



Pestivirus infection, an emerging threat to ruminants in India: A review

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ABSTRACT

The genus *Pestivirus* in the family *Flaviviridae* comprises 4 recognized species: Bovine viral diarrhoea virus type 1 (BVDV-1), BVDV-2, border disease virus (BDV) and classical swine fever virus (CSFV). Among ruminant pestiviruses, BVDV/BDV infections occur worldwide and due to its economic importance, BVD is listed by OIE as a priority cattle disease for international trade. Pestivirus antibodies were detected in several countries with prevalence rates varying from 0–90% in cattle and 0–50% in sheep. Ruminant pestiviruses are genetically and antigenically diverse, as displayed by identification of 16 subtypes within BVDV-1, 2 subtypes within BVDV-2 and 7 subtypes within BDV species. Heterogeneity in host spectrum, virulence and clinical signs provide further challenges in disease diagnosis and control.

Both large and small ruminants form backbone of the livestock sector in India. BVD was earlier considered exotic despite serological evidence of BVDV infection reported during 1980's and 90's. The first confirmatory evidence of BVD by virus isolation from cattle was reported in 2004, followed by its detection in sheep, buffalo, yak and goats. Later reports established the predominant occurrence of BVDV-1 and sporadic occurrence of BVDV-2, with BVDV-1b, BVDV-1c, BVDV-2a and BVDV-2b genotypes identified in various species of ruminants. Furthermore, moderate pathogenicity of BVDV-1 was demonstrated in experimentally infected cattle. A systematic surveillance of ruminant pestiviruses and their economic implications need to be taken up in future. In this review, we discuss the current status of ruminant pestivirus infections in India besides highlighting the gaps in current knowledge with regard to epidemiology, diagnosis and control.

Key words: Border disease, Bovine viral diarrhoea, BVDV, Pestivirus, Ruminants

Pestiviruses are animal pathogens of major economic importance for the livestock industry and have significant influence in cattle, pig and sheep production. Bovine viral diarrhoea virus type 1 (BVDV-1) and BVDV-2 along with border disease virus (BDV) of sheep and classical swine fever virus (CSFV) in pigs belong to the genus *Pestivirus* in the family *Flaviviridae* (Thiel *et al.* 2005). The giraffe pestivirus has tentatively been classified as a fifth species. However, several new pestiviruses identified in ruminants, both domestic as well as wild are yet to be classified. Bovine viral diarrhoea virus causes mainly alimentary, respiratory and reproductive problems like pneumonia, diarrhoea, mucosal disease or abortion in cattle and small ruminants besides haemorrhagic syndrome and thrombocytopenia (Baker 1987). BDV infects sheep and goats causing mainly reproductive disease (Nettleton *et al.* 1998). CSFV causes an acute

haemorrhagic disease in domestic pigs, and also infects wild boars. According to the OIE (2008), bovine viral diarrhoea/mucosal disease (BVD/MD) and classical swine fever (CSF) are notifiable diseases and hence eradication programmes are underway in several countries worldwide. Scandinavian countries were extremely successful in controlling BVDV infection while limited success was achieved in U.S.A. Development and increased use of newer molecular diagnostics and tools have enhanced the understanding of pestivirus genetic diversity and evolution besides helping in controlling the outbreaks. Introduction of exotic germplasm either through semen or livestock trade during last four decades for cross breeding programme to augment livestock production has introduced several exotic diseases into India. Most probably, the ruminant pestiviruses also were introduced during this period and remained undetected due to difficulty associated with the diagnosis and virus isolation. The present review presents the current scenario of pestivirus prevalence besides their genetic and antigenic diversity in various species of ruminants in India (Mishra *et al.* 2004, 2006, 2007a, 2007b, 2008b, 2008c, 2009, Behera *et al.* 2011).

