Antigenic and histopathologic evaluation, with molecular characterization and identification of BPIV3 in cattle with respiratory system infections

ALI KÜÇÜK[1*], YAKUP YILDIRIM[2]

[1] Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Animal Hospital, Burdur, Türkiye
[2] Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Virology, Burdur, Türkiye

SUMMARY

Parainfluenza virus is one of the viral agent- which is nowadays a major health issue worldwide causing respiratory system diseases in cattle. This study aimed to determine the role of Bovine Parainfluenzavirus 3 (BPIV3) in respiratory system diseases in cattle by molecular and virological analyses. A total of 570 nasal swabs, conjunctival swabs and leukocyte samples were taken from 190 cattle of varying races, ages, and genders. All Sampled cattle not vaccinated against the BPIV3 and have respiratory system infection symptoms. The inoculum prepared from swab samples were planted into MDBK and Vero cell lines using the adsorption method for virus isolation. Although the four blind passages were not isolated BPIV3, all samples underwent the RT-PCR technique in which the viral genome was detected in two nasal swab samples, while the viral nucleic acid could not be detected in conjunctival, leukocyte samples, or cell culture supernatants. As a result of the sequence analysis applied on one of these samples, the agent was proved as the BPIV3 genotype strain. Additionally, the DIF (Direct Immunofluorescence) test applied on samples prepared from 380 nasal and conjunctival swabs, BPIV3 antigenic structures were seen in six swab samples, while the virological diagnosis was not performed from conjunctival swab samples, the virological diagnosis was performed by the DIF test in two samples which the viral genome was detected. Histopathological studies were carried out on a lung tissue sample of an animal that was positive for molecular and virological BPIV3. These studies has been revealed that few histopathological findings, such as septal tissue thickening indigenous to pneumonia cases and inflammatory cell infiltration. Henceforth, it has been suggested that BPIV3 is a crucial etiological agent while its genotype C is in circulation among the cattle susceptible to respiratory system infections in southwest of Turkey.

KEY WORDS

Bovine Parainfluenza Virus Type 3, Histopathology, Phylogenetic Analysis, Prevalence.

INTRODUCTION

The *Bovine Parainfluenzavirus Type 3* (BPIV3), order *Mononegavirales* is an RNA virus in the *Respirovirus* genus of *Orthoparamyxovirinae* subfamily and *Paramyxoviridae* family[21,30]. Morphologically, they are enveloped viruses containing a non-segmented, negative-sense, single-stranded ribonucleic acid (RNA)[9] and encode six structural proteins, namely, nucleoprotein (N), matrix protein (M), phosphoprotein (P), fusion protein (F), hemagglutinin-neuraminidase (HN) and large protein (L) as well as three non-structural proteins V, C and D[10,34]. A study on genetic domains coding M and HN proteins revealed that BPIV3 had three different genotypes: BPIV3 genotype A, BPIV3 genotype B, and BPIV3 genotype C[9,44].

Although BPIV3 causes endemic infections in many animal species, especially cattle[9,10] certain immunosuppressive factors such as aerosol usage, stress, poor nutrition, and unhygienic living conditions predispose them to diseases in many direct or indirect ways[32,34]. Although BPIV3 might cause diseases in animals of all ages, its prevalence is higher in animals aged two to eight months[9]. When BPIV3 distribution was correlated with the seasonal variations, it was observed that as the virus mostly proceeded subclinically, it caused more frequent infections in the autumn and winter months. In contrast, in conjunction with other co-pathogens, it developed clinical symptoms such as dyspnea, cough, high fever as well as persistent nasal and conjunctival discharge[21].

Molecular techniques, such as RT-PCR, are frequently used with other serological tests, such as ELISA, immunofluorescence, hemagglutinin inhibition (HI), and virus neutralization, for diagnosing BPIV3. These methods are also coupled with the virus isolation method to confirm the diagnosis[10,34].

This study aimed to detect BPIV3 by using molecular techniques employed for nasal and conjunctival swabs along with the leukocyte samples taken from the non-vaccinated, affected animals with showing symptoms of respiratory infection. Antigen-based detection of conjunctival and nasal swab samples taken from the same animals was also carried out. In addition to that, the strain detected by sequence analysis and the phylogenetic tree illustration was genetically similar to other known strains.

