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First report of histopathological and molecular characterizations of bovine herpesvirus-1 from outbreak at dairy farm in India

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Abstract

Infectious bovine rhinotracheitis, caused by bovine herpesvirus-1 (BHV-1) is a highly contagious viral disease affecting bovines, and clinically characterized by pyrexia, inappetence, respiratory distress, dyspnoea, conjunctivitis, nasal discharge, and sometimes abortions. In the present study, buffalo dairy farm having high mortality was investigated. The buffaloes were suffering from high rectal temperature, conjunctivitis, severe respiratory distress, and nasal discharge. Tissue samples from upper respiratory tract were collected aseptically following post-mortem examination of died buffaloes. Tracheal tissue samples were then processed for histopathological examination and DNA isolation. The presence of BHV-1 in the tissue samples was confirmed by nested polymerase chain reaction using *glycoprotein B* gene primers. The present study reported for the first time the clinical signs, post-mortem lesions, histopathological evidence, and detection of DNA of BHV-1 *glycoprotein B* gene through nested polymerase chain reaction assay during an active outbreak in buffaloes in India. The findings of this study are crucial for improving the diagnosis of BHV-1 and ultimately reducing financial losses within dairy industry.

Introduction

Bovine herpesvirus-1 (BHV-1), classified as Varicello-virus, subfamily Alphaherpesvirinae, family Herpesviridae, and order Herpesvirales, is a major pathogen of bovines. The genome of the virus is double-stranded DNA of 135-140 kbps in size.[1] Based on genomic analysis and viral peptide patterns, BHV-1 is further categorized into sub-types namely BHV-1.1, -1.2a, and -1.2b.[2,3] The BHV-1 is known to cause infectious bovine rhinotracheitis (IBR), abortion, infectious pustular vulvo-vaginitis, and systemic infection in livestock, imposing significant economic losses to the livestock industry and farmers. The disease is clinically characterized by pyrexia, salivation, rhinitis, conjunctivitis, mucopurulent nasal discharge, inappetence, and dyspnea. Severe cases may exhibit open mouth breathing before death. Reproductive form is associated with infertility disorders, abortion, and birth defects. The BHV-1 is endemic in livestock of different parts of globe, including United States, Europe, Asia, Australia, and Africa.[3-6] The virus is excreted in the secretions, with or without clinical manifestations, and can become latent in sensory ganglia.[7] During the acute phase of infection, viremia occurs, and viral DNA can be isolated from body fluids, tissues, and fetus.

India, with the largest livestock population in the world, heavily relies on agriculture and livestock for the livelihood. While serological studies in India have reported the seroprevalence of IBR in livestock ranging from 14.75 to 68.90%,[8-10] there is limited information available regarding active outbreaks, as well as histopathological and molecular characterizations of BHV-1 in India. This study describes for the first time the detection of BHV-1, with histopathological and molecular characterizations of BHV-1 from buffaloes affected with IBR.

Materials and Methods

Study area and management practice. The affected buffalo dairy farm was situated in the Jola village, Budhana block, Muzaffarnagar district, Uttar Pradesh state, India. Plains agricultural fields make up the majority of the village's landscape. The buffaloes on the dairy farm were raised using semi-intensive farming and fed with wheat straw, green fodder, and concentrates. In late January 2024, a lactating buffalo died after the symptomatic treatment

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by local veterinarians. Within 4 - 5 days, two more buffaloes died even after receiving the treatment, including antibiotics and non-steroidal anti-inflammatory drugs. Recognizing the severity of the situation, district animal husbandry authorities sought assistance from the College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India, for the disease diagnosis and advising the line of treatment to save the remaining livestock.

Clinical signs and sample collection. On clinical examinations, the buffaloes were suffering from pyrexia, severe respiratory distress, dyspnea, nasal discharge, inappetence, and conjunctivitis. The blood samples were collected aseptically from jugular vein using heparinized vacuum blood collection vials. Necropsy was performed on two died buffaloes and tissue samples from respiratory tract were collected in two aliquots, one without preservative on cold ice pack for molecular diagnosis and the other in 10.00% neutral buffered formalin for histopathological examination.

