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Original Article

Investigating bovine coronavirus in Indian calves: Incidence, molecular evidence, and pathological role in bovine respiratory disease complex (BRDC)

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Abstract

Background: Bovine coronavirus (BCoV) and bacterial pathogens contribute to bovine respiratory disease complex (BRDC) in young calves. However, the role of BCoV in BRDC occurrence and site-specific respiratory pathology in India remains poorly explored. **Aims:** This study aimed to assess BCoV prevalence in BRDC cases. **Methods:** We investigated 406 weaner calves (166 cattle, 240 buffaloes) up to ≤1 year, with respiratory distress and pulmonary lesions. **Results:** BRDC cases exhibited 0.98% BCoV occurrence, confirmed by partial *N* gene amplification (172 bp) via RT-PCR and immunohistochemistry (IHC). Grossly, 4 BCoV positive cases showed variable degrees of consolidation of cranioventral lobes and non-collapsed caudodorsal lobes, associated with congestion and emphysema. Microscopically, the inflated sites of the lung tissue sections showed hallmark changes of interstitial pneumonia characterized by moderate infiltration with lymphocytes and increased numbers of fibroblasts in the interalveolar septa and the stroma of bronchioles and bronchi. In concomitant *Pasteurella multocida* infected cases, cranioventral lobes exhibited suppurative bronchopneumonia with neutrophilic exudate. The above lesions were well colocalized with BCoV antigen in the epithelial cells and in the debris of the lumen of the alveoli and the bronchi/bronchioles. The sequence comparison of the 172 bp amplicon with the published BCoV *N* gene showed close relatedness. **Conclusion:** The present study implicated BCoV as a component of BRDC in India that should be considered in the diagnosis of BRDC outbreaks.

Key words: BCoV, Bovine respiratory disease complex, IHC, Pneumonia, RT-PCR

Introduction

Bovine coronavirus (BCoV) of the genus *Betacoronavirus* belongs to the subgroup 2a of the *Coronaviridae* family under the Nidovirales order. It is an enveloped, single-stranded, non-segmented positive-sense RNA virus with a genome size of 32 kb. The other members of the family include HCoV-OC43, SARS-CoV, and MERS-CoV, which cause respiratory pathology in humans (Fehr and Perlman, 2015). BCoV is involved in the bovine respiratory disease complex (BRDC) as well as shipping fever in feedlot and dairy cattle, and causes significant economic losses (Hasoksuz *et al*., 2002; Ellis, 2019; Franzo *et al*., 2020; Hodnik *et al*., 2020; Vlasova and Saif, 2021). BCoV with pneumoenteric potential (Saif, 2010) induces three distinct clinical syndromes in cattle namely respiratory infection, calf diarrhea (Clark, 1993), and winter dysentery in adults (Traven *et al*., 2001).

After first described as a cause of calf enteritis by Mebus (1973), BCoV and its newer strains (Boileau and Kapil, 2010) have been increasingly implicated in the BRDC in association with other viruses, bacteria, and/or *Mycoplasma* spp. leading to reduced growth and production performances in feedlot cattle (Storz *et al*., 2000; Cho *et al*., 2001b; Gagea *et al*., 2006; Vlasova and Saif, 2021).

In India, prevalence of the BCoV has been reported to range from 1.76% to 27.3% from clinical cases of diarrhea or fecal samples in cattle/buffaloes from many States (Rai *et al*., 2011; Dash *et al*., 2012; Hansa *et al*., 2012; Suresh *et al*., 2012; Singh *et al*., 2019). However, despite being a recognized respiratory pathogen in bovines with documented mortality, morbidity, and

predisposition to secondary bacterial infections (Storz *et al*., 2000), no systemic studies from the country have investigated the role of BCoV in pneumonia development. Therefore, the present study was conducted in pneumonic lungs of fallen bovine calves (≤1 year) to describe the incidence and immunopathology caused by BCoV alone, or in combination with other bacteria by employing RT-PCR, immunohistochemistry (IHC), and pathomorphological techniques.

Materials and Methods

Tissue samples

A total of 406 (166 cattle and 240 buffaloes) calves ≤1 year of age, either sex, were selected for the study based on a history of respiratory signs and pulmonary lesions at necropsy. The samples were collected at the Postmortem Facility of the Division of Pathology, Indian Veterinary Research Institute, Bareilly, India (n=119), Municipality Abattoirs at Moradabad (n=33), Delhi (n=70), and Chandigarh (n=111); and also, from Private Dairy Farms (morbid tissues) in districts of Maharashtra (n=42), Andhra Pradesh (n=19), and Madhya Pradesh (n=12). The period of sample collection spanned from March 2016 to June 2018. Representative thin tissue pieces (5 × 10 × 20 mm dimension) from both upper and lower respiratory tracts of pneumonic lungs along with bronchial/mediastinal lymph nodes were collected in 10% neutral buffered formalin (NBF) for pathomorphology and immunohistochemistry (IHC) in wide mouth containers. The adjoining tissue samples from the lesion sites were also collected aseptically in sterile and screw-capped polypropylene vials containing RNA*later* (Thermo Fisher Scientific, USA) solution for molecular pathology work. All the tissue samples were properly labeled and stored at room temperature (RT) and -20°C till further processing.