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Structure of the genome and function of viral proteins:

The pestivirus genome consists of a single-stranded RNA of about 12.3 kb in length. Similar to Hepatitis C virus in humans, the genome lacks a 5' cap and 3' poly-A tract. A long ORF flanked by untranslated regions at both ends (5' and 3' UTR) is translated into a poly-protein of about 4000 amino acids and is cleaved into 4 structural [capsid (C) and 3 envelope (E^{ns}, E1 and E2) proteins] and 6–7 nonstructural proteins (N^{pro}, NS2-3 or NS2 and NS3, NS4A, NS4B, NS5A and NS5B) by viral and host cell proteases (Meyers and Thiel 1996). The humoral immune response allows detection of antibodies against the structural envelope proteins E2 and E^{ns} as well as against the highly conserved non-structural protein NS3 (p80). Antibodies against all 3 proteins are suited to indirectly demonstrate an infection with a pestivirus. For sero-diagnosis enzyme immunoassays and neutralization assays can be used. However, cross reacting antibodies elicited by ruminant pestiviruses are usually registered by these tests. E2-glycoprotein generates neutralizing antibodies and cross neutralization tests using all species of ruminant pestiviruses can definitively identify a particular type involved.

Two biotypes of pestiviruses, cytopathic (cp) and noncytopathic (ncp) are recognized based on their effect in cultured epithelial cells (Gillespie *et al.* 1960). Biotypes do not correlate to the virulence of the virus in field, as BVDV strains associated with severe acute outbreaks are all ncp-biotypes (Carman *et al.* 1998). The ncp-biotypes are preponderant in nature and responsible for majority of disease syndromes including establishment of persistent infection. The cp viruses are relatively rare and cytopathogenicity is correlated with the appearance of non-structural protein-NS3 (p80) (Meyers and Thiel 1996). However, this hypothesis needs reconsideration in light of the recent studies providing evidence that NS2–3 gets cleaved into NS3 also in leukocytes of animals infected with ncp isolates (Kameyama *et al.* 2008, Mishra *et al.* 2010). The generation of a cp strain in an animal already persistently infected with ncp BVDV is considered as the crucial step for pathogenesis of mucosal disease.

Genetic and antigenic typing

A more precise typing of pestiviruses was obtained from the application of molecular genetic methods such as, reverse transcription-polymerase chain reaction (RT-PCR) and sequencing coupled with phylogenetic analysis. Antigenic relationship of pestiviruses studied by cross neutralization using defined serological reagents has also been used as an additional criterion for demarcating pestivirus types. Although sequence analysis of 5'UTR can be used for the segregation of pestivirus isolates into 4 established species, segregation into types and subtypes is better accomplished by sequence analysis of N^{pro}, E2 and other coding sequences (Becher *et al.* 1999). However, there is little correlation between pestivirus subtypes and course of the disease.

Analysis of pestiviruses at antigenic and genetic level clearly distinguishes pestiviruses infecting domestic animals into 4 species: BVDV-1, BVDV-2, BDV and CSFV. Analysis of available BVDV-1 and BVDV-2 isolates collected at different countries around the world has divided them into 15 BVDV-1 subtypes (Vilcek *et al.* 2001, Jackova *et al.* 2008) and two BVDV-2 subtypes (Vilcek *et al.* 2004, Mishra *et al.* 2007b, 2008b). Sequence analysis of BDV isolates has divided them into at least 6 subtypes with an additional subtype reported recently (Becher *et al.* 2003, Ogozoglul *et al.* 2008). CSFV isolates of domestic pigs and wild boars were divided into 3 genogroups and 10 subgroups (Paton *et al.* 2000).

Pestivirus infections in cattle

The severe economic losses due to BVD in cattle usually occur through transplacental infections resulting in abortion, mummification, stillbirth and congenital defects. BVDV-1 was first isolated from cattle in 1940s and occurs worldwide. In contrast, BVDV-2 was identified in 1990s and was initially detected only in North America (Ridpath *et al.* 1994). However, recently it was also found in Europe (Vilcek *et al.* 2001, 2004), Asia (Nagai *et al.* 2004, Park *et al.* 2004) and South America (Flores *et al.* 2002, Pizarro-Lucero *et al.* 2006). Fifteen subtypes within BVDV-1, and 2 subtypes within BVDV-2 were identified with different subtypes predominating in various countries.

