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A study on co-infection of bovine parainfluenza 3 virus (BPI3V) and bovine respiratory syncytial virus (BRSV) in cattle

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Abstract
Background: Pneumonia in livestock is attributed for major economic loss to the poor and marginal farmers in terms of the expenditure on medicine, veterinary, reduced production by death of the animal. Bovine parainfluenza 3 virus (BPI3V) and bovine respiratory syncytial virus (BRSV) are the two main viral respiratory infections that cause substantial morbidity and death.
Aim: The aim of present study was to investigate co-infection of Bovine Parainfluenza 3 Virus (BPI3V) and Bovine Respiratory Syncytial Virus (BRSV).
Methods: Tissue samples from 32 cattle presented for post-mortem examination were collected in 10% NBF and processed for histopathological evaluation. The pathological lesions were observed for the severity and extent of lesions including inflammatory reaction. PCR assays were performed to detect the nucleic acid of BPI3V and BRSV in the tissue samples.
Results: Out of 32 tissue samples, 6.25% (2/32) were found to be affected with co-infection of Bovine Parainfluenza 3 Virus (BPI3V) and Bovine Respiratory Syncytial Virus (BRSV).
Conclusion: This finding concludes that co-infection of Bovine Parainfluenza 3 Virus (BPI3V) and Bovine Respiratory Syncytial Virus (BRSV) prevails and is one of the causes of pneumonia and production losses to cattle industry.

Keywords: Pneumonia, BPI3V, BRSV, cattle

Introduction
Livestock plays an important role in Indian economy. About 20.5 million people depend upon livestock for their livelihood. Livestock sector contributes 4.11% to GDP and is 25.6% of total Agriculture GDP. As per the 20th Livestock Census, 2019 total livestock population of India is 535.78 million. Domestic ruminant population (525.48 million), contributes more than 98% of total livestock. India ranks number one in milk production. Farmers face challenges due to diseases causing economic losses by reduced production and death of the animal.

Pneumonia is second most common causes of mortality in dairy calves after diarrhea. Among the viral respiratory pathogens, bovine parainfluenza 3 virus (BPI3V) is major pathogen along with bovine respiratory syncytial virus (BRSV) causing high morbidity and mortality. BPI-3 is a negative sense ssRNA virus of genus Respirovirus of Paramyxoviridae family. BPI3 has a global prevalence and has significant economic importance in farm animals (Martin and Bohac, 1986) [8]. BPI-3 infection induces broncho-interstitial pneumonia, and most frequently affected lobes are apical pulmonary lobes (Sharp and Nettleton, 2007) [11] marked by several areas of small reddish-grey discoloration, atelectasis leading to linear consolidated areas, found in bronchioles and also in segmental and tertiary bronchi (Hore *et al*., 1968) [5]. BRSV belongs to the genus Pneumovirus of the Pneumoviridae family earlier considered as subfamily of Paramyxoviridae of Mononegavirales order (Bunt *et al*., 2005) [2]. The distribution of BRSV is worldwide and is responsible for economic losses on account of prophylactic and therapeutic costs prevention and treatment costs, reduced production and deaths (Valarcher and Taylor, 2007) [12]. BRSV infection involves cranio-ventral and caudo-dorsal lobes of lungs showing varying degree of consolidation and emphysema of the lungs (Ellis *et al*., 2007) [3].

Materials and Methods

Sampling

The tissue samples were collected form 32 fallen animals brought to the postmortem facilities. Tissue samples were collected in 10% neutral buffered formalin (NBF) for histopathological evaluation. Tissue samples were also collected as in ice and for further storage kept at -20 °C for molecular biology work. Tissue samples were processed for molecular detection of BPI3V and BRSV nucleic acid by PCR.

Histopathology

Representative tissue samples (0.5 cm in size) were taken in 10% NBF from the region surrounding the gross lung lesions for microscopy and immunohistochemistry (IHC). Tissue sections were processed for haematoxylin & eosin staining by following standard protocol (Lillie, 1958) [6].

