



Prevalence of bovine respiratory viruses in cattle calves in Kangra district of Himachal Pradesh

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The livestock sector plays a crucial role in enhancing the income of farmers and contributes significantly to the overall Gross Domestic Product (GDP) of our nation. Dairy farming serves more than just a means for household consumption; it is a source of additional income for farmers, particularly those who are marginal. One of the key challenges faced by the dairy industry are the infectious diseases of bovines that lead to heavy morbidity and mortality, thereby causing huge economic loss. The potential of calves as the future of the dairy herd is often ignored since they do not yield any immediate economic return to the farmers. Pneumonia is the second-leading cause of mortality in calves after enteritis, causing up to 50 per cent of mortality (Veena and Sumathi 2011).

Bovine respiratory disease (BRD) is a complex of several infectious diseases caused by viral and bacterial agents and other factors like stress, transportation, etc. that affects bovines all around the globe and leads to huge economic losses (Van Leenen *et al.* 2020, Singh *et al.* 2023). The main respiratory viruses involved in BRD are bovine respiratory syncytial virus (BRSV), bovine herpes virus type 1 (BoHV-1), bovine parainfluenza virus 3 (BPI3), and bovine viral diarrhoea virus (BVDV) (Fulton 2020, Pardon *et al.* 2020). BRSV is a member of the family *Pneumoviridae*, and the genus *Orthopneumovirus*. The most common clinical manifestations of BRSV infection include fever up to 40 °C, anorexia, increased respiratory rate, coughing, and mucopurulent nasal discharge (Makoschey and Berge 2021). Similar but milder signs are shown by BPI3, which is a member of the family *Paramyxoviridae*, subfamily *Orthoparamyxovirinae*, and genus *Respirovirus*. BVDV is categorised under family *Flaviviridae* and genus *Pestivirus* and is primarily associated with diseases of the gastrointestinal system, but it can also affect the reproductive and respiratory systems

(Walz *et al.* 2020). The clinical signs of BVDV are usually very mild or absent and mainly include fever and coughing (Ridpath *et al.* 2020). BVDV is known to potentiate the BRD through synergism and immunosuppression (Ridpath 2010, McGill *et al.* 2016). BoHV-1 belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, and genus *Varicellovirus*. The virus causes huge economic losses, representing a wide spectrum of clinical manifestations, including periods of latency. The clinical symptoms include fever, conjunctivitis, lack of appetite or anorexia, serous nasal discharge, balanoposthitis, pustular vulvovaginitis, and abortions (Biswas *et al.* 2013, Jones 2019).

All the above-mentioned viral infections can be diagnosed using several tests like polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), enzyme-linked immunosorbent assay (ELISA), fluorescent antibody test (FAT), and serum neutralisation test (SNT) (Biswas *et al.* 2013, Veljović *et al.* 2016, Hanon *et al.* 2017, Kamdi *et al.* 2020, Makoschey and Berge 2021, İnce *et al.* 2022, Zhang *et al.* 2022). Virus isolation is considered a gold standard test for diagnosis but is an expensive and time-consuming process that requires specific laboratory facilities for handling the cell cultures (Fulton and Confer 2012). On the other hand, results with ELISA can be obtained quickly and are more efficient in differential diagnosis as well as for detecting concurrent infection of multiple viruses, thereby making it the test of choice for the detection of antigens and antibodies (Avci *et al.* 2014). The objective of the present study was to detect different respiratory viruses in the lungs of dead cattle calves using a commercially available multiscreen antigen ELISA kit and also to find out the occurrence/ co-occurrence of these viruses in cattle calves of Palampur region of Kangra district in Himachal Pradesh, India.

Collection of samples: A total of 30 cattle calves under twelve months of age received from the organised and unorganised dairy farms in and around Palampur region of Kangra district were necropsied at the Department of Veterinary Pathology, Dr. G. C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh

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Krishi Vishvavidyalaya, Palampur between June 2021 and June 2022. At necropsy, tissue samples of lungs and nasal turbinates were collected and stored at -20°C until processing.

