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# Investigation of bovine herpesvirus 1 and bovine parainfluenza virus 3 in bovine pneumonia using polymerase chain reaction, double immunohistochemistry, and double immunofluorescence

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**Abstract**

The aim of this study was to investigate the presence of bovine herpesvirus type 1 and bovine parainfluenza virus type 3 (PI-3) in cattle lungs exhibiting pneumonia symptoms observed during macroscopic examination. Polymerase chain reaction, double immunohistochemistry, and double immunofluorescence methods were used for viral detection. For this purpose, during the summer-autumn period of 2023, a total of 84 lung samples from cattle aged 1 year and older, exhibiting macroscopic signs of pneumonia, were collected in Kayseri province, Türkiye. Pneumonia lesions were recorded according to the anatomical locations. In virus detection, PI-3 was identified at a rate of 8.33% using double immunohistochemistry/immunofluorescence staining methods, while this rate was found to be 6.72% in polymerase chain reaction. Bovine herpesvirus type 1 was not detected by any of the three methods. In the cattle lungs, 44 lesions (52.38%) were detected in the apical lobe, while seven lesions (8.33%) were detected in the middle lobe, eight lesions (9.52%) in the accessory lobe, and 25 lesions (29.76%) in the basal lobe. The PI-3 immunopositivity was observed in alveolar epithelial cells, as well as mononuclear cells in the interstitial and peri-vascular regions. This study is the first to examine bovine herpesvirus type 1 and PI-3 presence in bovine lungs using three distinct validation techniques (polymerase chain reaction, double immunohistochemistry, and double immunofluorescence).



## Introduction

Animal proteins play a significant role in human nutrition today. When it comes to protein value, dairy products and red meat are considered the most important primary sources for humans. The provision of animal proteins largely depends on cattle farming, which is a major industry for many countries, and losses in this sector can result in economic damage to national economies.[1] The respiratory system, due to its large surface area and constant interaction with the external environment, is exposed to many pathogenic agents.[2] Respiratory diseases in cattle, particularly in young animals and feedlots, cause significant financial losses in cattle farming.[3] The majority of respiratory system diseases in cattle are caused by pneumonias.[4] Numerous studies on the etiology of pneumonia have shown that, in addition to infectious agents, such as viruses, bacteria, protozoa, fungi, and parasites, factors, such as cold barn conditions, allergic reactions, and the inhalation of excessive toxic gases in barns, also play a significant role.[5] Important viral agents causing pneumonias in cattle include bovine respiratory syncytial virus, bovine viral diarrhea virus, bovine parainfluenza virus type 3 (PI-3), and bovine herpesvirus type 1 (BHV-1).[6]

The PI-3 is classified within the *Mononegavirales* order, *Paramyxoviridae* family, *Orthoparamyxovirinae* sub-family, and *Respirovirus* genus. Since 2016, this virus has been referred to as bovine respirovirus-3 by the International Committee on Taxonomy of Viruses.[7] Among the viruses causing pneumonia in animals, infections caused by the PI-3 are the most commonly observed. Pneumonias caused by the PI-3 have been reported in various species, including cattle, sheep, buffaloes, deer, pigs, monkeys,

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dogs, cats, horses, and humans. When the PI-3 is present alone in a living organism, it typically causes mild or sub-clinical respiratory infections, but it also weakens the immune system, making the body susceptible to secondary bacterial infections.[8-10] The majority of para-influenza viruses primarily infect epithelial cells in the respiratory system. After entering the nasal cavity, the virus spreads to lymphoid tissues, especially the tonsils and respiratory mucosa, where primary viral replication occurs. The PI-3 replicates in both ciliated and non-ciliated epithelial cells, causing destruction of these cells and weakening the lung defense system.[11] In PI-3 infections, microscopic examination of the lungs reveals lymphocyte and macrophage infiltration in the peri-bronchial, bronchiolar, and peri-vascular regions, as well as inter-alveolar septa. Also, interstitial pneumonia characterized by thickening of the inter-alveolar septa is observed.[12]

The BHV-1 is classified within the *Herpesviridae* family, *Alphaherpesvirinae* sub-family, and *Varicellovirus* genus.[13] Since its identification, this virus has spread worldwide, and three genetic sub-types (1.1, 1.2a, and 1.2b) have been identified.[14] The BHV-1 is an important pathogen in cattle, causing both acute and latent infections.[15] The primary site of initial replication of BHV-1 in the body is the epithelial cells at the entry point. Following this replication, there are three main courses of infection, including local confinement, systemic spread *via* viremia, and neural spread.[16] Infection is more commonly observed in feedlot cattle, and the mortality rate is low unless secondary infections occur. Seropositivity is higher in male cattle compared to females. In operations with high animal density, male cattle aged 3 - 6 years are more susceptible to the BHV-1. Clinical signs may include nasal discharge, ranging from serous to mucopurulent, difficulty breathing in severe cases, and coughing.[17]

The aim of this study was to investigate the potential presence of PI-3 and BHV-1 in bovine pneumonia using three different confirmatory techniques, including polymerase chain reaction (PCR), double immunohisto-chemistry (IHC), and double immunofluorescence (IF).