Processing of tissues for histopathology

After proper fixation of tissues, 2-3 mm thin tissue pieces were cut, trimmed, and run through water, graded alcohol, xylene, xylene plus benzene mixture, benzene, and then finally in melted paraffin, using Tissue Processor (York Scientific Instruments, Pvt., Ltd., India). The paraffin-embedded tissues were then made into blocks in the Tissue Embedding Station (Unimeditreck Pvt., Ltd., India) and cut into 5-6 µm thick sections by using Microtome (MicroGmbH, Germany). The ribbons of paraffin sections were relaxed in a floatation water bath set at 55°C. Thin paraffin tissue sections were lifted out on clean glass slides and stained with hematoxylin and eosin (H&E) stains as per the standard procedures (Luna, 1972).

Reverse transcriptase polymerase chain reaction (RT-PCR)

TRIzol® Reagent (Thermo Fisher Scientific, USA) was used to extract total RNA from tissues of lungs and associated lymph nodes following the manufacture's protocol. The quantification of all extracted RNA samples was done by NanoVue plus spectrophotometer (Thermo Fisher Scientific, USA), and the purity of RNA was checked by $A_{260/280}$ and $A_{260/230}$ ratios. The amplification of partial fragment of the Nucleocapsid (*N*) gene of BCoV was carried out *via* reverse transcriptase PCR. High-capacity cDNA reverse transcription kits (Applied Biosystems, USA) were used to synthesize cDNA from total RNA. The synthesized cDNA was stored at -20°C until further use. In the PCR assay, the primer sequence (5´-3´), BCoV; F-TGA CGA GCC CCA GAA GGA TGT and BCoV; R-GAC CAC GCT GAC GCT GTG GTT with a predicted amplicon length of 173 bp was used (Singh *et al*., 2019). Briefly, PCR reaction was carried out in 0.2 ml PCR tubes containing a reaction mixture of 12.5 µL of Green Dream Taq Master mix (Genetix, Belgium), 0.5 µL of each primer (10 µM), 2 µL of cDNA (1500 ng/µL), and 9.5 µL of nuclease-free water. The amplification reaction was carried out in a thermocycler with the following cycling conditions; initial denaturation of 95°C for 5 min, followed by 35 cycles [denaturation (94°C, 30 s); annealing (58°C, 15 s), extension (72°C, 20 s)], and a final extension at 72°C for 8 min. An aliquot of 5 µL PCR product was resolved by agarose gel electrophoresis (1% w/v) at 95 V for 1 h in TAE buffer with 0.5 µg/ml ethidium bromide, and viewed under UV transilluminator (Geldoc, USA).

Sequencing and phylogenetic analysis

The amplified PCR products were purified by using the QIAEXII Gel extraction kit (QIAGEN, The Netherlands) as per the manufacturer's instructions and stored at -20°C till use. The purified PCR products were sequenced using pJET 1.2 forward (TGA CGA GCC CCA GAA GGA TGT) and reverse (GAC CAC GCT GAC GCT GTG GTT) sequencing primers (Eurofins Genomics Pvt., Ltd., Bengaluru, India). The nucleotide sequences of the BCoV *N* gene were initially verified through BLAST analysis (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and homologous sequences were retrieved from NCBI database for further analysis using 'EditSeq' program of 'Lasergene' version 6 (DNASTAR Inc., USA). ClustalW method of the 'MEGA6' program was used to align nucleotide sequences separately. Multiple sequence alignment was carried out using ClustalW program of MEGA v.6 software (Tamura *et al*., 2013). A phylogenetic tree was constructed based on the target genes using MEGA v.6 software with the Neighbour-joining method and p-distance as a nucleotide substitution model.

Immunohistochemistry (IHC)

The paraffin tissue sections with 4-5-micron thickness were taken on the aminopropyltriethoxysilane (APES) coated slides and incubated at 37°C and then rinsed thrice in 1X phosphate buffer saline (PBS) (pH 7.2) for 5 min. The paraffin sections were deparaffinized twice in xylene (10 min each) and rehydrated through descending grades of alcohol to distilled water.