RNA Extraction

Extraction of total RNA from tissue suspensions/ nasal swabs by RNeasy mini kit (Qiagen, Netherlands) was done as per the instructions from manufacturer with minor adjustments. For the extraction 200ul of tissue suspensions or 600 µL of 1x PBS with nasal swab were taken in a nuclease free 2ml micro centrifuge tube and about 600ul of RLT buffer was mixed and centrifuged at 12000 rpm for 3 min. The resultant supernatant of about 700 µL was transferred into genomic DNA elimination spin column and centrifuged at 1000 rpm for 30 sec. and in the next step the upper column was discarded and equal volume of 70% Ethanol (molecular grade) was added and vortexed to mix thoroughly, from which 700 µL was then transferred to RNeasy spin column and centrifuged at 10000 rpm for 30sec and the flow through was discarded. 700 µL of RW1 was pipetted into RNeasy spin column and centrifuged at 10000 rpm for 30 sec and the flow through was discarded. In the next step working solution of RPE, 500 µL was used to wash the spin column by centrifuging at 12000 rpm for 2 min. The spin column were then transferred to new sterile 1.5 ml collection tubes, where 30-50µL of nuclease free water was added and centrifuged at 12000 rpm for 2 min for elution of the total RNA. The concentration of the RNA extract was measured by NanoDrop Spectrophotometer (Thermo Scientific, USA), and then was stored at -80 °C until further need. The tubes and reagents during the entire process of extraction were maintained on ice to avoid degradation of RNA.

The RNA extracts were subjected to quantification by Nabi UV-Visual Nano Spectrophotometer (Micro digital Co., South Korea). The absorbance readings were taken at A260/280 for concentration and purity of the isolated RNA samples. Pure samples of DNA and RNA should have a ratio of 1.65 to 1.85 and 1.8 to 2.0 respectively. Mini cooler was used during the time of measurement to maintain the samples at -20 °C. Later the samples were stored at -80 °C for future use.

Complimentary DNA/cDNA Synthesis

After quantification, samples with suitable concentration were subjected to RT-PCR where cDNA was synthesized with the application of High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, USA) as per instructions by the Manufacturer.

Oligonucleotide primers

Published PCR primers pairs for BPI3V (F: 5'-CCTGCCCTTTGGAGTTATGCGA-3', R: 5'-GCATCACGTGCCACTGCTTG -3') and BRSV (F 5'-GCACCACACTGTCCCAACCA -3', R 5'-GGCAGAGTGGTGAACAGGCA -3') were used for the detection of BPI3V and BRSV nucleic acid, respectively in the lung tissue samples. The primers were expected to amplify the specific cDNA template of sizes 128 and 246 bp for BPI3V and BRSV, respectively (Neethu, 2018) [9].

Polymerase chain reaction assay

BPI3V & BRSV cDNA were detected using PCR in standard 12 µl reaction volumes in 0.2 ml tubes using sets of primers as specified. Green Dream Taq Master mix (Thermo Scientific, USA), was used to create a PCR reaction mix of 12 µl, consisting of 6µl of Green Dream Taq Master mix, 0.3 µl of forward and reverse primer for each, 3.4 µl of nuclease-free water, and 2 µl of cDNA template. The contents of the tubes were combined, then they were put in a thermocycler (Q-Cycler 96, Hains Lifesciences, Verti 96 well thermocycler), and they were exposed to the standardized thermal cycling conditions. The thermal cycling conditions were composed of initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C (BPI3V) and 56 °C (BRSV) for 15 s, with a 20 s extension at 72 °C and single cycle of final elongation of 8 min at 72 °C.

The amplified cDNA products were visualized by transillumination under UV light (Geldoc; c150Azure Biosystem) in 1.5% agarose containing ethidium bromide (0.5 g/mL) after 45 minutes of electrophoresis at 90 volts.

Results

This investigation presents study of mentioned pathogens affecting dairy calves. 32 cattle were presented for postmortem examination.

Pathological conditions in lung tissues

Grossly, marbling and consolidation of cranio- middle lobes of both lungs and froth in trachea were observed. Histopathological examination of lungs revealed congestion, atelectasis, emphysema, edema, and thickened inter-alveolar septa due to infiltration of inflammatory cells. In few cases, syncytia formation, and intracytoplasmic eosinophilic inclusion bodies were observed.