Pathomorphological changes. Severe pathological changes were observed in the buffalo carcass. Tissue was processed as per standard protocol and sections were made after paraffin embedding and stained with Hematoxylin and Eosin.[11]

Detection of viral nucleic acid. Tissue samples from both buffaloes were transported on ice, and manually homogenized by a sterile pestle and mortar and phosphate-buffered saline (pH: 7.40) to make the homogenized 10.00% (w/v) solution. Genomic DNA was isolated from tissues of tracheal lesions by DNeasy Blood and Tissue Kits (Qiagen, Hilden, Germany) following manufacturer's instructions. Isolated DNA was quantified using a Nano-spectrophotometer (NanoBio 3.0; Analytical Technologies Ltd., Baroda, India) by measuring absorbance at 260/280 nm. Nested polymerase chain reaction (PCR) was performed with pre-published primers.[12] Primary (external) PCR primer sequences were F 5'-CACGGACCTGGTGGACAAGAAG-3' and R 5'-CTACCGTCACGTGAGTGGTACG-3'. The internal (nested) primer sequences were F 5'-AGCCGAGTACCTGCGCAG-3' and R 5'-AGCCCTCGATCTGCTGGA-3'. The PCR cycle consisted of 35 cycles of 1 min at 95.00 ˚C, 1 min at 56.00 ˚C, and 1 min at 72.00 ˚C with 6 min final extension. Horizontal gel electrophoresis was conducted to determine the size of PCR products by 1.50% agarose gel, and the products were then visualized by Gel Documentation System (Universal Hood II; Bio-Rad, Hercules, USA).

Results

Clinical examination. The affected buffaloes were restless, and showed high temperature, inappetence, depression, congested mucous membrane, respiratory distress, dyspnea, nasal discharge, and conjunctivitis, all of which were suggestive of IBR.

Gross pathological examination. Upon examination, significant lesions were found in the upper respiratory tract and ocular tissue, while all other organs appeared normal except for mild congestion in gastro-intestinal tract. The ocular conjunctiva was highly congested with purulent exudation being noticed in all the animals (Fig. 1). The trachea was highly congested particularly at the luminal surface, along with marked necrotic debris, muco-purulent discharge, and pseudo-diphtheritic membrane formation (Fig. 2). Similar lesions, accompanied by froth extended to bronchi, while lung parenchyma was grossly normal except mild to moderate congestion. Larynx and pharynx were also highly congested. The gross alterations observed during post-mortem examination of the buffaloes indicated the possibility of IBR.

Fig. 1. Gross appearance of ocular tissue of infectious bovine rhinotracheitis affected animal, showing highly congested conjunctiva and purulent ocular discharge around the globe.

Fig. 2. Gross appearance of trachea of infectious bovine rhino-tracheitis affected animal, showing marked congestion, necrotic debris, and mucopurulent exudation.

Histopathological changes. Microscopic lesions consisted of marked thickening of mucosa due to the infiltration of mononuclear cells along with polymorphs, destruction and sloughing of ciliated mucosal epithelial cells, marked congestion, and exudation of trachea and bronchi (Figs. 3 and 4). Similar lesions were extended up to the bronchioles with characteristic infiltration of inflammatory cells around the peri-bronchiolar area (Fig. 5).

However, the lung parenchyma and alveoli were almost unaffected except some non-specific lesions, such as mild to moderate congestion and edema in localized areas. Intra-nuclear viral inclusion bodies were not seen in the respiratory epithelial cells.