The first serological evidence of BVDV infection in India was reported by Nayak *et al.* (1982) while studying the causes of abortion in cattle at agricultural university farm and Chiplima farm in Orissa. This was supported subsequently by prevalence of BVDV antibodies in cattle (40.6%) exhibiting a wide range of clinical signs including anorexia, chronic diarrhoea, buccal ulceration, conjunctivitis and abortion in Gujarat (Mukherjee *et al.* 1989). A sero-epidemiological study on sera collected from 17 states initially for rinderpest surveillance further demonstrated wide prevalence of BVDV antibodies in cattle (17%) in most parts of the country (Sudharshana *et al.* 1999). Then an overall BVD seroprevalence rate of 30% was found in cattle (1902) and buffaloes (498) belonging to 14 states during 1999 and 2004 (Sood *et al.* 2007). In a recent study, 37.6% of Indian cattle were found positive for BVDV antibodies indicative of widespread infection (Bhatia *et al.* 2008). However, a systematic sero-epidemiological study, covering cattle of all ages and breeds in various states is lacking.

Though the earlier studies indicated serological evidence of BVDV infection, the conclusive evidence of BVD in Indian cattle was provided only recently by virus isolation and subsequent phylogenetic analysis of BVDV isolates (Mishra *et al.* 2004, 2005). Thirteen BVDV isolates collected from cattle of different geographical regions of India between 2000 and 2002 were genetically typed in 5' UTR and N^{pro} region. The phylogenetic analysis revealed that all the cattle BVDV isolates originating from different herds in eastern,

northern and western India are closely related and belong to BVDV-1b, subtype. Despite long distance between the farms from which the viruses were isolated, higher genetic similarity was observed. This may be due to uncontrolled movement of cattle and use of semen for breeding local and farm cattle in different states of India. It was concluded that BVDV-1 in India originated from historical trade with countries of Europe and America in importing cattle germplasm for cross breeding programme for the last several decades.

Further genetic analysis in N^{pro} and entire gene region coding structural proteins (capsid, E^{ms}, E1, E2) of 3 selected cattle isolates allocated them inside BVDV-1 subtype b cluster to a separate clade with a longer distance (Mishra *et al.* 2006). Some unique mutations were observed in N^{pro} and E2 genes of Indian isolates. In another study, genetic typing of BVDV strains collected during 2001–05 revealed again that BVDV-1b is the absolutely predominant subtype in cattle (Mishra *et al.* 2007). BVDV-1 was identified also in cattle serum used for cultivation of cells in manufacturing veterinary vaccines which indicated the need for mandatory testing of bovine serum and cell lines for BVDV before being used for cell cultivation. The genetic analysis in NS3 gene region also typed the Indian BVDV cattle isolates as BVDV-1b (Mishra *et al.* 2008a).

Recently, BVDV-2 isolated from Indian cattle was characterized at antigenic and genetic level (Behera *et al.* 2011). The genetic analysis of the isolated virus in 5' UTR, N^{pro}, entire structural genes (C, E^{ms}, E1 and E2), nonstructural genes (NS2–3), besides 3' UTR demonstrated that the nucleotide and amino acid sequences showed highest similarity with BVDV-2. The phylogenetic analysis revealed that the cattle isolates and goat BVDV-2 isolates fall into separate clades within BVDV-2a subtype. Antigenic typing with monoclonal antibodies verified the cattle isolates as BVDV-2. In addition, cross-neutralization tests using antisera raised against Indian BVDV strains circulating in ruminants (cattle, sheep, goat and yak) displayed significant antigenic differences only between BVDV-1 and BVDV-2 strains. A more extended study can reveal the genetic diversity of BVDV-1 and BVDV-2 subtypes in Indian cattle. A map showing current status of pestivirus infections in various species of large and small ruminants in India is presented in Fig 1.