Processing of samples: The collected tissue samples were minced using sterile scissors and forceps. Approximately 1g of minced tissue sample was collected in a homogenizer tube, and 2 mL of working lysis buffer solution (5X lysis buffer diluted in double distilled water in 1:5) was added, followed by homogenization of the samples using a tissue homogenizer (FastPrep-24TM Classic Instrument, MP Biomedicals). Homogenised tissues were then centrifuged at 3000 rpm for 10 min, followed by the collection of the supernatant for using as a sample in the ELISA.

Protocol for sandwich ELISA: A commercially available multiscreen antigen ELISA kit (Bio-X Diagnostics, Rochefort, Belgium, Cat. No.: BIO K, 340/5) was used for the detection of respiratory viruses such as bovine herpes virus-1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), and bovine parainfluenza virus type 3 (BPI3). The standard protocol was followed as per the instructions given by the manufacturer. Afterwards, the optical density was measured using a microplate spectrophotometer (Thermo Fisher Scientific) at 450 nm.

Interpretation of ELISA results: The results of the ELISA were interpreted as per the formula (given below) and reference values provided with the kit. Formula for calculation of Val(ue) in percentage is given as follows.

$$\text{Val(ue)} = \frac{\Delta \text{OD Sample} \times 100}{\Delta \text{OD positive}}$$

In this study, a total of 30 necropsied samples were analysed for viral antigens. Overall, 7/30 (23.33%) samples were found to be negative for all four viruses tested. BoHV-1 and BRSV were the most prevalent viruses and were reported in 17/30 (56.67%) samples. BPI3 was detected in 14/30 (46.67%) samples, whereas BVDV was detected in 8/30 (26.67%) samples (Table 1). Mixed infection with different viruses was observed in cattle calves. A total of 5/30 (16.67%) samples were tested positive for all four tested viruses. Also, 4/30 (13.33%) samples were tested positive concurrently for the three viruses. Furthermore, 10/30 (33.33%) samples had two concurrent viruses, and only 4/30 (13.33%) samples had only one virus. The age-wise prevalence of different viruses in cattle calves is depicted in Table 1.

Viral agents are the primary cause of respiratory disease in bovine calves. They lower the local immunity, damage the respiratory tract epithelium, and hamper the mucociliary

clearance of the respiratory tract, thereby paving the way for the colonisation of opportunistic secondary bacteria (Cusack *et al.* 2008, Horwood *et al.* 2014, Murray *et al.* 2017). In the present study, we detected a high presence of respiratory viruses in cattle calves under one year of age. Multiple infections with different viruses were evident in a large number of dead calves, indicating complex dynamics involved in the pathogenesis of respiratory diseases.

BoHV-1 was found to be 56.67 per cent in the present study. Similarly, Gangil *et al.* (2020) used sandwich ELISA and reported the prevalence of BHV to be 1.8 per cent in the nasal samples of bovines. Singh *et al.* (2013) reported 11.1 per cent prevalence of BoHV-1 in nasal samples of bovine calves in Uttar Pradesh. Majumdar *et al.* (2015) and Patil *et al.* (2017) also reported the serological and virological evidence of BoHV-1 infection in bovines from different states of India. The BoHV-1 has a tendency to undergo latency in the ganglions and is difficult to detect because of the stoppage of shedding. But our study revealed a high prevalence of BoHV-1 infection, which suggests the reactivation of the virus under stressful and immunocompromised conditions in bovine calves (OIE 2008).

Similar to present findings, the high prevalence of BRSV in bovines has been reported by many researchers. İnce *et al.* (2022) reported 58.48 per cent seroprevalence of BRSV using Ab-ELISA. Avci *et al.* (2014) also reported the 16.6 per cent positivity of BRSV using direct ELISA. Yazici *et al.* (2020) reported the presence of BRSV in all three cattle tested using multiscreen sandwich ELISA.

Bovine parainfluenza infections are known to be one of the primary virological agents involved in BRD and predispose the animals to secondary bacterial infections. BPI3 has been highly reported in bovines from multiple studies across the world. Similar to current findings, Solís-Calderón *et al.* (2007) reported the high seroprevalence of BPI3 at 85.6 per cent in the samples collected from multiple cattle farms. Similarly, Noori *et al.* (2014) recorded a high prevalence of BPI3 infection in 20/100 (20%) pneumonic lung tissue samples using sandwich ELISA. However, Gangil *et al.* (2020) found a low prevalence of BPI3 at 5.4 per cent using sandwich ELISA.