## Materials and Methods

**Materials.** This study consisted of 84 lung samples collected from cattle aged 1 year and older, showing macroscopic signs of pneumonia, in abattoirs located in Kayseri province, Türkiye, during the summer-autumn period of 2023. The research was conducted with the approval of the Erciyes University Local Ethics Committee for Animal Experiments, Kayseri, Türkiye (EUHHADYEK-2023/156).

**Macroscopic examination.** The locations of pneumonia-affected areas in the cattle lung samples were recorded according to the shape and structure of lung anatomy, as described in study by Liebler-Tenorio *et al.*[18]

**Polymerase chain reaction**. For virological examination, the samples stored at – 20.00 ˚C were brought to room temperature and allowed to thaw. The thawed samples were homogenized using a homogenizer. The homogenized samples were diluted with phosphate-buffered saline (PBS) at a ratio of 1.00 g to 9.00 mL. After dilution, the tissue mixture was vortexed and centrifuged at 2,000 *g* for 5 min. After centrifugation, the supernatant was collected and proceeded with the extraction process. For extraction, a Viral Nucleic Acid Extraction Kit (Vivantis Malaysia, Subang Jaya, Malaysia) was used, and the kit procedure was followed as per the manufacturer's instructions. After extraction, the samples were stored at – 20.00 ˚C until the PCR was performed. The PCR tests targeting various gene regions have been performed for both detection and detection of differences in the virus in terms of BHV-1. In the study conducted by Fuchs *et al.*,[19] it was emphasized that detections were made by PCR using the *gB*, *gC*, and *gE* gene regions of BHV-1, respectively, and similarly, in a study conducted by Nišavić *et al.*,[20] similar results were obtained in the comparative detection of the *TK* and *gB* gene regions of BHV-1. Therefore, the *gB* gene, a conserved region of the virus commonly used for diagnostic purposes, was preferred in this study. For BHV-1, direct PCR was carried out using primers (477-gB-F-TACGACTCGTTCGCGCTCTC and 477-gB-R-GGTACG TCTCCAAGCTGCCC) and optimization conditions as described by Atasever *et al.*[21] For PI-3, being an RNA virus, the RNA was first converted to the complementary DNA using reverse transcriptase enzyme. After the samples were converted to complementary DNA, PCR was carried out. In a study conducted by Wen *et al.*,[22] they aimed to detect genotypes in Chinese isolates for PI-3 and reported that *HN* and *M* genes were suitable for virus detection. Similarly, Horwood *et al.*[23] used the *M* gene, a conserved gene region of the virus, to detect two different PI-3 genotypes. For this reason, primers previously used for detection in different studies in our country were preferred in this study. For PI-3, primers (328-M1-AGTGATCTAGATGATGATCCA and 328-M2-GTT EK GAT CCA EK GCT GT) and optimization conditions used in the study by Timurkan *et al.*[24] were employed. The thermal cycling conditions and optimizations for the PCR reaction (Table 1) were chosen based on the primers used in the referenced publications. The amplicons obtained from PCR were evaluated by gel electrophoresis (PowerPac Basic; Bio-Rad Laboratories, Subang Jaya, Malaysia). After conventional reverse transcription-PCR and PCR, agarose gels (1.50%) containing SYBR safe DNA gel stain were prepared to visualize potential PCR products (Table 1), and electrophoresis was performed under an electric current. After electrophoresis, the sizes of the samples were compared with the marker (100 - 3,000 bp; Thermo Fisher Scientific, Waltham, USA).

**Table 1.** The primers used in the study, and polymerase chain reaction (PCR) conditions.

| Primers | Sequences | Annealing tempereture (°C) | PCR amplicon size (bp) | References |
|---|---|---|---|---|
| **BHV-1/477-gB** | F: TACGACTCGTTCGCGCTCTC<br>R: GGTACGTCTCCAAGCTGCCC | 60.00 | 477 | 21 |
| **PI-3/328** | M1: AGTGATCTAGATGATGATCCA<br>M2: GTTATTGATCCAATTGCTGT | 50.00 | 328 | 24 |

BHV-1: Bovine herpesvirus type 1; PI-3: Bovine parainfluenza virus type 3.