When pathogenicity of an Indian BVDV-1b isolate was studied experimentally, the infected calves developed respiratory illness, biphasic pyrexia, diarrhoea, leukopaenia and mild thrombocytopaenia. The viraemia was detected between 3–15 days post infection (dpi) and seroconversion at 15 dpi. At necropsy, glomerulonephritis, interstitial pneumonia, testicular degeneration and mild gastroenteritis were prominent lesions. It was concluded that the isolate was moderately pathogenic and BVDV induced glomerulonephritis was recorded first time in acutely infected



Fig. 1. Prevalence of pestivirus infections among large and small ruminants in India

calves (Galav *et al.* 2007). The study indicated that the disease may be aggravated in field conditions in presence of other concurrent viral and bacterial infections and laboratory diagnosis is essential. Besides, in episodes of abortion and other reproductive disorders encountered in field, the involvement of BVDV should also be suspected along with other infections as about 40% cases of abortion in European and American countries have been incriminated for BVDV.

Pestivirus infections in buffaloes

Serological evidence of BVDV infection in buffaloes was reported from Brazil, Egypt and Pakistan (Akhtar *et al.* 1996, Zaghawa 1998). BVDV antigen and RNA was also detected in young water buffaloes suffering from clinical BVD in Brazil (Craig *et al.* 2008). The sequence analysis revealed closer relationship with reference strains of BVDV-1a and 1b while the buffalo isolate from Australia was typed as BVDV-1c (Becher *et al.* 1997).

A survey for BVDV antibodies in buffaloes showing gastrointestinal and reproductive disorders in Gujarat showed a prevalence rate of 65.9% (Mukherjee *et al.* 1989). However, the mean prevalence rate of BVDV antibodies in buffaloes of 9 states was 23.2% (Sudharsana *et al.* 1999). While a study carried out on buffalo sera collected from slaughter houses in Chennai, Tamil Nadu has shown a prevalence rate of 27.4% (Selvaraj *et al.* 2007), a later study showed a prevalence rate of 30.6% in buffaloes sampled from various states (Bhatia

et al. 2008). However, again systematic sampling and sero-surveillance for BVDV in buffaloes covering all states is yet to be carried out.

Since BVDV-1 was found prevalent in cattle, it was surmised that pestivirus infection might also be present in Indian buffaloes, as persistently infected cattle can transmit the infection during close contact with buffaloes. The detection and genetic typing of BVDV strains from buffaloes was reported recently by Mishra *et al.* (2007a) for the first time. Of 183 blood samples obtained from buffaloes having history of respiratory disorders, diarrhoea and reproductive problems from the states of Odisha, Gujarat, Maharashtra, Uttar Pradesh and Punjab, 4 were found positive for BVDV antigen. On the basis of sequence analysis in 5'UTR, of the 4 buffalo strains, 3 were typed as BVDV-1b and 1 as BVDV-1c. The Indian buffalo strain M 2014 grouped with recently identified Australian cattle strains VR 448-2, VR 449, VR 999 and earlier strains of Bega and Trangie in the BVDV-1c branch. Nucleotide sequence comparison revealed that M-2014 strain showed maximum homology (93.7%) with Bega followed by VR 448-2 (90.8%) and VR 449 (89.1%). The homology with other 3 buffalo BVDV-1b strains was about 80% and with cattle BVDV-1b strains in the range of 79.8 to 81.9%. The homology between buffalo BVDV-1b strains ranged between 98.7 and 99.2%, although they were from distant geographical locations suggesting a common origin. Interestingly, the only earlier report of buffalo A1-isolate originating from Australia, was also typed as BVDV-1c. The results indicated the probable presence of BVDV-1c strains in Indian cattle which remain undetected in the absence of a continuous surveillance programme. More extended genetic analysis of buffalo strains in future cannot exclude the possibilities of finding other BVDV-1 and BVDV-2 subtypes. Moreover, the pathogenicity of BVDV and the precise role of buffaloes in transmitting the infection to cattle is yet unknown and may be subject of future studies as buffaloes contribute equally to a sustainable milk production in India.