In the present study, BVDV was least reported. Similar to present findings, İnce and Ayaz (2023) reported an animal-level seroprevalence of 48.37 per cent using an indirect ELISA kit. However, Zhou *et al.* (2023) reported a low prevalence of 4.89 per cent in the lung tissues of

Table 1. Age-wise distribution of respiratory viruses in cattle calves

Age	BoHV-1	Per cent	BVDV	Per cent	BRSV	Per cent	BPI3	Per cent
<1 month (n=2)	1	3.33	0	0	0	0	0	0
1-3 months (n=4)	2	6.67	0	0	2	6.67	3	10
3-12 months (n=24)	14	46.67	8	26.67	15	50	11	36.67
Total (n=30)	17	56.67	8	26.67	17	56.67	14	46.67

Where, n = no of animals.

cattle. Maher *et al.* (2023) reported a positivity rate of 31.5 per cent for BVDV. Chicoski *et al.* (2023) reported a seropositivity rate of 51.8 per cent for BVDV in Brazilian cattle.

Mixed virus infection was the most common finding in the current study, and similar findings has been reported by Zhou *et al.* (2023). They reported BoHV-1 in 11.20 per cent (55/491), BRSV in 5.50 per cent (27/491), BVDV in 4.89 per cent (24/491) and BPI3 in 4.28 per cent (21/491) cases, with a mixed infection rate of 16.29 per cent (80/491). Chicoski *et al.* (2023) also reported multiple virus infections with an overall positivity rate of BoHV-1 at 92.1 per cent (1,154/1,253), BPI-3 at 86.6 per cent (1,100/1,270), BRSV at 77.1 per cent (959/1,244), and BVDV at 51.8 per cent (656/1,266) cases.

In the present study, high prevalence of different respiratory viruses was found, which might be attributed to the fact that most of the animals necropsied were from farms where animals were housed, watered, and fed together, which provides sufficient opportunities for close contact and subsequent transmission of these viruses among the animals.

In conclusion, the findings of the present study confirm the presence of antigens of four respiratory viruses' viz. BoHV-1, BVDV, BRSV and BPI3 in cattle calves of Himachal Pradesh. The presence of multiple viral infections in cattle calves underscores the complex viral dynamics associated with bovine respiratory diseases. However, further studies are required to understand the complex dynamics of viral infections that would contribute in providing insights for prevention and control of viral infections among bovine calves.

SUMMARY

Respiratory diseases causing pneumonia are the 2nd leading cause of mortality in bovine calves and are primarily caused by viral pathogens. The present study was undertaken in cattle calves under twelve months of age for detecting four respiratory viruses, viz. bovine herpes virus-1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), and bovine parainfluenza virus type 3 (BPI3), using a commercially available multiscreen antigen ELISA kit. A total of 30 cattle calves necropsied at the Department of Veterinary Pathology, DGCN COVAS, CSKHPKV, Palampur, from June 2021 to June 2022 were screened. The samples of lung tissues and nasal turbinates were collected and stored at -20°C until further processing. The collected tissue samples were homogenised and processed as per the standard protocol. Among the 30 necropsied samples, BoHV-1 and BRSV were detected in 17/30 (56.67%) samples, BPI3 was detected in 14/30 (46.67%) samples, and BVDV was detected in 8/30 (26.67%) samples. 5/30 (16.67%) samples were found positive for all four viruses. Moreover, three viruses were concurrently detected in 4/30 samples (13.33%), and two viruses were present in 10/30

samples (33.33%). Additionally, a single virus was detected in 4/30 samples (13.33%). In conclusion, the present investigation reveals the substantial presence of respiratory viruses in the respiratory tract of cattle calves. The result indicates a complex pattern of co-infections of different viruses, emphasising the need for effective surveillance and management strategies to address the diverse viral dynamics affecting bovine respiratory health.

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