HoBi-Like Pestivirus and Reproductive Disorders

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HoBi-like pestivirus (HoBiPeV) is an emerging group of pestiviruses that has been detected in cattle and other ruminants in South America, Europe, and Asia. Analogous to other bovine pestiviruses, namely bovine viral diarrhea (BVDV) 1 and 2, HoBiPeV is able to cause a variety of clinical forms that range from asymptomatic infections to fatal disease, having a great impact on cattle productions and causing substantial economic losses, mainly as a consequence of the occurrence of reproductive failures. The manuscript aims to provide an updated review of the currently available literature about the impact of HoBiPeV infection on cattle reproduction. The reproductive disorders observed in cattle due to natural and experimental infections caused by this virus are reported along with the few available in-vitro studies involving the reproductive tract. HoBiPeV should be considered among the bovine pathogens that impact on reproduction, but there is a need for more specific and sensitive diagnostic methods, while the cross-protection elicited by commercially available BVDV vaccines should be better investigated.

Keywords: HoBi-like pestivirus, cattle, reproduction, natural infections, experimental infections

INTRODUCTION

Pestivirus infections in cattle are associated with various clinical forms that include subclinical infections, immunosuppression, respiratory signs, gastroenteritis, reproductive failures, haemorrhagic and systemic disease, such as mucosal disease in persistently infected (PI) animals (1–3). Pestiviruses (family Flaviviridae, genus Pestivirus) are single-strand, positive-sense RNA viruses, whose genome, 12.3 Kb in size, encodes for a polyprotein that is cleaved by viral and cellular proteases in structural (C, E', E1, E2) and nonstructural (Npro, NS2-3, NS4A, NS4B, NS5A, NS5B) proteins (4). Based on the current classification of the International Committee on Taxonomy of Viruses (https://talk.ictvonline.org/taxonomy/), genus Pestivirus is composed of 11 recognized species, namely Pestivirus A (original designation Bovine viral diarrhea virus 1, BVDV-1), Pestivirus B (Bovine viral diarrhea virus 2, BVDV-2), Pestivirus C (Classical swine fever virus, CSFV), Pestivirus D (Border disease virus, BDV), Pestivirus E (pronghorn pestivirus), Pestivirus F (Bungowannah virus), Pestivirus G (giraffe pestivirus), Pestivirus H (Hobi-like pestivirus, HoBiPeV), Pestivirus I (Aydin-like pestivirus), Pestivirus J (rat pestivirus) and Pestivirus K (atypical porcine pestivirus) (4). At least three pestivirus species are infecting primarily cattle, BVDV-1 and BVDV-2, known for decades, are grouped in 21 and 3 subtypes, respectively (3, 5, 6). HoBiPeV was first isolated from a contaminated batch of fetal bovine serum (FBS) from Brazil (7) and later detected in a contaminated cell culture and the blood of a buffalo in South America (8). Further HoBiPeV infections were reported in Thailand, Italy, Bangladesh, India, Argentina, China, and Turkey (9–20). However, while viruses circulating in South America, Europe, Thailand and China are closely related, other Asian HoBiPeV strains are highly divergent and at least four different subtypes have been identified so far (3). HoBiPeV infection has the same outcomes reported for BVDV-1
and BVDV-2, including immunosuppression, respiratory disease, gastroenteritis, persistent infections, mucosal disease, and reproductive disorders (10, 15, 19–30). The most relevant economic losses associated with BVDV-1 and BVDV-2 infection are due to reproductive disorders. The infection of pregnant cows during the first trimester of gestation with noncytopathic (ncp) BVDV strains may lead to failure of fertilization, return to oestrus, abortion, congenital malformations, stillbirths, or the birth of persistently infected (PI) animals. PI calves are BVDV seronegative but virus-positive animals, which shed BVDV with their secretion lifelong. They may develop a severe, fatal form of the disease, referred to as mucosal disease, due to superinfection with a cytopathogenic (cp) strain (2, 3). While a plethora of reports are available about reproductive failures caused by pestiviruses belonging to either BVDV-1 or BVDV-2 species, little is presently known about the impact of HoBiPeV infection on cattle reproduction. Therefore, in this paper I provide an up-to-date literature review about the association of this emerging pestivirus to reproductive disorders in cattle.

**REPRODUCTIVE DISORDERS AFTER HoBiPeV NATURAL INFECTIONS**

The first report of HoBiPeV sequences detected in bovine fetuses dates back to 2006, when a study was published aiming to investigate the genetic variability of BVDV strains from archival samples (31). Two fetuses, aborted in 2002 and 2004, respectively, were later found to contain HoBiPeV strains, which clustered with the virus prototype D32/00_HoBi (7). The fetuses were from farms located in the State of São Paulo, about 100 km apart from each other, suggesting that the two strains were probably circulating among cattle and causing reproductive disorders in the region. However, no further details were reported about the herds of origin of the fetuses.