Pestivirus infections in sheep and goats

Similar to BVD in cattle, pestivirus infections in small ruminants can cause variety of clinical syndromes including reproductive failure, abortion, still birth, respiratory disease, poor growth rate, diarrhoea, nervous signs and muscular tremor resulting in economic losses (Nettleton *et al.* 1998). Transmission of infection between small ruminants and cattle in both ways was demonstrated though usually it is from cattle to sheep or goats. Historically, all pestivirus isolates from sheep and goats were referred as BDV. But it is now established that sheep and goats can be infected with BVDV-1, BVDV-2 and BDV producing similar clinical signs. Different species of pestiviruses predominate in different countries depend on the close contact among ruminants. Border disease is caused by BVDV-1 in Norway and Sweden (Sandvik *et al.* 2002), BDV in Australia, New Zealand and

Spain (Valdazo-Gonzalez *et al.* 2008), Vilcek *et al.* 1998), BDV and BVDV-1 in U K (Willoughby *et al.* 2006), BDV, BVDV-1 and BVDV-2 in USA (Sullivan *et al.* 1997).

The first serological evidence of pestivirus infection in Indian sheep and goats was published recently (Mishra *et al.* 2009). Pestivirus antibodies were found prevalent in exotic, local and crossbred sheep (Suffolk, Dorset, Soviet Merino, Rambouillet, Corriedale, Awassi, Karakul, Muzzafarnagri, Deccani, Nellore, Nali, Hissar Dale, Patanwadi, Marwari, Chokla, Malpura, Sonadi, Magra, Balangir, Ganjam, Garole, Bannur, Mecheri, Kilakarsal, Madras Red) and goats (Alpine, Saanen, Anglo Nubian, Barbari, Jamnapari, Sirohi, Marwari, Beetal, Chegu, Zalawadi, Kutchi, Black Bengal, Ganjam, Osmanabadi) of 13 states in both the farmers' flocks and organized farms. In some of the pestivirus seropositive sheep and goat flocks, clinical signs such as respiratory illness, pneumonia, enteritis, abortion and poor growth rate were evident.

The genetic and antigenic typing of pestivirus isolates obtained from Indian sheep and goats was reported recently (Yadav *et al.* 2004, Mishra *et al.* 2007b, 2008c). BVDV-1 was detected in an autopsied lamb showing clinical features of Rift Valley fever-like sickness in Tamil Nadu and the isolated virus was antigenically characterized (Yadav *et al.* 2004). Subsequently, BVDV-1 and BVDV-2 were isolated from native Indian goats for the first time (Mishra *et al.* 2007b). The virus isolated from them was confirmed antigenically on the basis of virus neutralization test and reactivity with BVDV-1 and BVDV-2 specific monoclonal antibodies. A comprehensive phylogenetic analysis of goat BVDV-2 isolates in 3 different genomic regions, 5'-untranslated region (5' UTR), E^{ms}-structural coding region and NS5B-nonstructural coding region typed Indian goat isolates as BVDV-2a having close similarity with strains from North America and Europe suggesting its probable introduction through trade (Mishra *et al.* 2007b). It was placed in a separate clade within the 2a branch having unique mutations in E^{ms} and NS5B region. This was only the second report of BVDV-2 in goat species.

BVDV-1 was also isolated from sheep belonging to various geographical regions of India showing clinical signs of pyrexia, abortion, muscular tremor and pneumonia. Recently, the first occurrence of BVDV-2 subtype b in sheep was described in India by extensive antigenic and genetic characterization, providing evidence that this subtype can infect species other than cattle (Mishra *et al.* 2008c). Antigenic characterization using polyclonal and monoclonal antibodies typed the sheep isolate as BVDV-2. When analyzed at genetic level in N^{pro} (N-terminal autoprotease) and entire gene region coding structural proteins, namely capsid (C) protein and envelope proteins-E^{ms} (ribonuclease secreted), E1 and E2 genomic organization was the same for all pestiviruses, the nucleic acid and amino acid sequences showed highest similarity with those of BVDV-2.

Phylogenetic analysis of nucleotide sequences in 5' UTR, N^{pro}, E2, NS3 and NS5B regions placed the sheep isolate in a separate clade within BVDV-2 subtype b. When compared with BVDV-2 reference isolate 890, the sequence homology was 83.7% for C, 84.6% for E^{rms} and E1, and 81.5% for E2. The identification of subtype-b of BVDV-2, for the first time in sheep extends our knowledge on the genetic heterogeneity of pestiviruses.