**Histopathological examination.** The pneumonia samples were kept in a 10.00% formalin solution for 48 hr for Hematoxylin and Eosin, double IHC, and double IF staining methods. Afterward, the tissues were washed for 12 hr. Following this, the tissues were treated with 1X 70.00% ethanol for 2 hr, 1X 80.00% ethanol for 2 hr, 1X 90.00% ethanol for 2 hr, 2X absolute ethanol for 1.5 hr, 2X xylene for 1.5 hr, xylene-paraffin for 1.5 hr at 60.00 ˚C, and paraffin for 1.5 hr at 60.00 ˚C. Then, the tissues were embedded in paraffin blocks. Sections of 5.00 µm were taken from the blocks using a microtome and placed on regular slides. To remove the paraffin from tissue sections, the slides were placed in an oven at 58.00 ˚C for 2 hr. After that, the sections were incubated twice in xylene for 5 min each, followed by incubation in graded alcohol series of 100, 96.00, 80.00, 70.00, and 60.00% for 5 min each. After these steps, the sections were placed in distilled water for 5 min and then treated with Hematoxylin solution for 8 min. The sections were washed with tap water and then rinsed with distilled water again. The tissue was stained in Eosin solution for 45 sec and placed in alcohol series of 70.00, 80.00, 96.00, and 100% for 3 min each. Thereafter, the sections were incubated twice in xylene for 3 min each. The stained sections were then covered with a coverslip and examined under a light microscope. During the histopathological examination, the presence of mono-nuclear cell infiltration, bronchial-bronchiolar epithelial hyperplasia, inter-alveolar fibrosis, desquamation of bronchial-bronchiolar epithelium, necrotic bronchiolitis, atelectasis, and lymphoid hyperplasia was investigated in the lung samples.[25,26]

**Double IHC**. Subsequently, the viral antigens of PI-3 and BHV-1 in the samples were examined using double IHC and double IF methods. For double IHC, 5.00 µm tissue sections mounted on poly-L-lysine slides were stained with the Goat X Mouse/Rabbit Double Stain Kit (Biotech, Taipei, Taiwan) following the manufacturer's instructions. The procedure was as follows: The sections were de-paraffinized by incubating for 5 min in xylene. Afterward, they were subjected to graded alcohol series for 3 min each and washed with distilled water. The sections were incubated in a 3.00% $H_2O_2$ solution for 10 min, followed by two washes with PBS. To unmask the antigens, an antigen retrieval solution containing ethylene-diaminetetraacetic acid was applied to the sections, and they were treated with heat at 500 W for 10 min and washed three times with PBS. To block non-specific binding, the sections were incubated for 20 min at room temperature with Hi-effect Immunoblock II solution and washed twice with PBS. The sections were then incubated with BHV-1 anti-serum (diluted 1:500; VMRD, Pullman, USA) for 30 min at room temperature in a humid environment, followed by three washes with PBS. The sections were then incubated with Goat Probe for 10 min at room temperature, washed three times with PBS, and then incubated with Goat Probe horseradish peroxidase (HRP) for 30 min at room temperature. After three washes with PBS, the sections were incubated with 3,3′-diamino-benzidine chromogen for 2 min, followed by three washes with PBS. The sections were then incubated for 10 min in a 3.00% $H_2O_2$ solution and washed twice with PBS. To prevent non-specific binding, the sections were incubated for 20 min at room temperature with Hi-effect Immunoblock solution and washed twice with PBS. Next, the sections were incubated with PI-3 Monoclonal Antibody (diluted 1:200; Biox, Rochefort, Belgium) for 30 min at room temperature in a humid environment and washed three times with PBS. The sections were then incubated with Mouse/Rabbit Probe HRP for 30 min at room temperature and washed three times with PBS. Hematoxylin counterstaining was performed, and the sections were washed three times with PBS. Afterward, the sections were incubated with HRP Green for 10 min, washed three times with PBS, and mounted with water-based adhesive for microscopic examination. Brown staining was considered as positive for BHV-1, and green staining was considered as positive for PI-3. This method of evaluation was implemented as recommended by the manufacturer.[27]

**Double IF.** For double IF, 5.00 µm tissue sections mounted on poly-L-lysine slides were stained using a modified version of the double IF protocol suggested by Stanford Medicine (https://med.stanford.edu-Double-ImmunofluorescenceProtocol). To begin, the sections were deparaffinized by incubating in xylene for 5 min and then rehydrated in graded alcohol series for 3 min each, followed by washing in distilled water. The sections were then incubated in 3.00% $H_2O_2$ solution for 10 min to block endogenous peroxidase activity and washed twice with PBS. For antigen retrieval, the sections were treated with ethylenediaminetetraacetic acid -containing antigen retrieval solution at 500 W for 10 min, followed by three PBS washes. To block non-specific binding, the sections were incubated for 5 min at room temperature with Ultra V Block (Thermo Fisher Scientific) solution and washed twice with PBS. The primary antibodies used in immuno-histochemical staining were applied to the sections. The

BHV-1 (1/200 dilution) and PI-3 (1/200 dilution) primary antibodies were mixed in equal volumes and applied to the sections. After incubation at room temperature for 1 hr, the sections were washed three times with PBS. The secondary antibodies used were Mouse Anti-Goat Immunoglobulin G Heavy and Light Chains - Texas Red (Novus Biologicals, Centtennial, USA) and Fluorescein Isothiocyanate-Goat Anti-Mouse Immunoglobulin G (Jackson Immunoresearch, West Grove, USA), each diluted at 1:100. These secondary antibodies were mixed in equal volumes and applied to the sections. After incubation for 1 hr in the dark at room temperature, the sections were washed three times with PBS. Finally, 4′,6-diamidino-2-phenylindole (DAPI) was applied to the sections for nuclear staining, and the samples were examined under a fluorescence microscope using fluorescein isothiocyanate, Texas Red, and DAPI filters. Nuclei were stained blue, BHV-1 positivity was indicated by red fluorescence, and PI-3 positivity was indicated by green fluorescence. This method of evaluation was conducted based on the study by Atasever *et al.*[21]

## Results

**Macroscopic examination.** In the macroscopic examination, the anatomical distribution of lesions in the lungs of 84 pneumonic cattle, which constituted the study material, was as follows: 44 lesions (52.38%) in the apical lobe, seven lesions (8.33%) in the middle lobe, eight lesions (9.52%) in the accessory lobe, and 25 lesions (29.76%) in the basal lobe (Fig. 1).