Hydrogen peroxide in methanol (0.3%) was used to quench the endogenous peroxides for 20 min. Antigen retrieval was performed in a Coplin jar containing 0.01 M citrate buffer (pH 6.0) for 15 min by subjecting slides to microwave irradiation. Normal goat serum (5%) (200-400 µL) (Sigma Chemicals, USA) in PBS was used to block non-specific binding sites for 30 min at RT incubation. The slides were rinsed with PBS thrice for 5 min after each treatment with reagents. For immunostaining, an antibody was titrated for optimum concentration and sections were covered with 100-150 µL of primary monoclonal antibodies (1:100, mouse monoclonal IgG2a coronavirus capsid antigen, Santa Cruz Biotechnology, USA) in PBS containing 1% BSA (Sigma Chemicals, USA). The negative controls were covered with 1% BSA in PBS only. The slides were washed with PBS with continuous stirring. After wiping around the sections carefully, HRP-conjugated Goat anti-mouse IgGs secondary antibodies (Invitrogen, India) diluted at 1:500 were applied to cover the moist section and incubated for 1 h followed by PBS washing. The sections were covered with 3, 3-diaminobenzidine (DAB, Sigma Chemicals, USA) as a substrate, which gave a dark brown color product. DAB (3,3′-Diaminobenzidine) substrate solution was prepared by adding 1 drop of DAB chromogen to 1 ml of DAB buffer. The reaction was terminated before generalized background staining appeared in the negative controls by rinsing in double distilled water. Sections were then counter-stained for 1-2 min with Mayer's hematoxylin. After dehydrating in ascending grades of alcohol, sections were mounted with coverslips using DPX mountant.

Results

In the present study, BCoV was detected in four out of 406 calves (0.98%) containing 2 buffalo and 2 cattle calves. Two BCoV-positive cases were coinfected with *Pasteurella multocida*. The detection was based on the BCoV *N* gene-specific amplification, yielding a product size of 172 bp (Fig. 1) as well as on the localization of the BCoV capsid antigen in the tissue sections as strong brown color positive signals (Fig. 1). Ante-mortem history of these cases revealed signs of fever, dyspnoea, and nasal mucopurulent discharge (2 animals). Additionally, bilateral conjunctivitis was reported in three of the cases. All the 4 BCoV positive cases had no diarrheic history. A gross examination of the lungs showed multifocal and coalescing dark red consolidations (8-10 cm diameter) in the cranio-ventral lobes accompanied by moderate to severe emphysematous and focal hemorrhagic lesions in diaphragmatic lobes. In one of the cases, the multifocal to diffuse areas of consolidations were seen in the whole lung (Fig. 2). In 2 cases, yellowish dull mat-like fibrin was observed on the parietal pleura, in association with pulmonary lesions, while in one case, lungs had extensive plural hemorrhages and cranio-ventral consolidations accompanied with emphysema. Moreover, the tracheal mucosa was invariably involved in all the cases showing diffuse congestion and the presence of whitish frothy exudates in the respiratory airways. Tracheobronchial and mediastinal lymph nodes were edematously swollen.

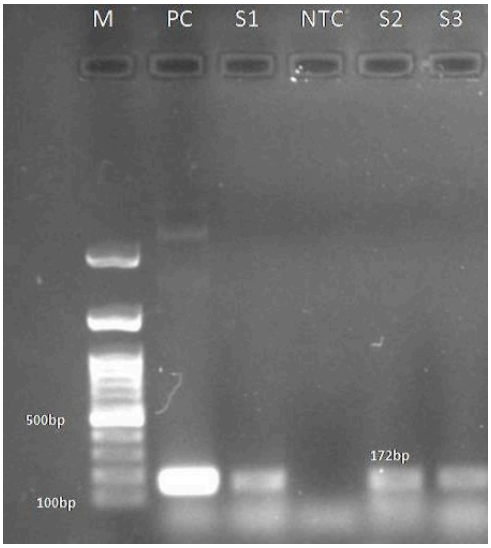

Fig. 1: Ethidium bromide stained agarose gel electrophoresis (2% agarose): positive samples (S1, S2, S3) for BCoV (172 bp) with negative control (NTC), positive control (PC), 100 bp DNA ladder (M)

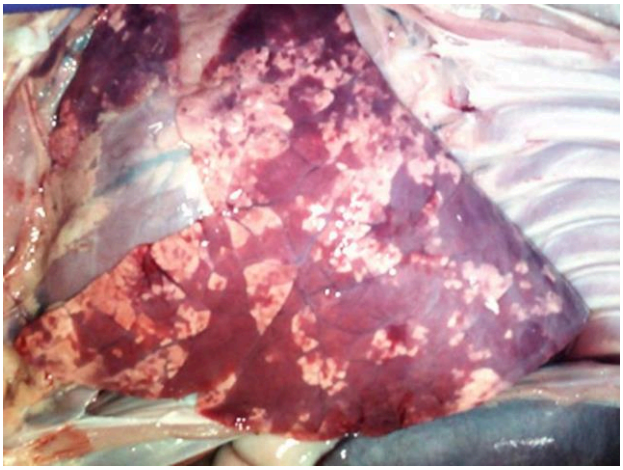

Fig. 2: Dark brown areas of multifocal to diffuse areas of consolidation with multiple areas of emphysema