MATERIALS AND METHODS

Preparing the animals and samples

Our study included a total of 570 nasal swabs (NS) and conjunctival swabs (CS) along with samples taken from 190 non-vaccinated cattle of different races, ages, and genders showing clinical findings of respiratory infection (Table 1). Bovine blood

Corresponding Author:
Ali Küçük (alikucuk@mehmetakif.edu.tr).

Table 1 - General information about sampled animals.

Data of Sampled Animals	
Number of Animal Sampled (n)	190
Age	
< 1 age	137
> 1 age	53
Gender	
Female (♀)	92
Male (♂)	98
Races	
Montofon (Brown Swiss)	40
Simmental	78
Holstein	72

samples from *Vena jugularis* were kept in EDTA (5 mL) tubes, while nasal and conjunctival swab samples were kept in dulbecco's modified eagle medium (DMEM, 1.5 mL) mixed with penicillin (500 IU/mL) and streptomycin (20 mg/mL) according to the cold chain procedure.

After centrifugation for ten minutes at 1200 rpm and 4ºC, leukocyte layer was dispensed by a sterile Pasteur pipette into sterile tubes (2 mL) containing phosphate-buffered saline (PBS, 1 mL). Leukocyte (250 µL) was centrifuged at 2000 rpm at 4 ºC for ten minutes was taken out and put into RNase DNase free tubes (2 mL), which was later added to total RNA isolation commercial solution (750 µL) and kept under –80ºC for further molecular studies. The NS and CS samples, after evaluation by vortex, were centrifuged at 3000 rpm and 4ºC for 20 min followed by the supernatant (250 µL) extraction. The samples were transferred into RNase DNase free tubes (2 mL), and total RNA isolation commercial solution (750 µL) was added to and was kept at –80ºC until the nucleic acid extraction. The remaining supernatant was kept at –80ºC in sterile tubes (2 mL) for further usage in cell culture studies. The NS and CS samples, were centrifuged at 1200 rpm and 4ºC for ten minutes. Obtained pellet was diluted with DMEM (50 µL) and spread of microscope slide. Smear of cells were dried at room temperature and cooled in chilled-acetone at –20ºC for 15 min. Simultaneously, a necroscopic sample was collected for histopathological examination from an animal that died one week after the sample collection and detection of BPIV3 by molecular and antigenic analysis.

Cell line and virus isolation

The virus was isolated from madine-darby bovine kidney (MDBK) and African green monkey kidney (Vero) cell lines. The cells were grown in 24-well microplates in 10% fetal bovine