Reproduction failures associated with natural infection with HoBiPeV were later reported in Italy in 2011 (20). An abortion storm occurred in a cattle herd of southern Italy, which included about 600 Holstein cattle, involving eight multiparous cows at 4–6 months of pregnancy out of 98 pregnant animals. Affected cows did not display any relevant clinical signs before nor any sequelae after the abortion. Two aborted fetuses were submitted to laboratory investigations, displaying high titres of HoBiPeV RNA in their tissues, ranging from $4.31 \times 10^5$ (kidney of one fetus) to $5.78 \times 10^4$ (lung of the other fetus) RNA copies per µl of template, by a specific real-time RT-PCR assay. No additional pathogens were detected by molecular methods for abortogenic agents of cattle. By immunofluorescence assay using an NS3 5’UTR, N antigen was obtained from the same fetuses. Sequence and phylogenetic analysis of pestivirus’s most informative genomic regions (E2, 5’UTR, Npoly) revealed that the abortogenic HoBiPeV strain was closely related to the virus which had been responsible for the respiratory outbreak a few months before (10).

More data are available about the generation of HoBiPeV PI calves under natural conditions. Pestiviruses can induce immune tolerance in calves born to cows infected in the first 3–4 months of pregnancy. These immune tolerant calves are PI and may have congenital malformations or appear clinically normal and show immunosuppression, thus developing respiratory and gastroenteric disease often caused by opportunistic pathogens (1–3). PI animals have been frequently reported due to BVDV-1, BVDV-2, BDV, CSFV, and, only recently, HoBiPeV infections (22). A calf born in October 2011 in a cattle herd with active HoBiPeV circulation in southern Italy displayed a low birth weight and a decreased growth rate, and later developed respiratory disease with cough and nasal discharge and ruffled hair with alopecic areas in different parts of the body caused by cutaneous mycosis. Laboratory investigations revealed that the calf was pestivirus antibody negative and virus positive, with the strain being characterized as ncp HoBiPeV. In March 2013, at the age of 17 months, the PI calf developed severe clinical signs and subsequently died. A ncp/cp HoBiPeV pair was isolated from the dead animal, which was highly suggestive of mucosal disease (23).

Subsequent reports of mucosal disease in calves in Brazil account for the generation of PI animals as a consequence of congenital infection with HoBiPeV (25, 29), analogous to what was reported for BVDV-1 and BVDV-2 (3). The identification and elimination of PI animals is the main direct prophylactic measure to achieve the eradication of pestivirus infections in cattle herds (32). This strategy has been proven to be useful also against HoBiPeV. In a cattle herd with large economic losses due to reproductive disorders caused by HoBiPeV, the application of an intensive eradication program, based on the detection and slaughtering of PI animals, resulted in a marked improvement of the productive performances (26).

**REPRODUCTIVE DISORDERS AFTER HoBiPeV EXPERIMENTAL INFECTIONS**

Several experimental infections have been carried out to better understand the impact of HoBiPeV infection on cattle reproduction.

In one of these studies, eight pregnant heifers were infected at around day 70 of gestation by intranasal instillation of either Brazilian HoBi_D32/00 or Italian Italy-1/10-1 HoBiPeV isolate (24). The experimental infection was successful in all animals but one inoculated with the Brazilian strain. Another heifer inoculated with HoBi_D32/00 aborted at 8 months of pregnancy, and the fetus was positive for HoBiPeV by RT-PCR. Of the remaining six animals, two heifers infected with the Italian strain gave birth to weak calves infected by HoBiPeV and died within 36 h of birth. In comparison, calves born to the other four animals (two inoculated with the Brazilian strain and two inoculated with the Italian strain) were diagnosed as PI animals by consecutive positive results throughout the study. These PI calves were later housed with pestivirus seronegative calves, sheep, goats, and pigs, resulting in successful infection of all exposed animals (33). Analogous to BVDV-1 and BVDV-2 PI animals, HoBiPeV PI calves displayed extensive depletion in the thymus, with PI calves surviving <5 weeks having lower corticomедullary ratios and greater depletion (34).
In a subsequent study by the same research group (35), cows that had previously calved BVDV-1 (four cows) or BVDV-2 (two cows) PI calves and had high antibody titres against the homologous BVDV strains, were inoculated with HoBiPeV at 85 days of gestation. At 30 days after the challenge, one inoculated cow had no fetus, while the fetuses harvested from 5 of the exposed dams (three BVDV-1 and two BVDV-2 cows) were all positive for HoBiPeV RNA. Therefore, there was no fetal cross-protection elicited by the previous infection with BVDV-1 or BVDV-2 against this emerging group of viruses.

