

Emerging HoBi pestiviruses: impact on animal health and their importance as contaminants of biotechnological inputs

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ABSTRACT

HoBi-like viruses are a group of emerging Pestiviruses affecting cattle and water buffaloes and which are common contaminants of biological products. After the first detection in Germany from Brazilian samples in 2004, several HoBi-like isolates have been reported in Brazil, Italy and some countries of Asia. Thus, this pathogen has concerned cattle and buffalo farmers and veterinarians as it has been involved in events of severe respiratory symptoms, abortions and persistently infected animals.

Due to the lack of an active surveillance system for HoBi-like viruses and since infections can be mistaken with bovine viral diarrhea, the true impact on cattle populations is unknown. In Argentina, the existence of HoBi-like viruses has not been studied, neither from an epidemiological point of view nor in terms of its potential as an adventitious agent in biological products and cell lines. The aim of this article is to provide an analysis of the worldwide epidemiological situation of HoBi-like viruses, in order to generate an approach and to awaken interest in veterinarians, virologists and health authorities for this emerging pathogen. It is essential to achieve greater investment and participation in interdisciplinary research to clarify basic aspects of the epidemiology of this new pathogen and to establish its possible impact on the productive systems of the South American countries.

Keywords: *Virology, animal health, contaminant agents.*

INTRODUCTION

The genus *Pestivirus*, from the *Flaviviridae* family, includes the Bovine Viral Diarrhea Virus type 1 and 2 (BVDV 1 and BVDV 2), Classical Swine Fever Virus (CSFV) and Border Disease Virus (BDV) (Collett et al., 2005). BVDV 1 and BVDV 2 are important pathogens that mainly affect cattle and the circulation of this virus in productive systems results in significant economic losses worldwide (Houe, 2003).

BVDV infection results in a wide range of manifestations, ranging from mild respiratory or gastroenteric disease to hemorrhagic syndromes, depending on the virus' virulence and the animal's immunological and reproductive status. BVDV can cross the placenta and infect the fetus at any

stage of gestation causing transient infertility, miscarriages, congenital malformations or the birth of persistently infected (PI) animals. The latter are the main source of virus spread and, as they are susceptible to reinfection by a cytopathic strain, animals may suffer from the picture known as "mucosal disease" (Baker, 1995).

In Argentina BVDV is endemic and the strategy used to control it is based on the vaccination with inactivated formulations. As for CSFV, this virus was eradicated from the country after intense vaccination campaigns. Finally, the circulation of BDV in Argentina has not been investigated.

This article presents a review of the emerging HoBi viruses that have been recently reported in several countries of

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the world, but have not been studied in Argentina or in most Latin American countries. We consider that to study these viruses is important for animal health of ruminants and for the safety of the biological products that include fetal bovine serum in their composition.

Atypical Pestiviruses HoBi

In addition to the abovementioned viral agents of the genus Pestivirus, there are other tentative species that have been proposed, such as the group of viruses known as "HoBi" or "Atypical Pestivirus" (Bauermann and Ridpath, 2015). Recently, in Italy, retrospective studies analyzing lots of fetal bovine serum (FBS) from 1992 were performed and the agent was identified. This would probably mean that this pathogen has been circulating in cattle for more than 20 years.

HoBi viruses are often involved in clinical cases similar to BVDVs and this is why some authors call them BVDV type 3 or BVDV-3. The tentative name "HoBi" comes from the first letters of the names of a German researcher (Horst Schirrneier) and his assistant (Birgit Meinke), who obtained one of the first isolates of this Pestivirus from a sheep cell line whose culture medium was supplemented with Brazilian FBS - known as "batch 547" - contaminated with this agent (Schirrneier et al., 2004).

HoBi viruses have been isolated from clinical cases in several countries such as Italy, Bangladesh, India and Brazil (Figure 1). In Thailand, seroconversion to the HoBi virus has been demonstrated in four bovine herds and a viremic animal was detected using ELISA and RT-PCR (Kampa et al., 2009; Liu et al., 2009).

