1 to 98.9%, respectively.

Consequently, a diagnosis of Hobi-like PI was suggested, which was confirmed by further testing carried out 1 month later. On March 2012, the calf was moved to the Infectious Diseases Unit of the Animal Hospital of the University of Bari (Italy) and was monitored for its clinical, hematological, biochemical, virological, and serological conditions until September 2012, when it was still alive. After 2 days, the onset of diarrhea was observed, whereas cough and nasal discharge persisted. Fecal examination by Ziehl-Neelsen staining and flotation with saturated sodium chloride solution revealed the presence of Cryptosporidium parvum, which was detected for more than 1 month despite a 2-week treatment with halofuginone lactate (500 μg/kg, orally [p.o.], every 24 h [q24h]). The calf was also administered enrofloxacin (10 mg/kg, subcutaneously [s.c.,] q24h for 10 days) for the respiratory signs and iodine solution added with 2% salicylic acid (topical medication for 15 days) for the dermatophytosis. At the end of April, respiratory signs were exacerbated and a mycoplasma was isolated in Hayflick broth (13) from the nasal secretions, which was characterized as Mycoplasma bovippinnis by sequence analysis of the product obtained with a Mycoplasma sp. PCR protocol (14). Consequently, oxytetracycline hydrochloride was administered at the dose of 11 mg/kg, orally [p.o.,] q24h for 7 days. These treatments were repeated periodically when the different clinical signs were more severe. The clinical conditions improved shortly after the therapy, but when this was discontinued, the calf slowly regressed to its previous clinical status and no permanent improvement was evident. At the age of 1 year, the calf still had small size and ruffled hair. Its body weight and withers height were 204 kg and 102 cm, respectively, whereas the mean measurements of 10 coetaneous male animals of the same farm were 380 kg and 135 cm, respectively.

EDTA-blood, serum, and urine samples were collected at 2-week intervals, from March to September 2012, for laboratory investigations. Despite the PI status, no significant change in the hematological and biochemical parameters was evident, with the
exception of a moderate lymphopenia which occurred in May, with $2.6 \times 10^9$ lymphocytes liter$^{-1}$ (lower reference limit, $3.0 \times 10^9$ lymphocytes liter$^{-1}$).

Hobi-like pestivirus was detected continuously during the entire observation period in all clinical samples collected from the PI calf, whereas pestivirus antibodies were not demonstrated at any time by means of ELISA and VN tests. Surprisingly, the specimen containing the highest viral load was represented by urine (median titers of $2.01 \times 10^6$ RNA copies liter$^{-1}$ of template), followed by blood ($8.51 \times 10^5$ RNA copies) and nasal swabs ($2.92 \times 10^5$ RNA copies), whereas rectal swabs were found to contain small amounts of virus, with a median titer of $6.06 \times 10^3$ RNA copies liter$^{-1}$ of template (Fig. 1).

Pestiviruses are able to induce immunotolerance when fetuses are infected in utero at 2 to 4 months of pregnancy. PI animals are constantly viremic and unable to produce antibodies against the virus causing immunotolerance. These animals may be apparently healthy or develop multiple clinical pictures, including lower birth weight, decreased growth rates, ruffled hair, respiratory and gastroenteric signs, or neurological disorders. Clinical signs are determined directly by pestivirus replication or by concurrent infections consequent to the virus-induced immunosuppression (15). Persistent infections have been reported for BVDV-1, BVDV-2, border disease virus, and classical swine fever virus. So far, Hobi-like pestivirus has been associated with respiratory disease (6) and reproductive disorders (8). A Hobi-like virus CP-NCP pair was recently isolated from the lungs of a heifer dead as a consequence of respiratory disease, but the PI status of this animal could not be assessed (7). Thus, the present study reports for the first time a Hobi-like pestivirus persistent infection, showing that its clinical course is similar to that observed in BVDV-1/BVDV-2 PI calves. Importantly, the calf was shedding the largest amount of virus through urine, a finding that, if confirmed by future studies, may have useful implications for diagnosis and control of this emerging infection.

PI calves may develop a deadly clinical form known as mucosal disease (MD), when a CP BVDV strain originates from the NCP virus infecting the animals (16). Although the Hobi-like PI calf has not displayed any sign of MD during a period of more than 6 months, it is still under observation and will be monitored until clinical signs are compatible with the animal well-being.

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