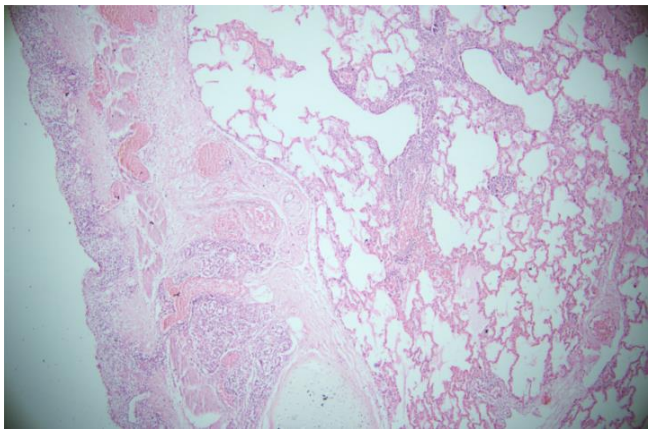

Fig. 3. Micrograph showing marked congestion, exudation, infiltration of inflammatory cells, and thickening of bronchial mucosa (Hematoxylin and Eosin staining, 40×).

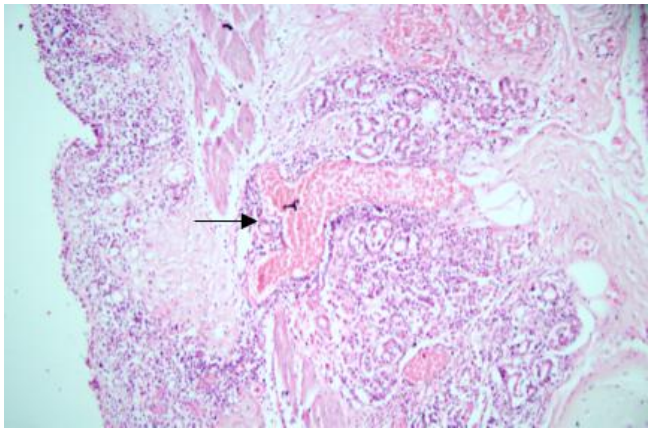

Fig. 4. Arrow shows peri-bronchial infiltration of mononuclear as well as polymorphonuclear cells, and congestion of mucosa (Hematoxylin and Eosin staining, 100×).

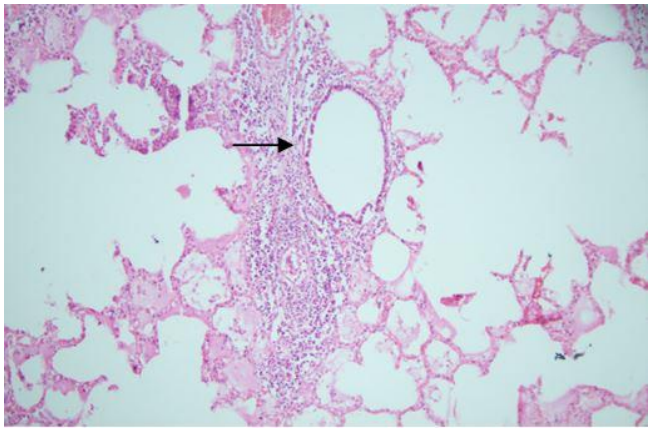

Fig. 5. Arrow shows peri-bronchiolar infiltration of monnuclear as well as polymorphonuclear cells (Hematoxylin and Eosin staining, 100×).

Molecular characterization. After primary PCR, nested PCR was performed using internal primers, which confirmed the presence of viral DNA showing the amplicon of 344 bp (*glycoprotein B* gene) in Gel Documentation System (Fig. 6).