A comprehensive study was carried out in pregnant sheep to evaluate their suitability as a model for vaccination trials of HoBiPeV experimental formulation for cattle (36). In fact, the HoBiPeV challenge of cattle to evaluate the fetal protection induced by pestivirus vaccines require great efforts due to handling a high number of large-size animals, adopting adequate biosecurity measures and spending much money for purchasing and feeding cattle. In the sheep study, ten ewes at different pregnancy stages (30 or 50 days) were experimentally infected with the Italian HoBiPeV prototype isolate Italy-1/10-1. All but one infected ewes underwent reproductive disorders, including abortion (n = 3), stillbirth (n = 4) or generation of PI lambs (n = 4). Aborted fetuses, stillborn and dead lambs displayed extensive histopathological changes, including hemorrhages, congestion and mononuclear infiltration in internal organs, and immunohistochemical detection of pestiviral antigens in affected tissues. PI lambs were constantly viremic, shed the virus through the nasal secretions and feces and, with one exception, were HoBiPeV seronegative. The single seropositive infected lamb showed low-titer viremia and viral shedding that ceased only several weeks after the 3-month observation period. Therefore, the sheep model reproduced the reproductive disorders observed in cattle due to natural or experimental infection with HoBiPeV and represents a suitable model for the evaluation of the fetal protection induced by pestivirus vaccines.

**IN-VITRO STUDIES INVOLVING THE BOVINE REPRODUCTIVE TRACT**

A study was carried out to evaluate the growth of different bovine pestivirus species in testicle cell cultures obtained from taurine (Bos taurus taurus), indicine (Bos taurus indicus), and mixed taurine and indicine (Bos taurus taurus × Bos taurus indicus) breeds (37). In this experiment, the HoBiPeV isolate Italy 1/10-1 was found to replicate efficiently in primary bovine testicle cells, and no significant differences were observed based on breed, whereas, there were significant differences in growth rate among animals within each breed group.

Another study aimed to evaluate the effect of pestivirus infection on embryo development as a consequence of exposure of bovine oocytes to different pestivirus species (38). Bovine oocytes were collected after slaughtering and treated with different concentrations of pestiviruses (BVDV-1, BVDV-2 and HoBiPeV) during their in-vitro maturation. After in-vitro fertilization, zygotes were cultured for seven days, and the developed embryos were evaluated according to the guidelines of the International Embryo Transfer Society and submitted to pestivirus detection by real-time RT-PCR. The obtained results showed that in-vitro produced embryos from BVDV-1 and BVDV-2 infected oocytes developed normally, but were infected, thus carrying the virus. In contrast, HoBiPeV infected oocytes had reduced cleavage and cause pre-implantation embryo loss, but viable embryos did not carry the virus, suggesting that HoBiPeV infection may cause embryo loss before blastocyst development. Therefore, the study demonstrated that oocyte exposure to HoBiPeV resulted in reduced embryo development, having the potential to impact more severely on cattle reproduction than other bovine pestiviruses.

**DISCUSSION**

Pestivirus infections cause economically significant diseases affecting the cattle industry, with losses stemming from decreased production and reproductive performance, and control costs. Economic losses associated with reproduction are relevant since these viruses may have negative effects on all phases of bovine reproduction. Reduced conception rates, early embryonic death, abortion, congenital defects, and weak calves have all been associated with pestivirus infection of susceptible cows. Besides, the birth of PI calves as a consequence of in utero fetal exposure is critical in the perpetuation of the virus in an infected herd or spread to other susceptible herds (39, 40). All studies reporting the outcomes of naturally or experimentally occurring HoBiPeV infection in cattle account for a high pathogenic potential of this virus on the reproduction, with the development of the same reproductive disorders that are commonly observed during BVDV-1 and BVDV-2 infection (20, 22, 26, 35). In addition, the virus affected embryo development as a result of oocyte exposure.

Consequently, this group of emerging viruses should be considered among bovine pathogens that impact reproduction and be included in the diagnostic algorithms of reproductive disorders in cattle. A major issue in HoBiPeV detection is that antigenic, molecular, and serological tests commercially available for BVDV-1 and BVDV-2 may not react or react at a lower extent with this emerging virus (24, 41). Also, even assays specifically developed for HoBiPeV detection may fail to detect the more divergent strains identified in recent times (42). Therefore, there is a need in the improvement of HoBiPeV specific diagnostic assays in order not to misdiagnose the reproductive disorder caused by this virus, especially in areas with low virus circulation. Nevertheless, vaccination trials are also required to assess the efficacy of BVDV currently available vaccines against HoBiPeV infections, since there are some concerns that those vaccines may not be completely protective against this pestivirus (35, 43).

**AUTHOR CONTRIBUTIONS**

ND conceived and wrote the article.

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**Conflict of Interest:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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