**Polymerase chain reaction findings.** This study investigated the BHV-1 and PI-3 in bovine lung samples showing macroscopic signs of pneumonia. Molecular PCR analysis of a total of 84 pneumonic lung samples revealed no detection of the partial *gB* gene of the BHV-1. However, the partial *M* gene of the PI-3 was detected in 6.72% of the samples (Fig. 2).

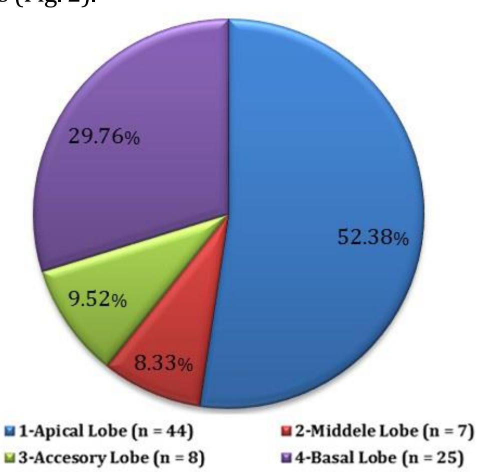


**Fig. 1.** Anatomical locations of pneumonia lesions in 84 bovine lungs used in the study.

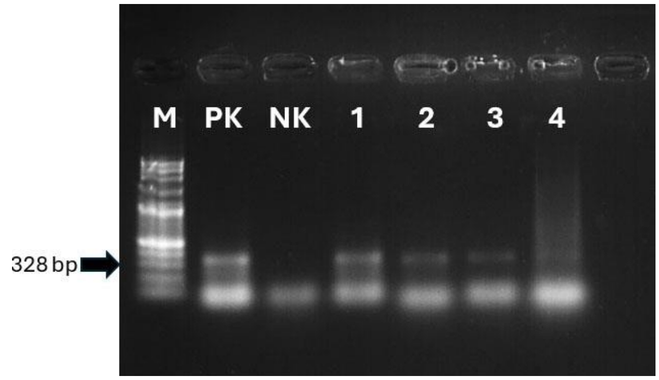


**Fig. 2.** Agarose gel electrophoresis of the partial *M* gene (328 bp) polymerase chain reaction products of bovine parainfluenza virus type 3. M: Marker; PK: Positive control; NK: Negative control; Lane 1: Sample 8; Lane 2: Sample 11; Lane 3: Sample 14; Lane 4: Sample 46.

**Histopathological findings.** In the histopathological examination of 84 cattle pneumonia samples, the following findings were observed: Mononuclear cell infiltration in all samples, bronchial-bronchiolar epithelial hyperplasia in 95.23% of the cases, inter-alveolar fibrosis in 86.90%, desquamation of bronchial-bronchiolar epithelium in 57.14%, necrotic bronchiolitis in 46.42%, atelectasis in 96.42%, and lymphoid hyperplasia in 11.90%. In the samples tested positive for PI-3, all of the aforementioned histopathological findings were present (Fig. 3).

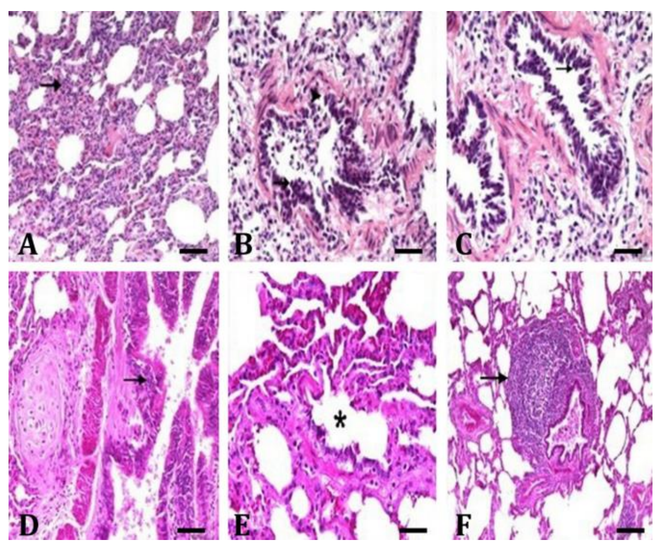


**Fig. 3.** Histopathological images of bovine parainfluenza virus type 3 positive (A-C) and pneumonia (D-F) samples (Hematoxylin and Eosin staining; bars = 50.00 µm in A and E, 20.00 µm in B, C, and D, and 100 µm in F). **A)** Mononuclear cell infiltration (arrow); **B)** Desquamation (arrowhead) and hyperplasia (arrow) in the bronchiol epithelium; **C)** Necrotic bronchiolitis (arrow); **D)** Bronchiol epithelium hyperplasia (arrow); **E)** Emphysema (asterisk); **F)** Hyperplasia in the bronchus-associated lymphoid tissue (arrow).

