HoBi-like pestivirus and its impact on cattle productivity

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Summary

The clinical features and economic impact of the infection caused by an emerging group of pestiviruses, namely HoBi-like pestivirus, in a cattle herd of southern Italy are reported. In 2011, the virus was first associated with respiratory disease, causing an abortion storm after one year and apparently disappearing for the following three years after persistently infected calves were slaughtered. However, in 2014 reproductive failures and acute gastroenteritis were observed in the same herd, leading to a marked decrease of productivity. A HoBi-like strain closely related to that responsible for previous outbreaks was detected in several animals. Application of an intensive eradication program, based on the detection and slaughtering of HoBi-like pestivirus persistently infected animals, resulted in a marked improvement of the productive performances.

Key words: Cattle; pestiviruses; HoBi-like strains; productive performances.

Introduction

Pestiviral infection in cattle herds is associated with severe economic losses in terms of milk production, reduced reproductive performance, growth retardation, increased occurrence of other diseases, unthriftiness, early culling and increased mortality among young stock (Ridpath and Neil, 2015). The genus *Pestivirus* consists of four recognised species, bovine viral diarrhoea virus (BVDV) 1, BVDV-2, border disease virus (BDV) and classical swine fever virus (CSFV) and some additional members that remain officially unrecognised, including a group of viruses variously referred to as HoBi-like, BVDV-3, or atypical pestiviruses (Bauermann et al., 2013). HoBi-like pestiviruses are an emerging group of pestiviruses infecting cattle, which have been detected in various parts of the world and are currently distributed in at least three continents. The first European Hobi-like virus, strain Italy-1/10-1, was isolated from calves with severe respiratory disease in southern Italy (Decaro et al., 2011, 2012b). Additional Hobi-like viruses were associated to reproductive disorders (Decaro et al., 2012a), generation of persistently infected (PI) calves (Decaro et al., 2013a), and occurrence of mucosal disease (Decaro et al., 2014; Weber et al., 2014).

Here, we report an outbreak of HoBi-like pestivirus infection in Italy that was responsible for abortion in pregnant cows and acute gastroenteritis in calves, causing severe economic losses in terms of productivity.

Case report

Between December 2009 and February 2010, a severe outbreak of respiratory disease occurred in a cattle herd in southern Italy (Decaro et al., 2011). Clinical signs appeared in 26 6-7-month-old calves and consisted of fever (39.4-40.1°C), coughing, accelerated pulse and breath, seromucoid nasal discharge, and moderate leukopenia. A HoBi-like pestivirus, strain Italy-1/10-1 was recognised as causative agent of the outbreak. The virus continued to

circulate in the herd, which one year later was affected by an abortion storm involving eight multiparous cows at 4-to-6-month of gestation. HoBi-like pestivirus was detected in two aborted foetuses (Decaro et al., 2012a).

The farmer was convinced to carry out an eradication program aimed to detect and slaughter PI animals. For this purpose, all the 752 animals of the herd were bled by the farm personnel and EDTA-blood samples were submitted to nested-PCR (Decaro et al., 2012d) and real-time RT-PCR assays (Losurdo et al., 2015; Mari et al., 2016) for detection and characterization of ruminant pestiviruses. A total of 9 PI animals were detected that were immediately slaughtered. All cows that were pregnant at the moment of sampling were monitored until they delivered and pestivirus testing was carried out on EDTA-blood collected from newborn calves. Additional 6 PI calves were detected following this strategy and the herd was finally considered to be free from pestiviruses. In the following months, the farm productivity increased markedly in terms of milk production, reproductive performance, growth rates, thriftiness, lower occurrence of other diseases, and decreased mortality among young stock. The productive performances remained high for three years, but in 2014 a new abortion storm was observed, with 14 abortions occurring between August and September (Fig. 1A-B). As observed in the previous abortion outbreak (Decaro et al., 2012a), reproductive failures were observed at 4–6 months of gestation.

In the same period, acute gastroenteritis was observed in 30 1-6-month-old calves (Fig. 1C-D). The herd productivity was again affected showing decreased milk production and increased repeat breeders, neonatal mortality and growth rates. Between August and November 2014, the average milk production per cow per day was 22.12 ± 0.42 kg, whereas it had been 24.35 ± 0.36 kg in the same quarter of the previous year. In the same quarter, the newborn calf mortality rates were 2.05% in 2013 and 6.76% in 2014 and repeat breeding rates were 8.23%

in 2013 and 15.82% in 2014. Analogously, the mean weaning weight of calves was 243 ± 36 kg and 226 ± 29 kg in 2013 and 2014, respectively.

Tissues from aborted foetuses and faeces from diarrhoeic calves were submitted to molecular investigations and standard protocols for the detection of the main abortogenic and enteric pathogens, as previously described (Decaro et al., 2008, 2012a).

HoBi-like pestivirus RNA (strain Italy-558/14) was detected in fetal tissues by nested-PCR (Decaro et al., 2012d) and real-time RT-PCR (Mari et al., 2016).