Microscopic examination of H&E sections in two cases of BCoV infection revealed thickening of interalveolar septa with infiltration of varying numbers of neutrophils, fibroblasts, and mononuclear cells and hyperplasia/hypertrophy of Type 2 pneumocytes (Figs. 3 and 4). These changes were accompanied by hyperemia and edema. Whereas, in the other two cases of the BCoV, wherein the *P. multocida* was also a concomitant pathogen, the lung sections had abundant presence of neutrophils, few mononuclear cells, and degenerated/ necrotic epithelial cells within the lumen of the airways and alveolar spaces (Fig. 5). The airways mucosal lining cells were degenerated and desquamated at places. The

interstitial connective tissue around the bronchioles infiltrated with inflammatory cells and the lumen was plugged with purulent exudates. The adjoining tissue parenchyma had changes of emphysema and atelectasis.

The duplicate tissue sections of all four RT-PCR positive cases were also got confirmed by immunostaining using BCoV specific antibody rose in mice. The BCoV capsid antigen was detected as a dark brown color in the bronchiolar and alveolar epithelium of the lungs (Fig. 6). To confirm the 172 bp amplified product of BCoV, the sequencing and phylogenetic analysis was carried out. Phylogenetic analysis of the partial *N* gene sequences of three amplicons revealed that the sequence of all the PCR amplicons was homologous to each other, was closely related to the sequences of the isolates reported from the USA and Thailand, and was distantly related to those of the isolates reported from France, Korea and Camel Coronavirus strain from Saudi Arabia (Figs. 7A and B).

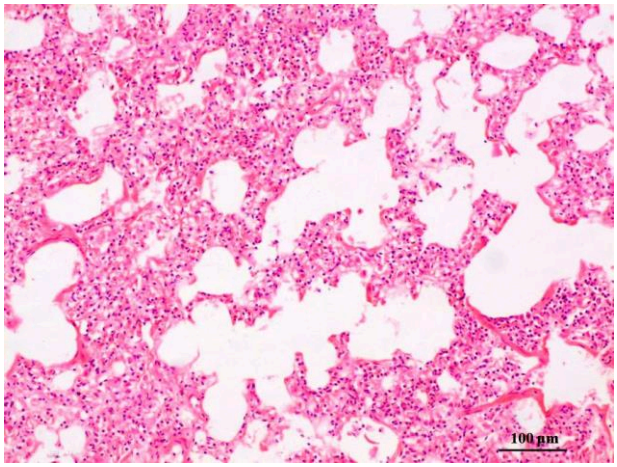

Fig. 3: Photomicrograph of the lung after hematoxylin and eosin staining showing diffusely enlarged alveolar septae due to capillary engorgement, fibroblast proliferation, macrophage infiltration, and type II pneumocyte hyperplasia (scale bar, 100 µm)

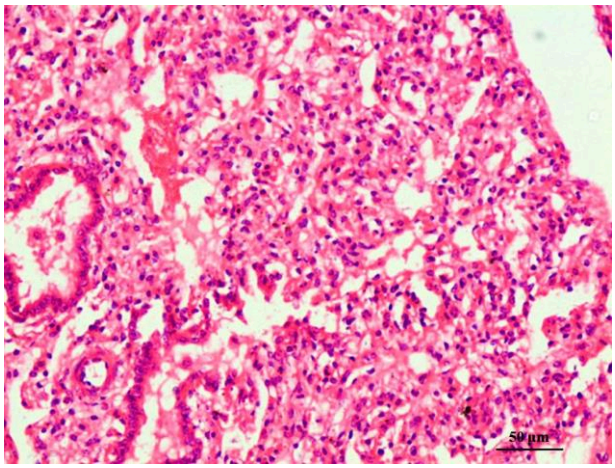

Fig. 4: Photomicrograph of the lung after hematoxylin and eosin staining showing diffusely thickened alveolar septa by edema, infiltration of MNCs, and type II pneumocyte hyperplasia (scale bar, 50 µm)

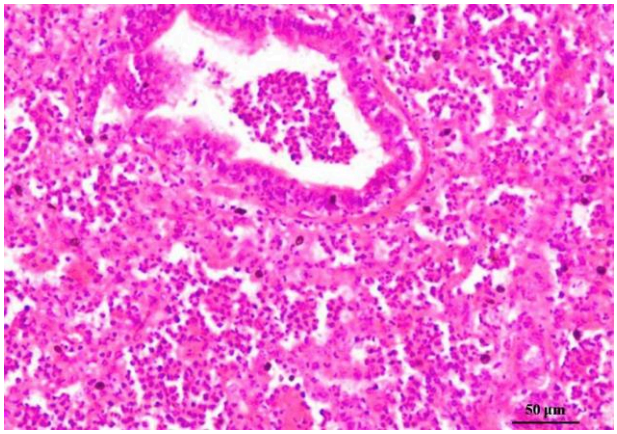

Fig. 5: Photomicrograph of the lung after hematoxylin and eosin staining showing abundant neutrophils in the bronchiolar and alveolar lumen (scale bar, 200 µm)