**Immunohistochemical and IF findings.** A total of 84 pneumonia samples were thoroughly examined under the microscope using immunohistochemical and IF staining methods. As a result, 8.33% of the samples tested positive for PI-3, while no positive results were observed for BHV-1. The PI-3 immunopositivity was observed in the alveolar epithelial cells and mononuclear cells found in the interstitial and peri-vascular areas (Fig. 4).

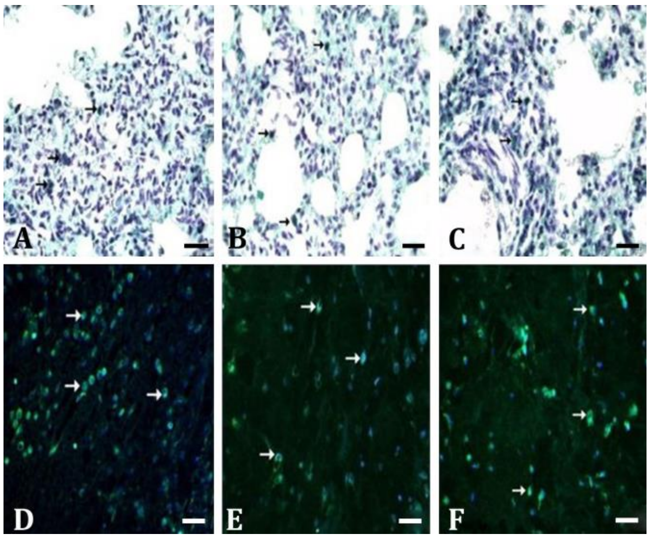


**Fig. 4.** Dual immunohistochemistry (A-C) and immunofluorescence (D-F) images (Bars = 20.00 µm). **A)** Bovine parainfluenza virus type 3 (PI-3) immunopositivity in mononuclear cells located in the interstitial area (arrows); **B)** The PI-3 immunopositivity in alveolar epithelial cells (arrows); **C)** The PI-3 immunopositivity in mononuclear cells located in the perivascular area (arrows); **D)** The PI-3 immunopositivity in mononuclear cells located in the interstitial area (arrows); **E)** The PI-3 immunopositivity in alveolar epithelial cells (arrows); **F)** The PI-3 immunopositivity in mononuclear cells located in the perivascular area (arrows).