Particularly in Italy, in 2009, HoBi viruses caused an outbreak of respiratory diseases affecting 6-7 month old calves (Decaro et al., 2011). In this episode, these viruses were detected from samples of nasal swabs and also in lungs in the necropsies of two dead animals. The clinical signs associated with these pictures were hyperthermia, cough, seromucose nasal discharge, leucopenia and accelerated respiratory rate. In 2011, also in Italy, HoBi viruses were found to cause bovine abortion (Decaro et al., 2012a).

In India and Bangladesh isolates of HoBi virus were obtained from cattle of farms with histories of respiratory, enteric or reproductive problems (Haider et al., 2014; Mishra et al., 2014).

In Brazil, HoBi viruses are endemic in cattle and probably also in water buffaloes. Natural cases of abortion, respiratory disease and mucosal disease have been reported (Cortez et al., 2006; Bianchi et al., 2011; Weber et al., 2014; Bauermann and Ridpath, 2015). One of the earliest isolates of HoBi virus, "BrazBuf9" (Stalder et al., 2005) was identified in the 1990s in water buffaloes.

In Argentina, HoBi viruses have not been studied in bovine herds or in buffaloes, nor have diagnostic techniques been standardized for their detection. There is no information available on the situation in the rest of the Spanish-

speaking Latin American countries, which makes the epidemiological situation completely unknown.

Genetic diversity

The comparison with the rest of the Pestiviruses shows that the identity of the sequences of the 'Npro' viral protease and the immunodominant protein "E2" of HoBi viruses is less than 70%, which indicates important gene differences (Liu et al. 2009). Similarly, seroneutralization and characterization studies with monoclonal antibodies revealed marked antigenic differences between HoBi viruses and the other Pestiviruses (Bauermann et al., 2012).

It is suggested that there are four clades of HoBi virus that group the Brazilian, Thai, Italian and Indian/Bangladeshi strains. Recently, it has been reported that in India and Bangladesh there are strains of HoBi virus belonging to more than one clade (Haider et al., 2014, Mishra et al., 2014, Bauermann and Ridpath, 2015). This would open the possibility that in fact the HoBi viruses have emerged in Asia. Since the main buffalo breeds originated in these regions, from which they were exported to other continents, some suspect HoBi viruses have been primarily in these animals and then spread imperceptibly (Bauermann et al., 2013b). So, are the HoBi viruses really emerging, or there were not adequate techniques to detect them?

Clinical presentation of HoBi virus in natural cases and in experimental infections

As for the pathogenic potential of HoBi viruses, they have been isolated from natural clinical cases of respiratory disease, diarrhea and reproductive problems including abortions. However, these Pestiviruses have also been detected in apparently healthy animals (Schirrneier et al., 2004; Decaro et al., 2011; Decaro et al., 2012a; Mishra et al., 2014). The fact that HoBi viruses coexist with BVDVs in certain regions hampers the investigations on their specific impact and estimates of economic losses.

Experimental investigations managed to infect calves using different strains of HoBi virus, which caused respiratory problems as well as hyperthermia, leucopenia and, later, seroconversion (Table 1). Pregnant cows have also been infected, which led to abortions and the birth of persistently infected animals (Bauermann et al., 2014a).

Infections with BVDV and BDV are not restricted to cattle and sheep, respectively, but also cattle can be infected with BDV and vice versa. There is only one etiological restriction in CSFV infections in ruminants.

It was also reported that it was possible to successfully infect lambs with HoBi viruses, causing respiratory signs (nasal secretion), moderate hyperthermia, leucopenia, viremia and viral excretion through nasal secretions and fecal matter.