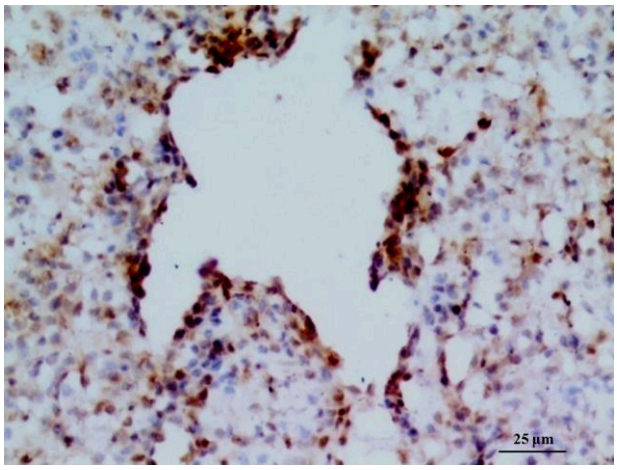

Fig. 6: BCoV-positive immuno-signals were seen in the interstitial cells and respiratory bronchiolar lining cells (IHC, DAB, scale bar, 25 µm)

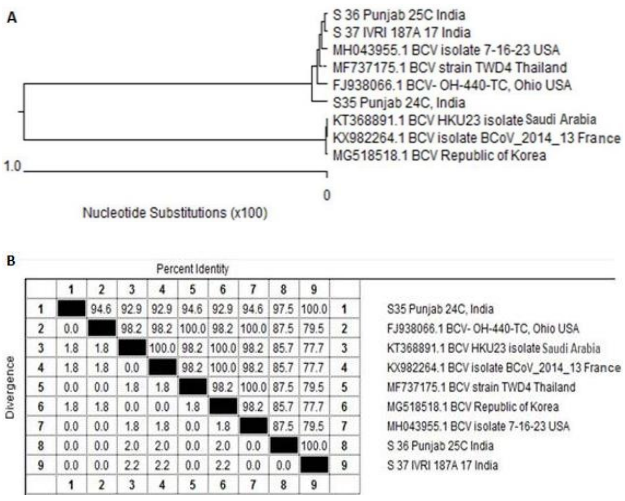

Fig. 7: Neighbor-Joining phylogenetic tree, the bar at the bottom of the figure denotes distance (**A**); sequence divergence (lower left of the table) and percent identity (upper right of the table) **(B)** of 172-nt BCoV *N* gene sequences 03 from the current study (S35, S36, and S37) and 06 sequences retrieved from NCBI Gen bank

Discussion

The BRDC is a polymicrobial and multifactorial disease condition of significant economic concern to the farming industry worldwide (Griffin, 2010; Ellis, 2019; Hodnik *et al*., 2020). Approximately 60-90% of the morbidity and mortality in feedlots has been attributed to BRDC (Baptista *et al*., 2017). Several reports on BRDC worldwide have found concomitant involvement of an array of bacterial and viral pathogens in the causation of pneumonia in bovines (Ames, 1997; Radostits *et al*., 2007; Griffin, 2010; Bednarek *et al*., 2012; Baptista *et al*., 2017; Murray *et al*., 2017). In India, numerous studies have indicated importance of bacterial pathogens in the causation of bovine pneumonia, and *Pasteurella* spp., considered to be the most frequently detected organism (Goswami *et al*., 2015; Choudhary *et al*., 2019). Although respiratory viruses like-BCoV, BRSV, parainfluenza viruses, and herpes viruses are important pathogens of bovine pneumonia, those also pave the way for the growth and development of other bacterial pathogens (Lojkic *et al*., 2015; Kamdi *et al*., 2020). The role of BCoV in BRDC has been earlier reported to be controversial and was not very clear, however recently its role has been reviewed and discussed to be significant with the evidence re-addressed implicating BCoV as an important respiratory pathogen that could lead to secondary infections, and emphasized the need of more explorative studies on BCoV to be conducted with regards to mixed infections and BRDC (Ellis, 2019). Furthermore, there are no reports of BCoV as a cause of pneumonia in young cattle and buffaloes in our country, albeit it is widely detected in diarrheic cases (Hansa *et al*., 2012; Singh *et al*., 2020). Such kind of data on viral and bacterial synergism in the causation of bovine pneumonia is meager in the Indian context for a better understanding of the BRDC for the development of diagnostic and therapeutic measures. Therefore, the present study describes the incidence and pathology of BCoV in young bovines that died of respiratory signs and pneumonia.