## Discussion

Respiratory diseases in cattle farming are among the most economically significant health issues. The economic losses caused by the respiratory diseases have exceeded the costs of many other diseases.[28] Despite detailed investigations and efforts to prevent diseases beforehand, respiratory system diseases in cattle continue to pose significant problems in cattle farming.[29] A large portion of respiratory system-related diseases in cattle is caused by infections resulting from pneumonia.[4] Viral agents, such as BHV-1, PI-3, and bovine respiratory syncytial virus, affecting the respiratory system, are considered significant causes of interstitial pneumonia in cattle.[30] The increased prevalence of BHV-1 and PI-3 infections can be attributed to the factors, such as seasonal transitions, sudden temperature changes in the environment, inadequate hygiene and sanitation in farms, low ventilation, high animal density, and stress factors caused by various reasons. In studies conducted on bovine pneumonia in Türkiye, the rates of interstitial pneumonia have been reported as 20.93% by Ortatli,[31] 42.00% by Altun,[32] and 36.24% by Yaman and Gülcü.[33] This study aimed to investigate the potential presence of PI-3 and BHV-1, being important viral agents causing interstitial pneumonia in cattle, in Türkiye. In the study, the presence of PI-3 was detected at a rate of 6.72% in PCR tests and 8.33% in double IHC/IF staining methods, while the presence of the BHV-1 was not detected. In field studies on PI-3, Trigo Tavera *et al.*[34] reported a positivity rate of 66.66% in fattening cattle. In Türkiye, some studies have been conducted on the PI-3 in cattle lungs, and it has been reported that PI-3 infection is commonly observed among cattle. In a study covering the northeastern Anatolian border provinces, Yildirim *et al.*[35] reported a seroprevalence of 55.84%. In a study conducted on cattle in the Eastern Anatolia region, Çeribaşi *et al.*[36] reported an immunopositivity of 6.88%. In another study conducted on cattle, Çomakli *et al.*[37] reported a seropositivity rate of 9.33% in the Erzurum region of Eastern Anatolia. In our study, PI-3 was detected in cattle at abattoirs in Kayseri province in the Central Anatolia region with a prevalence of 6.72% in PCR tests, and 8.33% in double IHC and double IF staining methods. When compared to the studies conducted in Türkiye, the reported positivity rates for PI-3 are similar to the PI-3 positivity rate found in cattle pneumonias in the Kayseri region. Serological studies on BHV-1 have been conducted in various countries around the world, and the results of these studies show differing seropositivity rates. In countries where cattle farming plays a significant role in the national economy, such as Canada, Australia, and the United States, the seropositivity rates are reported to be variable and high.[38] In Belgium, the BHV-1 seroprevalence was reported to be 67.00% in a study conducted by Boelart *et al.*[39] In many countries, such as Switzerland, Sweden, Austria, Denmark, and Finland, a successful eradication program has managed to reduce the BHV-1 prevalence in animal husbandry to the minimal levels. Countries in Europe where vaccination is prohibited are considered seronegative for BHV-1. In Poland, where vaccination programs are implemented, the seroprevalence rate has been reported to be 20.00 - 38.00%, in Lithuania it is 17.00%, and in Italy, it ranges from 65.00 to 82.00%.[26] In Türkiye, there have been numerous serological studies conducted on infections caused by BHV-1 in cattle. Generally, the seroprevalence of BHV-1 in cattle in Türkiye ranges between 9.25% and 74.00%.[40] In a detailed study conducted on sheep, Albayrak *et al.*[41] reported a seroprevalence rate of 1.74% for the BHV-1. In the present study, the BHV-1 was not detected in bovine lung samples using three different methods. This finding is attributed to the low concentration of BHV-1 in the collected bovine pneumonia samples. A detailed literature review on the localization of bovine pneumonia lesions in the lungs reveals that there are very few studies conducted in this area. When examining previous studies on the anatomical localization of bovine pneumonia lesions, it has been reported that lesions typically form in the cranial lobes, with fewer occurring in other lung lobes.[24,42] The anatomical distribution of lesions in our study shows similarities with the limited number of previous studies. In our study, although pneumonia lesions were pre-dominantly localized in the cranial and caudal regions of the apical lobe, lesions were also found in the middle, accessory, and basal lobes of the lungs. Various techniques are used for the detection of viral agents. In the diagnosis of BHV-1, the commonly used methods include PCR, western blot, fluorescent antibody testing, enzyme-linked immuno-sorbent assay, and immunoperoxidase staining.[43,44] The diagnosis of PI-3 is performed using methods, such as virus isolation in cell cultures, PCR, enzyme-linked immunosorbent assay, and IHC.[45,46] In our study, both pathogens were investigated in a confirmatory manner using PCR, as well as double IHC and double IF methods. To the best of our knowledge, no study has been conducted to detect the presence of BHV-1 and PI-3 in cattle lung tissue using PCR and double IHC/IF methods. From this perspective, the present study stands out as the first research to use three different confirmatory techniques in the diagnosis of BHV-1 and PI-3. In studies examining interstitial pneumonia in cattle caused by PI-3, histopathological changes in the lungs have been described, including degeneration of alveolar and bronchial epithelia, acidophilic intra-cytoplasmic inclusion bodies in the alveolar epithelium, mononuclear cell infiltration, lymphoid tissue hyperplasia, and necrotic bronchiolitis.[47,48] In our study, the histopathological findings in PI-3 positive interstitial pneumonia included mononuclear cell infiltration, bronchial-bronchiolar epithelial hyperplasia, inter-alveolar fibrosis, desquamation of bronchial-bronchiolar epithelium, necrotic bronchiolitis, atelectasis, and lymphoid hyperplasia, exhibiting similar characteristics. One of the specific histopathological findings in PI-3-induced pneumonia in cattle is the presence of acidophilic intra-cytoplasmic inclusion bodies in the respiratory tract epithelium. It has been reported that inclusion bodies are not observed in histopathological examinations of viral pneumonia contaminated with secondary bacterial pathogens.[49] In our study, the absence of inclusion bodies in PI-3 positive samples suggests the possibility of secondary bacterial infections.

In conclusion, this study demonstrates the presence of two major viral etiological agents, BHV-1 and PI-3, in bovine pneumonia. While no cases of BHV-1 were detected in the pneumonia samples, PI-3 was identified in 6.72% of samples by PCR and 8.33% by double IHC/IF staining techniques. This study is significant as it is the first to investigate the presence of BHV-1 and PI-3 in bovine pneumonia using three different methods and establish their seroprevalence. Additionally, the findings are crucial for future research and efforts aimed at combating respiratory infections in cattle in the region.

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## Conflict of interest

We declare that there are no conflicts of interest among the authors.