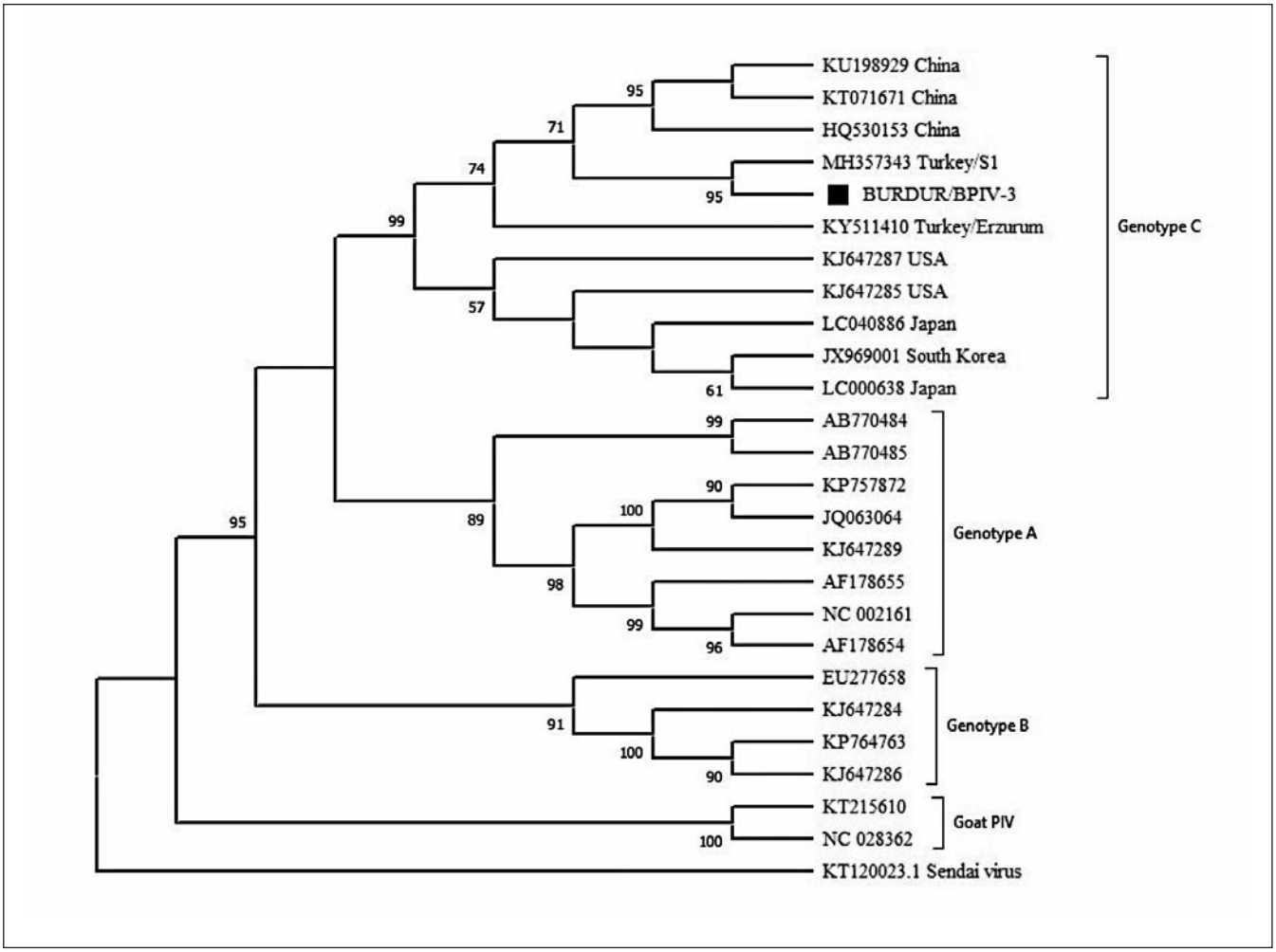

Figure 1 - Phylogenetic tree generated based on the nucleotide sequence alignment of the BPIV3 matrix (M) gene region of the virus obtained in the study (BURDUR/BPIV-3/ Feb.2019) and viral strains from Genbank, unrooted.

serum (FBS) and 1% DMEM containing penicillin/streptomycin. A mixture of cell culture suspension (1 mL) and DMEM was added into each well, followed by the incubation of cells in an incubator (Nüve, Turkey) with a 5% CO_2 at 37°C.
The plates were subcultured when 80% of the plate base was covered.
Swab samples kept in a deep freezer (–80 °C) were thawed in a 37°C water bath, and then inoculum was prepared by passing them through 0.22 µm pore size filters. A total of 400 µL solution was inoculated into each microplate well, and the place was transferred to a 5% CO_2 incubator at 37°C. After incubation for one hour, the inoculum was removed and mixed with non-serum DMEM (2 mL) and then reincubated with the same volume, followed by the examination of the plate for 4–10 days with an inverted microscope for detecting probable cytopathogenic effects appearing in cells. In spite of four blind passages acquired using microplate supernatants, non-cytopathogenic effect was observed in the cells. Thus, the samples subjected to PCR analysis were further confirmed.