The present study could figure out a 0.98% (4/406) incidence of BCoV from the morbid lungs of young bovines. Similar studies had also reported a 3.7% incidence of BCoV in pneumonic calves from Ireland (Murray *et al*., 2017), and incriminated the same in calves from Italy (Decaro *et al*., 2008). The BRDC outbreaks in calves due to BCoV and *P. multocida* (Storz *et al*., 2000) have also been reported in the USA. Our study also observed 2 cases of concomitant infection of BCoV and *P. multocida* in the pneumonic lungs of calves. It signifies the potential role of BCoV in causing bovine pneumonia in our country as well. The anamnesis of respiratory signs - like fever, mucopurulent nasal discharge, and conjunctivitis corroborated well with the findings described earlier in the BCoV-affected animals (Hick *et al*., 2012; Kalkanov *et al*., 2019). Gross lesions of bronchopneumonia and interstitial pneumonia were also in line with the earlier description by Saif (2010), Hick *et al*. (2012) and Murray *et al*. (2017). The BCoV virus multiplies in the respiratory epithelium, and causes damage to the respiratory escalator system, to favor the growth of opportunistic pathogens to produce sever pneumonia (Boileau and Kapil, 2010).

The interstitial pneumonia with hyperplasia of type II pneumocyte (2 cases) in the present study has similarly been described earlier (Park *et al*., 2007). Hyperplasia of type II pneumocytes indicates extensive damage to Type I cells as type II cells act as progenitors for the Type I cells in the lung (Fehrenbach, 2001). Whereas, in 2 cases suppurative bronchopneumonia characterized by the presence of neutrophilic and necrotic exudate in the airways, alveolar spaces, and their walls were in agreement with the findings of Saif (2010). It may be due to a superimposed bacterial infection after the BCoV infection. Kapil *et al*. (1991) and Hansa *et al*. (2012) described the extensive pleural and pulmonary hemorrhages in a diarrheic calf affected with BCoV, which is similar to the findings of the present study in one case.

As the virus has been shown to replicate more specifically in the respiratory tract epithelium of calves, immunosignals for BCoV antigens in the present study were well supported by immunohistochemistry. The same was previously described by Mebus *et al*. (1975) with the help of an immunofluorescence assay.

Further confirmation of BCoV infection in tissues was achieved by partial *N* gene nucleotide sequencing. The phylogenetic analyses showed close relatedness of our sequences with those of the isolates reported from the USA and Thailand; and were distantly related to those of the isolates reported from Riyadh, France, and Korea. Similarly, Lojkic *et al*. (2015) described the partial *N* gene-based BCoV phylogenetic analysis. As the *N* gene is the most conserved region in the genome of BCoV, all the samples group together in the phylogenetic tree and used in the viral detection assays (Cho *et al*., 2001a). This confirms the circulation of BCoV in the Indian bovine population. Moreover, further studies considering larger fragments and the complete genome are needed to identify various strains and genotypes circulating in the country. This will help to design preventive strategies and develop appropriate diagnostics.

In conclusion, the present study implicated BCoV as a component of BRDC in India and should be considered in the diagnosis of BRDC outbreaks. BCoV infection might have predisposed the animals to develop pasteurellosis in two cases of the present study. However, more molecular epidemiological studies are needed to elucidate the contribution of BCoV in the development of BRDC in India.

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Conflict of interest

The authors have declared no conflict of interest in the publication and authorship of the manuscript.

References

Ames, TR (1997). Dairy calf pneumonia: the disease and its impact. Vet. Clin. North Am. Food Anim. Pract., 13: 379-391. doi: 10.1016/S0749-0720(15)30303-0.

Baptista, AL; Rezende, AL; de Almeida, FP; Massi, RP; Nogueira, GM; Magalhães, LQ; Headley, SA; Menezes, GL; Alfieri, AA and Saut, JPE (2017). Bovine respiratory disease complex associated mortality and morbidity rates in feedlot cattle from southeastern Brazil. J. Infect. Dev. Ctries., 11: 791-799. doi: 10.3855/jidc.9296.

Bednarek, D; Szymańska-Czerwińska, M and Dudek, K (2012). Bovine respiratory syndrome (BRD) etiopathogenesis, diagnosis and control. In: Perez-Marin, CC (Ed.), *A bird's-eye view of veterinary medicine*. (2nd Edn.), Rijeka, Croatia, InTech. PP: 363-378. doi: 10.5772/33465.

Boileau, MJ and Kapil, S (2010). Bovine coronavirus associated syndromes. Vet. Clin. North Am. Food Anim. Pract., 26: 123-146. doi: 10.1016/j.cvfa.2009.10.003.

Cho, KO; Hasoksuz, M; Nielsen, PR; Chang, KO; Lathrop, S and Saif, LJ (2001a). Cross-protection studies between respiratory and calf diarrhea and winter dysentery coronavirus strains in calves and RT-PCR and nested PCR for their detection. Arch. Virol., 146: 2401-2419. doi: 10.1007/s007050170011.