Recent investigations revealed that clinical signs of border disease observed in sheep and goats were mostly due to BVDV-1 and BVDV-2 infections. However, the surveillance for BDV should be continued in un-sampled and imported flocks of small ruminants in light of the emergence of new BDV genetic subtypes and high mortalities observed in sheep and chamois populations in several countries of Europe. The identification of BVDV-2 earlier in goats and now in sheep reiterates the need for intensive surveillance in cattle in India.

Pestivirus infections in yaks

In India, domesticated yaks [*Bos* (now *poephagus*) *grunniens*] are reared mostly in mid Himalayan zone in the states of Jammu and Kashmir, Arunachal Pradesh, Sikkim, Himachal Pradesh and high hills of Uttarakhand for their meat, milk, wool, hair and hide besides being used for transport in hilly terrains. Since cattle are widely infected by BVDV in India, a study was undertaken to investigate pestivirus infections in yaks, which are related to cattle. Pestivirus infection was detected and BVDV was isolated from 3 Himalayan yaks having a flock history of abortion (Mishra *et al.* 2008b). The 5' UTR, N^{pro} and E2 regions were sequenced and used for genetic typing. Phylogenetic analysis revealed that pestiviruses detected in 3 Himalayan yaks were similar genetically, belonging to BVDV-1c (Mishra *et al.* 2008b). Similar viruses at the genetic level were detected in buffalo and deer from New Zealand (Becher *et al.* 1997). BVDV-1c subtype isolates were also detected in cattle originating from Germany (Becher *et al.* 1997) and Mozambique (Vilcek *et al.* 2001) as well as Australia, where this pestivirus subtype is absolutely predominant (Mahony *et al.* 2005). Antigenic characterisation of yak pestivirus also confirmed the typing as BVDV-1. This is the first report in the world on the identification and isolation of BVDV-1 in yaks. As cross breeding of domesticated yaks and hill cattle has been practiced for several years for increased milk yield and draught power, the historical and present contact between yaks and cattle is presumable. On the other hand, it is possible that the infection might have been transmitted from Tibet of China, Nepal or Bhutan which have traditional trade links with yak breeders in India.

Conclusions and future directions

The serological and virological studies indicated widespread ruminant pestivirus infections in India. The molecular characterization of pestiviruses revealed genetic

and antigenic heterogeneity of BVDV in ruminants while BDV remains exotic. The genetic diversity of pestiviruses identified in various species of ruminants included BVDV-1b, BVDV-1c, BVDV-2a and BVDV-2b. A more extended study can unravel presence of other subtypes or species of pestiviruses in future. Monitoring and genetic characterization of pestivirus isolates from ruminants further can improve our understanding of virus epizootiology and evolutionary history. Though pestiviruses do not cause human infections, inter animal species transmission is common and due to close relationship with Hepatitis C virus in humans BVDV has often been used as a surrogate model for studying antiviral drugs and unraveling the mysteries of persistent infection pathogenesis. The pronounced antigenic differences between BVDV-1 and BVDV-2 isolates is important for diagnosis and control as prior exposure with BVDV-1, either through infection or through vaccination, does not protect against infection with BVDV-2. The occurrence of BVDV specific antibodies and isolation of virus from wild animals has lead to the speculation that BVDV harboured in free ranging animals may be a hidden reservoir for transmission to cattle and sheep. This is important for BVD control programmes since domestic and free-living animals co-graze on common pastures. However, more evidence has been provided regarding transmission of BVDV from farm animals to free living animals. Although reported in other countries, the status of pestivirus infection in wild ruminants in India is not yet known. Similarly, domestic pigs can be infected with BVDV producing clinical signs similar to low or moderately pathogenic CSFV strains. Therefore, differentiation of ruminant pestiviruses from CSFV is of paramount importance also for CSF control programmes. A concerted effort with regard to the genetic and antigenic typing as well as surveillance for pestiviruses in ruminants and domestic pigs should provide meaningful answers for control of both BVD and CSF in India.

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