RNA extraction, RT-PCR and sequencing

All samples underwent RNA extraction using a commercial RNA isolation kit (Wizbiosolutions, WizolReagent, Korea), following the manufacturer's instructions. In order to detect BPIV3 by molecular technique, specific primers were synthesized targeting the gene, coding for M protein, with a length of 328 base pair (bp) (Mforw: AGTGATCTAGATGATGATCCA Mrev: GTTATTGATCCAATTGCTGT)[22]. All samples were treated with the one-step RT-PCR kit (Geneall®HyperScript™one-step RT-PCR master mix, Korea) in which RNA (5 µL) was added to RNAse-Free Water (2,5 µL), BPIV3 M for (1 µL), BPIV3 M rev (1.5 µL) and a master mix (10 µL) made up of (dNTP mix (10 mM), $MgCl_2$ (2.5 mM), Hyperscript™ reverse transcriptase, AmpONE™ HS-Taq DNA polymerase) in a total reaction volume (20 µL). Firstly, a complementary DNA (cDNA) was synthesized at 55°C for 30 min followed by viral genome amplification in 35 cycles comprising of 95°C for 15 min predenaturation, 94°C for 1 min denaturation, 1 min annealing at 50°C, and 72°C for 1 min extension. The amplicons were resolved in 1.2% agarose gel containing SafeView™ Classic (0.07 µL/mL, ABM, Canada) and were sequenced (Letgen Biotechnology, Turkey).
The PCR products were directly sequenced by Microsynth AG (Balgach, Switzerland) using the PCR primers. The nucleotide sequencing results were analyzed using DNA Dynamo DNA sequence analysis software along with the comparison of the consensus nucleotide sequences obtained by the Basic Local Alignment Search Tool (BLAST) software of the National Center for Biotechnology Information (NCBI)[3]. The M gene region's phylogenetic analysis targeting a 328-bp region was performed using 26 BPIV-3 sequences obtained from the GenBank database. The phylogenetic analyses were conducted using the MEGA X version[20,35] with the maximum likelihood (ML) evo-

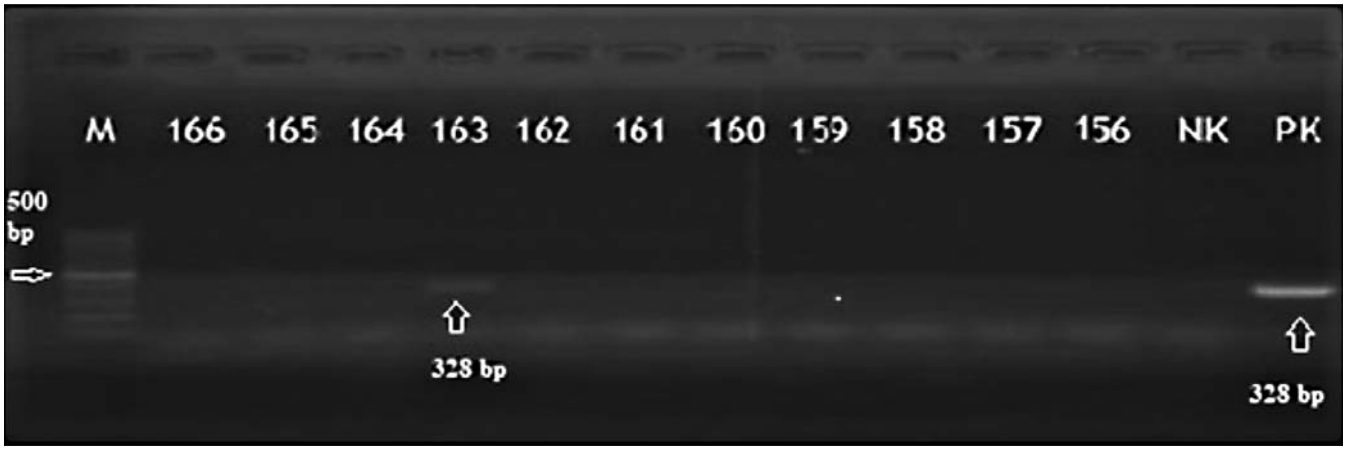

Figure 2 - Gel electrophoresis image of BPIV3 RT-PCR products, 156-166 field nasal swab samples, 163 positive nasal swab samples.