Also, it was experimentally verified that these viruses can generate abortions, malformations and even persistently

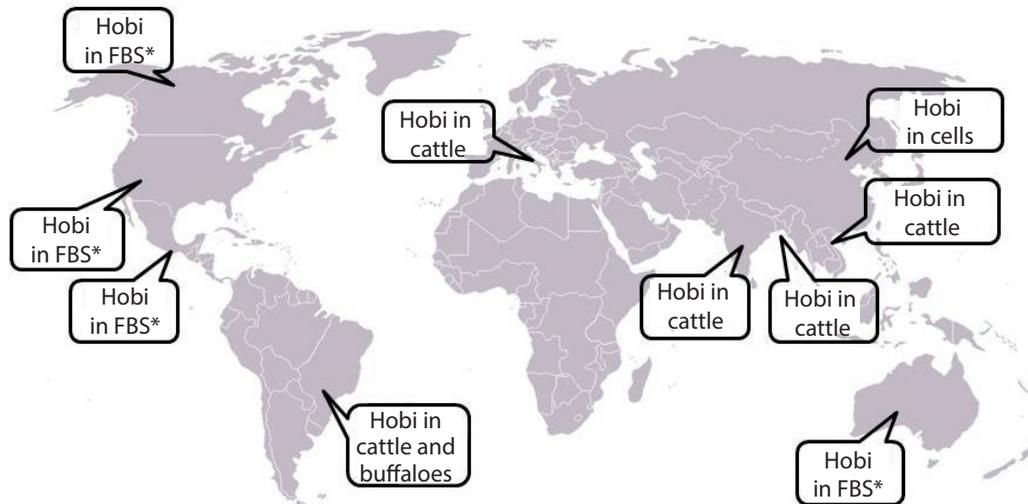


Figure 1. Distribution of the HoBi viruses isolated to date. HoBi viruses have been detected in batches of fetal bovine serum (FBS) generated in Brazil, Mexico*, Canada*, the United States* and Australia* (Xia et al., 2011). However, of these 5 countries, the virus was isolated from natural infections in cattle and water buffaloes only in Brazil (Bianchi et al., 2011; Cortez et al., 2006). In Europe, HoBi virus was isolated in Italy from clinical cases in cattle (Decaro et al., 2011; Decaro et al., 2012a). In India, Thailand and Bangladesh, HoBi viruses were isolated in farms with histories of respiratory and reproductive problems (Mishra et al., 2014; Kampa et al., 2009). In China, HoBi virus has been isolated from a bovine cell line (Mao et al., 2012).

infected animals in pregnant sheep. In contrast, pigs inoculated with the same virus presented no clinical signs, leucopenia or positive PCR days after infection (Decaro et al., 2012b; Decaro et al., 2015).

Diagnostic and prophylaxis tools for HoBi viruses

Diagnostic tests designed to detect BVDVs that are currently used often fail to detect HoBi viruses. To date, no specific monoclonal antibodies have been developed for this group of Pestiviruses and it is not possible to differentiate them from BVDVs using techniques such as viral isolation. In molecular biology several primers able to specifically detect HoBi viruses have been described in RT-PCR reactions (Bauermann and Ridpath, 2015). Recently, a real-time Multiplex PCR has been developed in Italy that allows the detection and differentiation of HoBi, BVDV-1 and BVDV-2 viruses (Mari et al., 2015).

In the absence of commercial diagnostic kits for detecting specific antibodies against HoBi viruses, some investigators choose classical seroneutralization assays against these viruses and against BVDV in parallel and then compare the neutralizing antibody titres of the animals against those pathogens (Bauermann et al., 2013b).

As for prophylaxis tools, both attenuated and inactivated BVDV vaccines have been shown to produce weak protection against HoBi viruses (Bauermann et al., 2013a). On the one hand, this low protection conferred by the commercial vaccines formulated with BVDV 1 and 2 against HoBi viruses could affect a high number of susceptible animals, with the generation of persistently infected animals that excrete

the virus in most of their fomites. There are currently no commercial vaccines in the world designed to protect livestock from HoBi viruses. On the other hand, this lack may be useful for serological surveillance tasks.