## References

1. Martin SW, Lumsden JH. The relationship of hematology and serum chemistry parameters to treatment for respiratory disease and weight gain in Ontario feedlot calves. Can J Vet Res 1987; 51(4): 499-505.
2. Parent RA. Comparative biology of the normal lung. 2nd ed. San Diego, USA: Academic Press 2015; 747-788.
3. Ertürk A, Çizmeci ŞG, Barut F. Viral Etiology of Bovine Respiratory System Infections (2002-2005), [Turkish]. Etlik J Vet Microbiol 2006; 17(1-2): 29-42.
4. Virtala AM, Mechor GD, Gröhn YT, et al. Epidemiologic and pathologic characteristics of respiratory tract disease in dairy heifers during the first three months of life. J Am Vet Med Assoc 1996; 208(12): 2035-2042.
5. Metin N, Özer H, Çiftçi MK. Pathological investigation on pneumonia in goats in Elâziğ [Turkish]. Selçuk Üniv Vet Fak Dergisi 1988; 4(1): 231-237.
6. Grissett GP, White BJ, Larson RL. Structured literature review of responses of cattle to viral and bacterial pathogens causing bovine respiratory disease complex. J Vet Intern Med 2015; 29(3): 770-780.
7. Sozzi E, Lelli D, Barbieri I, et al. Isolation and molecular characterisation of respirovirus 3 in wild boar. Animals (Basel) 2023; 13(11): 1815. doi: 10.3390/ani13111815.
8. Fenner F, Bachmann PA, Gibbs EPJ, et al. 27. Paramyxoviridae In: Veterinary virology. 1st ed. London, UK; Academic Press 1987; 473-492.
9. Yener Z, Sağlam YS, Timurkaan N, et al. Immunohistochemical detection of parainfluenza type 3 virus antigens in paraffin sections of pneumonic caprine lungs. J Vet Med A Physiol Pathol Clin Med 2005; 52(6): 268-271.
10. Özdarendeli A, Bolat Y, Bulut H, et al. Development and utilization of an enzyme-linked Immunosorbent assay for detection of bovine anti-parainfluenza-3 antibodies [Turkish]. Firat Univ J Health Sci 1997; 11(2): 277-282.

11. Clark RK, Jessup DA, Kock MD, et al. Survey of desert bighorn sheep in California for exposure to selected infectious diseases. J Am Vet Med Assoc 1985; 187(11): 1175-1179.
12. Dungworth DL. The respiratory system. In: Jubb KVF, Kennedy PC, Palmer N (Eds). Pathology of domestic animals. 4th ed, Vol 2. San Diego, USA: Academic Press 1993; 589-613.
13. Petrini S, Curini V, Righi C, et al. Genomic characterization of a wild-type bovine alpha herpesvirus 1 (BoAHV-1) strain ısolated in an outbreak in central Italy. Viruses 2024; 16(1): 150. doi: 10.3390/v16010150.
14. d'Offay JM, Fulton RW, Fishbein M, et al. Isolation of a naturally occurring vaccine/wild-type recombinant bovine herpesvirus type 1 (BoHV-1) from an aborted bovine fetus. Vaccine 2019; 37(32): 4518-4524.
15. Roizmann B, Desrosiers RC, Fleckenstein B, et al. The family Herpesviridae: an update. The Herpesvirus Study Group of the International Committee on Taxonomy of Viruses. Arch Virol 1992; 123(3-4): 425-449.
16. Engels M, Ackermann M. Pathogenesis of ruminant herpesvirus infections. Vet Microbiol 1996; 53(1-2): 3-15.
17. Muylkens B, Thiry J, Kirten P, et al. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. Vet Res 2007; 38(2): 181-209.
18. Liebler-Tenorio EM, Lambertz J, Ostermann C, et al. Regeneration of pulmonary tissue in a calf model of fibrinonecrotic bronchopneumonia ınduced by experimental ınfection with *Chlamydia psittaci*. Int J Mol Sci 2020; 21(8): 2817. doi: 10.3390/ijms 21082817.
19. Fuchs M, Hübert P, Detterer J, et al. Detection of bovine herpesvirus type 1 in blood from naturally infected cattle by using a sensitive PCR that discriminates between wild-type virus and virus lacking glycoprotein E. J Clin Microbiol 1999; 37(8): 2498-2507.
20. Nišavić J, Knežević A, Stanojević M, et al. Molecular detection of bovine herpesvirus 1 (BoHV-1)in cattle in Serbia. Revue Med Vet 2018; 169(7-9): 180-184.
21. Atasever A, Mendil AS, Timurkan MO. Detection of bovine viral diarrhea virus and bovine herpes virus type 1 in cattle with and without endometritis. Vet Res Forum 2023; 14(10): 541-548.
22. Wen X, Sun J, Zhang Y, et al. Identification and genetic characterization of bovine paraifluenzavirus type 3 genotype c strain isolated from cattle in western China. Turk J Vet Anim Sci 2017; 41(2): 180-186.
23. Horwood PF, Gravel JL, Mahony TJ. Identification of two distinct bovine parainfluenza virus type 3 genotypes. J Gen Virol 2008; 89(Pt 7): 1643-1648.
24. Timurkan MO, Aydin H, Sait A. Identification and molecular characterisation of bovine parainfluenza virus-3 and bovine respiratory syncytial virus - first report from Turkey. J Vet Res 2019; 63(2): 167-173.
25. López A, Martinson SA. Respiratory system, mediastinum, and pleurae. Pathol Basis Vet Dis 2017: 471-560.
26. Çiftçi MK, Ortatli M, Erer H, et al. Veterinary systemic pathology [Turkish]. Vol 1. Konya, Turkey: Selcuk University Press 2015: 21-36.
27. Biotna website. Evaluation method for double immunohistochemistry (IHC). Available at: https:// biotna.net/wpx/wp-content/uploads/2022/02/TA DS02_Goat-X-Mouse.Rabbit-Double-Stain-Kit-New.pdf. Accessed April 25, 2025.
28. Highlander SK. Molecular genetic analysis of virulence in Mannheimia (Pasteurella) haemolytica. Front Biosci 2001; 6: D1128-D1150.
29. Taylor JD, Fulton RW, Lehenbauer TW, et al. The epidemiology of bovine respiratory disease: what is the evidence for preventive measures? Can Vet J 2010; 51(12): 1351-1359.
30. Panciera RJ, Confer AW. Pathogenesis and pathology of bovine pneumonia. Vet Clin North Am Food Anim Pract 2010; 26(2): 191-214.
31. Ortatatli M, Çiftçi MK. The incidence and pathology of pneumonia in beef cattle slaughtered in Konya Province [Turkish]. Veterinarium 2005; 16(1): 24-35.
32. Altun S. Investigation of mycoplasma bovis infection in bovine lungs with pneumonia by pathological and molecular methods. PhD Thesis, Atatürk University Institute of Health Sciences. Erzurum, Turkey: 2015.
33. Yaman I, Gülcü HB. Pathological and bacteriological investigation of pneumonia in fattened calves. Eur J of Vet Sci 2002; 18(3-4): 99-108.
34. Trigo Tavera FJ, Chávez Gris G, Juárez Barranco F, et al. Viral participation in respiratory disease in feedlot cattle, as identified by immunohistochemistry. Veterinaria Mexico 2009; 34(1): 1-12.
35. Yildirim Y, Yilmaz V, Faraji Majarashin AR. Seroprevalance of viral respiratory system ınfections in cattlein the border provinces of north-east Turkey [Turkish]. Kafkas Univ Vet Fak Derg 2009; 15(4): 601-606.
36. Çeribaşi AO, Özkaraca M, Ceribasi S, et al. Histopathologic, immunoperoxidase and immuno-fluorescent examinations on natural cattle pneumonia originated from Parainfluenza type 3, respiratory syncytial virus, adenovirus type 3 and herpesvirus type 1. Rev Med Vet 2014; 165: 201-212.
37. Çomakli S, Sağlam YS, Özkaraca M, et al. The determination of parainfluenza-3 viral antigens using immunohistochemical methods in cattle lungs in Erzurum region [Turkish]. Atatürk Univ J Vet Sci 2018; 13(3): 309-316.
38. Ackermann M, Engels M. Pro and contra IBR-eradication. Vet Microbiol 2006; 113(3-4): 293-302.