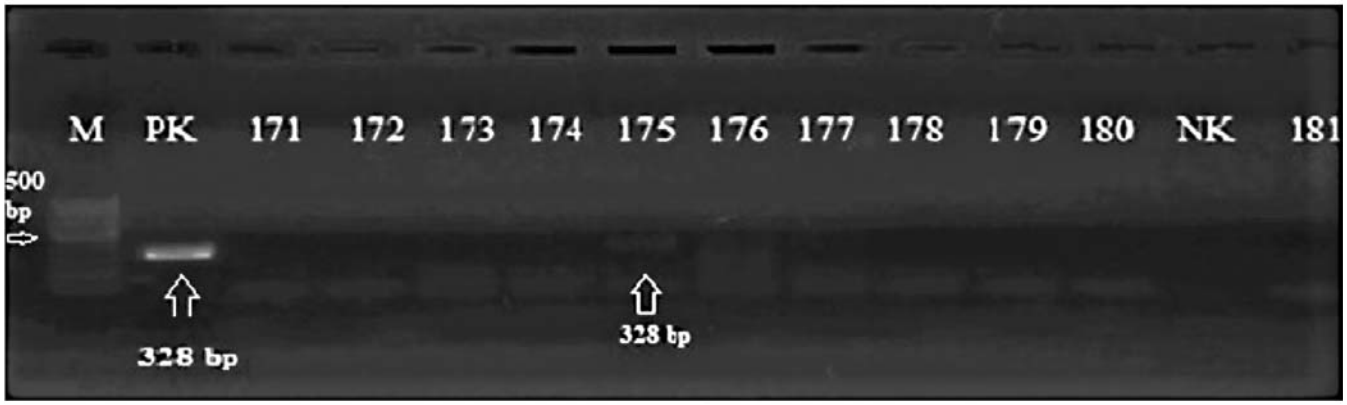

Figure 3 - Gel electrophoresis image of BPIV3 RT-PCR products, 156-166 field nasal swab samples, 175 positive nasal swab samples.

lutionary tree based on the Tamura-Nei (TN92G+I) model[20,35] with 1,000 bootstrap replicates for the genetic distance determination between the associated nucleotide sequences.

Antigenic detection using direct immunofluorescence

All conjunctival and nasal swab samples underwent DIF in which the pre-fixed cells were stained with polyclonal antiserum conjugate (VMRD, USA) marked with a fluorescent dye, Fluorescein isothiocyanate (FITC). This assay revealed the presence of antigen after incubating at 37°C for 30 min in a humid environment. The cells were evaluated under a fluorescent microscope (Olympus Corp., Tokyo, Japan).

Histopathology

A lung tissue sample taken from an animal that underwent necropsy was used for histopathologic examination. The samples were blocked with 10% formaldehyde and fixed using paraffin, alcohol, and xylene. The samples were cut into 5-micron sections and transferred on slides. The sections were stained with hematoxylin and eosin and examined under a microscope (Olympus Co., Tokyo, Japan). A morphometric analysis was also carried out by Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

RESULTS

Virus isolation

All nasal and conjunctival swab samples taken from the 190 study animals were planted on the MDBK and Vero cell lines for isolating BPIV3. Despite four blind passages, non- cytopathological event was observed in the microscopic examinations.

Molecular and sequence analysis

Of the nasal and conjunctival swabs along with leukocyte samples obtained from 190 study animals, the RT-PCR technique detected BPIV3 viral nucleic acid only two nasal swab samples (sample no: 163 and 175, Table 2). The positive nasal swab samples were obtained in the months of December-February when respiratory disease symptoms were frequently observed for 3–7 days, such as abdominal respiration, fever >39°C, mucopurulent nasal flow, and cough. The phylogenetic analysis of the 163NS sample yielded an etiologic agent BPIV3, by RT-PCR technique using primers specific to a gene for M protein. Additionally, this agent was identified in the GenBank database with accession no. MT949524. In contrast, the other positive sample, 175NS showed no sequence similarity in the database. The reason for this unusual fact might be that due to its fragile structure, the extracted viral RNA got degraded during storage or transportation. The sequence similarity of BPIV3 based on M gene amplicons (328 bp) is given in Table 3.

Table 2 - Comparison of positivity rates according to test results.