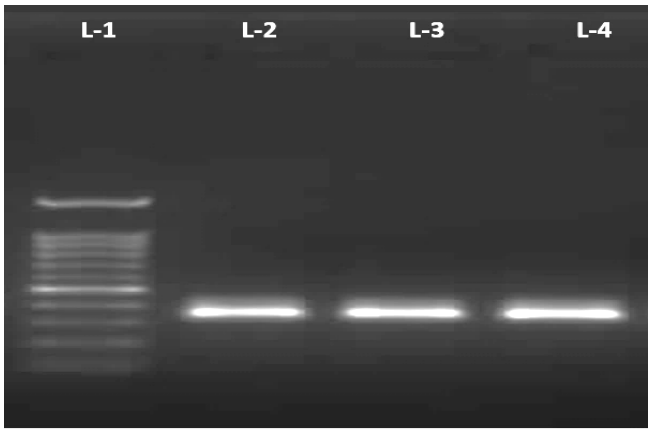

Fig. 6. Electrophoresis gel shows lane L-1 as a ladder 100 bp+, and lanes L-2, L-3 and L-4 as positive polymerase chain reaction amplification results of infectious bovine rhinotracheitis virus infection.

Discussion

Infectious bovine rhinotracheitis, also known as rednose, is a disease of major significance in the bovine industry due to the IBR virus synergy with *Mannheimia haemolytica* in causing pneumonia. Clinical forms of IBR can range from respiratory distress, abortion, and infectious pustular vulvo-vaginitis to the systemic infection in bovines.[13] In the present study, animals showed inspiratory dyspnea as a result of upper respiratory tract infection, distinguishing it from hemorrhagic septicemia, a leading cause of mass mortality in buffaloes, typically showing expiratory dyspnea due to the lower respiratory tract involvement. Similar clinical signs and pathological lesions were seen in buffaloes affected with BHV-1 infection. This study reports for the first time, the detection of BHV-1, as well as histopathological and molecular characterizations of BHV-1 *glycoprotein B* gene from buffaloes affected with IBR.

The respiratory form of IBR is characterized by severe hyperemia and widespread necrosis of the nasal, pharyngeal, laryngeal, tracheal, and occasionally bronchial mucosae. The IBR lesions, like those seen in other respiratory viral infections, are characterized microscopically by ciliated epithelium necrosis and exfoliation, followed by healing. Secondary bacterial infections at the necrosis sites lead to the thick coating of fibrino-necrotic (diphtheritic) material in the nasal, tracheal, and bronchial mucosae. Intra-nuclear inclusion bodies, commonly found in herpesvirus infections, were not visible in the present case as these were rarely found in field cases because they typically appear only early in the disease.[14] However,

another study has documented eosinophilic viral inclusions in placenta, liver, brain, and abomasum of aborted fetuses from IBR infected animals.[15] Occasional bronchitis and bronchiolitis with spared lung parenchyma in uncomplicated cases were also recorded. Thick yellowish mucopurulent exudates from nostrils were evident in previous study.[14] The limited involvement of alveoli and lung parenchyma in this case may be attributed to the early administration of antibiotics, as the owner has already lost several animals and promptly treated the remaining affected animals.

The BHV-1 becomes latent in sensory ganglia and typically sheds during the acute phase of the disease or under stress conditions. Consequently, viral antigens can be detected in whole blood, tissues, and secretions during the acute stage of the infection. In the present study, tracheal tissue samples tested positive for BHV-1 DNA using nested PCR. Nested PCR is widely used for the diagnosis and confirmation of BHV-1 infection across a variety of sample types. In India and abroad, most researchers have conducted sero-prevalence study,[9,10] with a few focusing on histological examination of cases. As a result, there is a paucity of data regarding gross and histopathological aspects of the respiratory form of disease, particularly in India. To the best of author's knowledge, the present study addresses this gap by presenting necropsy case report from an outbreak of IBR in western Uttar Pradesh, India, highlighting the gross and microscopic findings of the respiratory form of IBR in India.

In conclusion, this is the first report detailing the gross lesions, as well as histopathological and molecular characterizations of the BHV-1 from an active outbreak in buffaloes of Uttar Pradesh, India. This study highlighted the importance of utilizing molecular methods, such as nested PCR for the accurate diagnosis of IBR in order to safeguard the dairy industry and farmers.

Acknowledgments

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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