Sequence analyses carried out on a fragment spanning from 5'UTR to the E2 5' end of the viral genome (GenBank accession number KX011411) showed that the virus responsible for the novel outbreak was highly related (more than 99% of nucleotide identity) with the HoBi-like strains that had been previously detected in the same herd (Table 1).

Phylogenetic analysis based on comparison of the same nucleotide sequences grouped strain Italy-558/14 into the same phylogenetic branch as other HoBi-like strains, clustering with other viruses of Italian origin (Fig. 2).

In September 2014, a more extensive eradication program was started which involved again the entire cattle herd. In order to bleed all animals of the herd, samples collection was performed under our supervision. Virological investigations were carried out on a total of 780 animals that were sampled twice at 30 days apart by collecting EDTA-blood samples. All blood samples were tested for HoBi-like pestivirus by nested-PCR (Decaro et al., 2012d) and real-time RT-PCR (Losurdo et al., 2015; Mari et al., 2016). Animals were considered PI when they were found to be viremic at both sampling times; in contrast, acutely infected (AI) animals were viremic only at the first sampling, while after 30 days they were expected to become virus negative. Using this strategy, no AI and 18 PI animals were detected, which were immediately slaughtered. All calves born from September 2014 to May 2015 were tested for HoBi-like pestivirus within 10-20 days of age, so that additional 16 PI and 7 AI calves were

identified. The latter animals also included 3 calves that were still viremic after 30 days but yielded very low viral titers as calculated by real-time RT-PCR. In fact, these animals were virus negative at a third bleeding carried out after further 30 days. After PI animals were slaughtered, no additional pestivirus-infected animal was detected and a marked improvement of the productive performances was subsequently observed. Between August and November 2015, the average milk production per cow per day was 23.97 ± 0.31 kg, the newborn calf mortality rates were 2.37%, repeat breeding rates were 7.03% and the mean weaning weight of calves was 245 ± 33 kg.

Discussion

The present study suggests that HoBi-like pestivirus introduction into a cattle herd has the same outcome as the other, more widespread, pestiviruses, BVDV-1 and BVDV-2, whose impact on animal productivity is well recognised. Recent studies have shown that, analogous to extant pestiviruses, HoBi-like strains can cause respiratory distress (Decaro et al 2011, 2012b, 2013b), reproductive failures (Cortez et al., 2006; Decaro et al., 2012a), generation of PI animals (Decaro et al., 2013a; Bauermann et al., 2014), occurrence of mucosal disease (Decaro et al., 2014; Weber et al., 2016), severely affecting the productive performances of the cattle industry. In the outbreak described in the present report, the virus re-emerged four years after its first detection in 2010, causing both reproductive and enteric disease. The high genetic relatedness with HoBi-like strains circulating previously in the same herd accounts for a viral persistence in the herd rather than for a new introduction. This hypothesis is also supported by the lack of active viral circulation in neighbouring farms (N. Decaro, personal observation). Virus re-emergence in the farm after four years was likely due to an inappropriate approach of the first eradication program, which was supervised by the farm veterinarian and did not include long-term surveillance of calves born in the subsequent

months. Therefore, some cows, which were carrying infected foetuses at the time of first testing, could have generated PI calves that led to HoBi-like pestivirus persistence in the herd. To avoid further failure of the eradication attempts, the 2014 program was carried out under our direct supervision, so that all calves born in the following 8 months were tested virologically. Consequently, several PI animals were detected and removed from the farm, which resulted in the complete suppression of the virus circulation.

HoBi-like pestiviruses are spreading worldwide and their presence has been already reported in three continents (Bauermann et al., 2013). Natural infections by HoBi-like pestiviruses have been reported in South America (Cortez et al., 2006; Weber et al., 2014), Asia (Kampa et al., 2010; Haider et al., 2014; Mishra et al., 2014) and Europe (Decaro et al., 2011, 2012a, 2012b, 2013a, 2013b, 2014). However, virus neutralization assays showed a limited cross-reactivity of HoBi-like pestivirus with BVDV-1 and BVDV-2 (Bauermann et al., 2012; Decaro et al., 2012c; Larska et al., 2012). Therefore, if in-vitro findings are confirmed by in-vivo vaccine trials, the need to develop specific vaccines should be carefully considered to prevent the negative economic impact of this group of emerging pestiviruses.

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Figure legends.

Fig. 1. Gross lesions in aborted foetuses (**A, B**) and calves with gastroenteritis (**C, D**). **A.** Haemorrhagic pleural effusion. **B.** Enlargement and congestion of the spleen. **C.** Catarrhal enteritis. **D.** Enlargement and congestion of a mesenteric lymph node.

Fig. 2. Phylogenetic tree based on the genomic 5' end (3,018 nucleotides) of members of the genus *Pestivirus*. For phylogenetic tree construction, pestivirus sequences reported in Table 1 were used. The tree was generated using the Bayesian inference with Generalized Time-Reversible (GTR) model and gamma rate variation and supplying statistical support with subsampling over 200 replicates. The scale bar indicates the number of nucleotide substitutions per site.