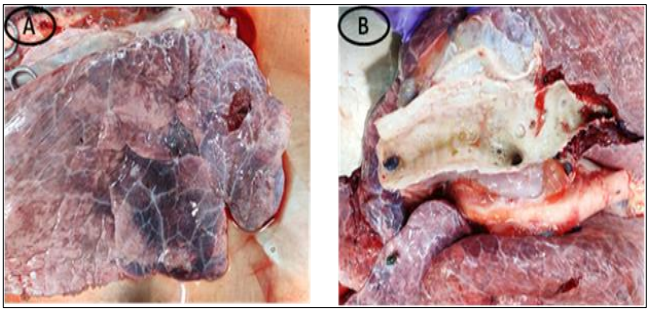

Fig 1: (A) Lungs: Marbling and scattered areas of consolidation of right cranial and intermediate lobe; (B): Presence of frothy fluid in the tracheal lumen

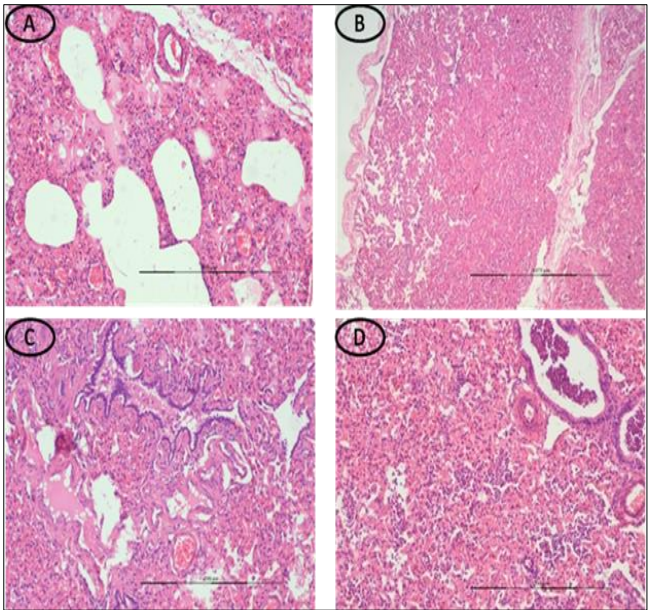

Fig 2: (A) Lungs tissue sections showing congestion, emphysema, alveolar oedema and thickening of inter-alveolar septa; (B) Lungs tissue sections showing thickening of pleura and alveolar collapse (atelectasis) with congestion; (C) Lungs tissue sections showing alveolar edema and Congestion along with accumulation of exudate in the bronchiole; (D) Lungs tissue sections showing Infiltration of inflammatory cells in interstitial alveolar spaces (H&E)

Detection of BPI3V and BRSV

For confirmation of BPI3 and BRSV, PCR amplification of specific genes for both agents from lung samples was done. It revealed positive amplification of matrix glycoprotein (M) gene of BPI3V and glycoprotein (G) gene of BRSV with band size of amplicons 128 bp and 246 bp, respectively.

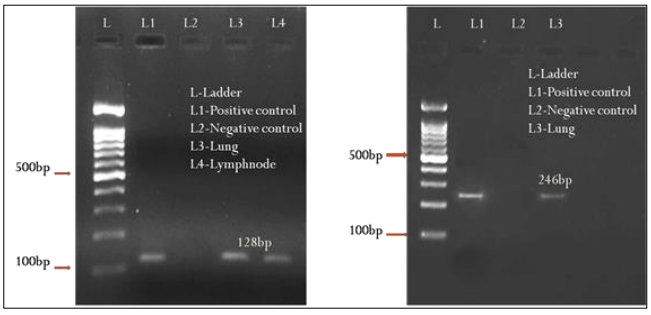

Fig 3: Agarose gel electrophoresis (1.5%) showing specific PCR amplified products 128bp (A) and 246bp (B) of BPI3V and BRSV

On the basis of gross, microscopic and molecular findings, out of 32, 2 cases (6.25%) were diagnosed as co- infection of BRSV and BPI3V.