Cho, KO; Hoet, AE; Loerch, SC; Wittum, TE and Saif, LJ (2001b). Evaluation of concurrent shedding of bovine coronavirus via the respiratory tract and enteric route in feedlot cattle. Am. J. Vet. Res., 62: 1436-1442. doi: 10.2460/ajvr.2001.62.1436.

Choudhary, M; Choudhary, BK; Ghosh, RC; Bhoyar, S; Chaudhari, S and Barbuddhe, SB (2019). Cultivable microbiota and pulmonary lesions in polymicrobial bovine pneumonia. Microb. Pathog., 134: 1-8. doi: 10.1016/j.micpath.2019.103577.

Clark, MA (1993). Bovine coronavirus. Braz. Vet. J., 149: 51-70. doi: 10.1016/S0007-1935(05)80210-6.

Dash, SK; Kumar, K; Goel, A and Bhatia, AK (2012). Detection of coronavirus antigen by ELISA from diarrhoeic cow calves in Mathura, India. Vet. World. 5: 166-168. doi: 10.5455/vetworld.2012.166-168.

Decaro, N; Mari, V; Desario, C; Campolo, M; Elia, G; Martella, V; Greco, G; Cirone, F; Colaianni, ML; Cordioli, P and Buonavoglia, C (2008). Severe outbreak of bovine coronavirus infection in dairy cattle during the warmer season. Vet. Microbiol., 126: 30-39. doi: 10.1016/j.vetmic.2007.06.024.

Ellis, J (2019). What is the evidence that bovine coronavirus is a biologically significant respiratory pathogen in cattle? Can. Vet. J., 60: 147-152.

Fehr, AR and Perlman, S (2015). Coronaviruses: an overview of their replication and pathogenesis. Methods Mol. Biol., 1282: 1-23. doi: 10.1007/978-1-4939-2438-7_1.

Fehrenbach, H (2001). Alveolar epithelial type II cell: defender of the alveolus revisited. Respir. Res., 2: 33-46.

Franzo, G; Drigo, M; Legnardi, M; Grassi, L; Pasotto, D; Menandro, ML; Cecchinato, M and Tucciarone, CM (2020). Bovine coronavirus: variability, evolution, and dispersal patterns of a no longer neglected betacoronavirus. Viruses. 12: 1-17. doi: 10.3390/v12111285.

Gagea, MI; Bateman, KG; van Dreumel, T; McEwen, BJ; Carman, S; Archambault, M; Shanahan, RA and Caswell, JL (2006). Diseases and pathogens associated with mortality in Ontario beef feedlots. J. Vet. Diagn. Inves., 18: 18-28. doi: 10.1177/104063870601800104.

Goswami, P; Banga, HS; Deshmukh, S; Mahajan, V; Singh, ND and Brar, RS (2015). Pathological investigation of bovine lungs in naturally acquired *Pasteurella multocida* infection by immunohistological technique. Indian J. Vet. Pathol., 39: 304-310.

Griffin, D (2010). Bovine pasteurellosis and other bacterial infections of the respiratory tract. Vet. Clin. Food Anim., 26: 57-71. doi: 10.1016/j.cvfa.2009.10.010.

Hansa, A; Rai, RB; Dhama, K and Wani, MY (2012). ELISA and RT-PCR based detection of bovine coronavirus in Northern India. Asian J. Anim. Vet. Adv., 7: 1120-1129.

Hasoksuz, M; Hoet, AE; Loerch, SC; Wittum, TE; Nielsen, PR and Saif, LJ (2002). Detection of respiratory and enteric shedding of bovine coronaviruses in cattle in an Ohio feedlot. J. Vet. Diagn. Invest., 14: 308-313. doi: 10.1177/104063870201400406.

Hick, PM; Read, AJ; Lugton, I; Busfield, F; Dawood, KE; Gabor, L; Hornitzky, M and Kirkland, PD (2012). Coronavirus infection in intensively managed cattle with respiratory disease. Aust. Vet. J., 90: 381-386. doi: 10.1111/j.1751-0813.2012.00978.x.

Hodnik, JJ; Jezek, J and Staric, J (2020). Coronaviruses in cattle. Trop. Anim. Health Prod., 52: 2809-2816. https://doi.org/10.1007/s11250-020-02354-y.

Kalkanov, I; Dinev, I and Zarkov, I (2019). Etiological and pathomorphological investigations in calves with coronaviral pneumoenteritis. Maced. Vet. Rev., 42: 43-49. https://doi.org/10.2478/macvetrev-2018-0028.

Kamdi, B; Singh, R; Singh, V; Singh, S; Kumar, P; Singh, KP; George, N and Dhama, K (2020). Immuno-fluorescence and molecular diagnosis of bovine respiratory syncytial virus and bovine parainfluenza virus in the naturally infected young cattle and buffaloes from India. Microb. Pathog., 145: 1-7. https://doi.org/10.1016/j.micpath.2020.104165.