39. Boelaert F, Speybroeck N, de Kuif A, et al. Risk factors for bovine herpesvirus-1 seropositivity. Prev Vet Med 2005; 69(3-4): 285-295.
40. Aslan ME, Azkur AK, Gazyagci S. Epidemiology and genetic characterization of BVDV, BHV-1, BHV-4, BHV-5 and *Brucella* spp. infections in cattle in Turkey. J Vet Med Sci 2015; 77(11): 1371-1377.
41. Albayrak H, Yazici Z, Okur-Gumusova S. Seroprevalence to bovine herpesvirüs type 1 in sheep inTurkey. Vet Arhiv 2007; 77(3): 257-263.
42. Breeze R. Respiratory disease in adult cattle. Vet Clin North Am Food Anim Pract 1985; 1(2): 311-346.
43. Keuser V, Schynts F, Detry B, et al. Improved antigenic methods for differential diagnosis of bovine, caprine, and cervine alphaherpesviruses related to bovine herpesvirus 1. J Clin Microbiol 2004; 42(3): 1228-1235.
44. Majumder S, Ramakrishnan M, Nandi S. Infectious bovine rhinotracheitis: an Indian perspective. Int J Curr Microbiol Appl Sci 2015; 4(10): 844-858.
45. Alkan F, Ozkul A, Bilge-Dagalp S, et al. Virological and serological studies on the role of PI-3 virus, BRSV, BVDV and BHV-1 on respiratory infections of cattle. I. The detection of etiological agents by direct immunofluorescence technique. Dtsch Tierarztl Wochenschr 2000; 107(5): 193-195.
46. Grubor B, Gallup JM, Meyerholz DK, et al. Enhanced surfactant protein and defensin mRNA levels and reduced viral replication during parainfluenza virus type 3 pneumonia in neonatal lambs. Clin Diagn Lab Immunol 2004; 11(3): 599-607.
47. Aly NM, Shehab GG, Abd el-Rahim IH. Bovine viral diarrhoea, bovine herpesvirus and parainfluenza-3 virus infection in three cattle herds in Egypt in 2000. Rev Sci Tech 2003; 22(3): 879-892.
48. Çeribasi S, Özkaraca M, Özer H, et al. Prevalence of parainfluenza type 3 virus antigens in pneumonic caprine lungs by direct immunofluorescence and immunoperoxidase techniques. Small Rumin Res 2012; 108(1-3): 127-132.
49. Viuff B, Uttenthal A, Tegtmeier C, et al. Sites of replication of bovine respiratory syncytial virus in naturally infected calves as determined by in situ hybridization. Vet Pathol 1996; 33(4): 383-390.