Discussion

According to reports, bovine respiratory syncytial virus (BRSV) and BPI3V are two of the main primary pathogens among all respiratory viral diseases that have a high rate of morbidity and death in young animals. Worldwide prevalence of BPI3V among agricultural animals has been shown by seroprevalence investigations (Autio *et al.*, 2007) [1]. Nevertheless, the reports are few since they are seldom identified during normal examination. Furthermore, there are few reports about the virus's isolation from PI3 pneumonia outbreaks. (Ellis, 2010) [4]. Apical pulmonary lobes are the most commonly afflicted lobes in broncho-interstitial pneumonia caused by BPI-3 infection (Sharp and Nettleton, 2007) [11]. In bronchioles as well as segmental and tertiary bronchi, there are several spots of little reddish-grey discolouration and atelectasis resulting in linear consolidated regions (Hore *et al.*, 1968) [5]. Because BRSV predominantly damages the bronchiolar epithelium, it impairs the local pulmonary defense and promotes bacterial growth, which results in broncho interstitial pneumonia (Schrijver, 1998) [10]. In the present study the lesions found were coinciding with those of previous workers and were in accordance with those described earlier. There are little reports on the prevalence of BPI3V and BRSV in household ruminants in India. According to the data, BRSV and BPI3V may have been two of the main viruses that caused respiratory diseases in ruminants.

Conclusion

In the present study BPI3V genome could be detected in lungs tissues of animals in co-infection with BVDV in 2 (6.25%) out of 32 cases studied. This finding concludes that co-infection of Bovine Parainfluenza 3 Virus (BPI3V) and Bovine Respiratory Syncytial Virus (BRSV) prevails and is one of the causes of pneumonia and production losses to cattle industry. More detailed study, including large number of samples need to be done to know the correct present scenario of these diseases and their impact on dairy industry. Knowledge of common diseases, agents causing them and their lesions and confirmation can help in selecting preventive and control measures.

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References

1. Autio T, Pohjanvirta T, Holopainen R, Rikula U, Pentikäinen J, Huovilainen A, *et al*. Etiology of respiratory disease in non-vaccinated, non-medicated calves in rearing herds. Vet Microbiol. 2007;119(2-4):256-265.
2. Bunt AA, Milne RG, Sayaya T, Verbeek M, Vetten HJ, Walsh JA. *et al*, editors. Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier, Academic Press; c2005. p. 655-671.
3. Ellis J, Gow S, West K, Waldner C, Rhodes C, Mutwiri G, *et al*. Response of calves to challenge exposure with virulent bovine respiratory syncytial virus following intranasal administration of vaccines formulated for parenteral administration. J Am Vet Med Assoc. 2007;230(2):233-243.
4. Ellis JA. Bovine parainfluenza-3 virus. Vet Clin Food Anim. 2010;26:575-593.
5. Hore DE, Stevenson RG, Gilmour NJL, Vantsis JT, Thompson DA. Isolation of parainfluenza virus from the lungs and nasal passages of sheep showing respiratory diseases. J Comp Pathol. 1968;78(2):265-264.
6. Lillie RD. Manual of Histologic and Special Staining Techniques. Am J Clin Pathol. 1958;30:265–266.
7. Livestock Census. Data on Indian livestock. Govt. of India; 2019.

8. Martin SW, Bohac JG. The association between serological titers in infectious bovine rhinotracheitis virus, bovine virus diarrhoea virus, parainfluenza-3 virus, respiratory syncytial virus and treatment for respiratory disease in Ontario feedlot calves. Can J Vet Res. 1986;50(3):351-358.
9. Neethu G. Pathomorphology of pneumonia in sheep and goat with special reference to parainfluenza virus. Thesis, MVSc. Deemed University, IVRI, Izatnagar, India; 2018.
10. Schrijver RS. Immunobiology of bovine respiratory syncytial virus infections. Tijdschr Diergeneeskd. 1998;123:658-662.
11. Sharp JM, Nettleton PF. Acute respiratory virus infections. In: Aitken ID, editor. Diseases of Sheep. 4th ed. Blackwell Publishing; 2007. p. 207-208.
12. Valarcher JF, Taylor G. Bovine respiratory syncytial virus infection. Vet Res. 2007;38:153-180.