Findings in cell lines and biotechnological inputs

Pestiviruses in general, and HoBi viruses in particular, are a threat to the safety of cell lines and biologicals products. To date, several studies reported that they are able to determine the presence of HoBi viruses in batches of FBS, suggesting the spread of the agent through cell lines (Mao et al., 2012; Giangaspero, 2013; Xia et al., 2011; Xia et al., 2013). The dissemination of HoBi viruses not only affects animal production, but also threatens the status of “Pestivirus free” that some European countries have achieved after immense efforts.

In the laboratory, FBS is used for the amplification of cells in cultures to propagate viruses used in research and for the formulation of vaccines, and in other processes such as embryonic transplantation. It has also been suggested that it is the main “scapegoat” to explain the widespread dissemination of HoBi viruses (Giangaspero, 2013). Consequently, the detection of genetic material of HoBi virus has been reported in lots of FBS from countries such as Brazil, Australia, Canada, Mexico and the United States (Figure 1).

However, it was not possible to determine if contamination with these agents occurred in the countries of origin or during the mixing of lots of FBS from different sources, which is often done in European countries (Bauermann et al., 2014b). Faced with this problem, there are some points to empha-

Reference strain	Rectal temperature	Respiratory signs	% of leukopenia	Viremia	Viral excretion	Seroconversion
Hobi D32/00	Slight increase	sin signos clínicos	Mild leukopenia	(+) 5 dpi	(+) 3-6 dpi in nasal swabs	Verified
Italy-1/10-1	40 °C 3 and 7 dpi	Seromucous nasal secretion	50% 3-10 dpi	(+) 5-24 dpi	(+) 5-21 dpi in nasal secretions (+) 7-21 dpi in stool	Verified
Th04_Khonkean	39 °C and 7-10 dpi	Nasal and ocular secretions, cough and conjunctivitis	40% 5-7 dpi	(+) 5-9 dpi	(+) 5-7 dpi en secreciones nasales	Verified
Hobi_D32/00	Slight increase	Without clinical signs	65% 9 dpi	(+) 3-9 dpi intermittent	(+) 3-9 in intermittent nasal secretions	Verified

Table 1. Description of the clinical manifestations after experimental infections of calves with HoBi virus (adapted from Bauermann et al, 2013b); Dpi: days post infection.

size: first, the dissemination of Pestiviruses could be reduced or even avoided if the FBS was to be commercialized only if irradiated. Secondly, determination of HoBi virus is not currently required in routine studies for the use of FBS, cell lines or vaccine strains. And last but not least, it should be clarified that the filtration process that is commonly used during the manufacture of FBS does not prevent the presence of Pestivirus due to the small size of the viral particles.

All of this must be taken into account when working with embryo transplant techniques and with biological inputs both at research and industrial scales, in order to ensure the quality of the products.

In summary, as Dr. Schweizer and Peterhans have pointed out, “there are likely to be more scientists working with Pestivirus than scientists who consciously work with Pestiviruses” (Schweizer and Peterhans, 2014).

CONCLUSIONS

The increasing number of reports on Pestivirus HoBi raises two main concerns: one related to the emergence and

spread of these pathogens, with consequences in animal health and disease control. The second concern is related to the problem of contamination of lots of FBS, with implications for the biosecurity of the biological products that contain it, such as vaccines, diagnostic reagents, kits and other bio-industry products.

In animal health, specifically, it is important to determine if the HoBi viruses circulate in our herds and to know if both the prophylactic tools and those designed to detect BVDV in our country are able to detect and combat this viral agent.

Also, we must acknowledge the relevance of the quality of the biological inputs we use, not only for research but also for the use of cell lines used for diagnostic services and vaccine formulation in industries. In this regard, it is important to raise awareness about the impact of adventitious viruses.

Finally, we consider that this problem requires the deepening of research at national and international level to know which is the real situation against HoBi viruses, their economic impact and if they are truly emerging viruses.