Kapil, S; Pomeroy, KA; Goyal, SM and Trent, AM (1991). Experimental infection with a virulent pneumoenteric isolate of bovine coronavirus. J. Vet. Diagn. Invest., 3: 88-89. doi: 10.1177/104063879100300123.

Lojkic, I; Kresic, N; Simic, I and Bedekovic, T (2015). Detection and molecular characterization of bovine corona and toroviruses from Croatian cattle. BMC Vet. Res., 11: 1-6. https://doi.org/10.1186/s12917-015-0511-9.

Luna, LG (1972). *Histological staining methods of the armed forces institute of pathology*. 3rd Edn., New York, McGraw Hill Book Co., PP: 12-159.

Mebus, CA (1973). Neonatal calf diarrhea: propagation, attenuation, and characteristics of a coronavirus-like agent. Am. J. Vet. Res., 34: 145-150.

Mebus, CA; Newman, LE and Stairjr, EL (1975). Scanning electron, light, and immunofluorescent microscopy of intestine of gnotobiotic calf infected with calf diarrheal coronavirus. Am. J. Vet. Res., 36: 1719-1725.

Murray, GM; More, SJ; Sammin, D; Casey, MJ; McElroy, MC; O'Neill, RG; Byrne, WJ; Earley, B; Clegg, TA; Ball, H and Bell, CJ (2017). Pathogens, patterns of pneumonia, and epidemiologic risk factors associated with respiratory disease in recently weaned cattle in Ireland. J.

Vet. Diagn. Invest., 29: 20-34. doi: 10.1177/1040638716674757.

Park, SJ; Kim, GY; Choy, HE; Hong, YJ; Saif, LJ; Jeong, JH; Park, SI; Kim, HH; Kim, SK; Shin, SS and Kang, MI (2007). Dual enteric and respiratory tropisms of winter dysentery bovine coronavirus in calves. Arch. Virol., 152: 1885-1900. doi: 10.1007/s00705-007-1005-2.

Radostits, OM; Gay, CC; Hinchcliff, KW and Constable, PD (2007). *Veterinary medicine*. 10th Edn., London, W. B. Saunders Company Ltd., PP: 1286-1287.

Rai, RB; Hansha, A; Rai, S; Singh, B; Kumar, H; Singh, AK; Damodaran, T and Dhama, K (2011). Prevalence of rota and coronavirus infections in calves of Barabanki and Raebareli districts of Uttar Pradesh. Indian J. Vet. Pathol., 35: 73-74.

Saif, LJ (2010). Bovine respiratory coronavirus. Vet. Clin. North Am. Food Anim. Pract., 26: 349-364. doi: 10.1016/j.cvfa.2010.04.005.

Singh, S; Singh, R; Singh, KP; Singh, V; Malik, YPS; Kamdi, B; Singh, R and Kashyap, G (2019). Prevalence of bovine coronavirus infection in organized dairy farms of central and north regions, India. Biol. Rhythm Res., 50: 1-7. https://doi.org/10.1080/09291016.2019.1629093.

Singh, S; Singh, R; Singh, KP; Singh, V; Malik, YPS; Kamdi, B; Singh, R and Kashyap, G (2020). Immunohistochemical and molecular detection of natural cases of bovine rotavirus and coronavirus infection causing enteritis in dairy calves. Microb. Pathog., 138: 1-16. https://doi.org/10.1016/j.micpath.2019.103814.

Storz, J; Lin, X; Purdy, CW; Chouljenko, VN; Kousoulas, KG; Enright, FM; Gilmore, WC; Briggs, RE and Loan, RW (2000). Coronavirus and *Pasteurella* infections in bovine shipping fever pneumonia and Evans' criteria for causation. J. Clin. Microbiol., 38: 3291-3298. doi: 10.1128/JCM.38.9.3291-3298.2000.

Suresh, T; Rai, RB; Dhama, K; Bhatt, P; Sawant, PM and Sharma, AK (2012). Prevalence of rotavirus, coronavirus and *Escherichia coli*: the main agents responsible for calf diarrhoea. Vet. Pract., 13: 160-165.

Tamura, K; Stecher, G; Peterson, D; Filipski, A and Kumar, S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol., 30: 2725-2729.

Traven, M; Naslund, K; Linde, N; Linde, B; Silvan, A; Fossum, C; Hedlund, KO and Larsson, B (2001). Experimental reproduction of winter dysentery in lactating cows using BCV-comparison with BCV infection in milk-fed calves. Vet. Microbiol., 81: 127-151. doi: 10.1016/s0378-1135(01)00337-6.

Vlasova, AN and Saif, LJ (2021). Bovine coronavirus and the associated diseases. Front. Vet. Sci., 8: 1-14. doi: 10.3389/fvets.2021.643220.