Samples	Number of Samples (*n*)	Number of Positive Samples (%)	
		RT-PCR	DIF
Leukocyte	190	%0 (0/190)	-*
Nasal swab	190	%1,05 (2/190)	%3.15 (6/190)
Conjunctival swab	190	%0 (0/190)	%0 (0/190)

* No Test Available

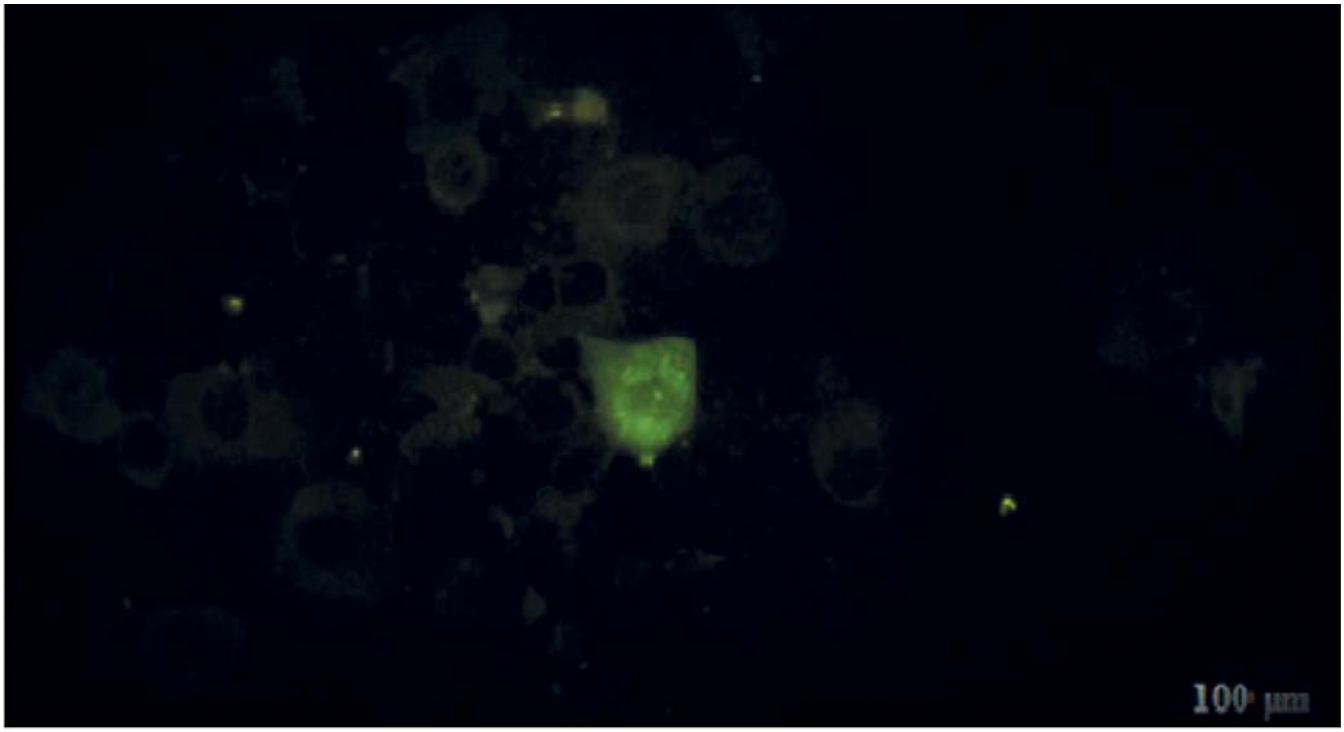

Figure 4 - DIF Positive nasal swab sample (sample number 163).

Table 3 - The nucleotide similarity of the sequence obtained in the study and the sequences referenced from Genbank.

Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1 KT215610	-																								
2 NC028362	100	-																							
3 EU277658	66,33	66,33	-																						
4 KJ647284	67,27	67,27	89,16	-																					
5 KP764763	66,62	66,62	89,98	99,35	-																				
6 KJ647286	66,62	66,62	89,98	99,35	100	-																			
7 KY511410	62,58	62,58	82,74	80,28	80,28	80,28	-																		
8 MH357343	62,74	62,74	85,36	82,5	82,5	82,5	97,99	-																	
9 BUR/BPIV3	62,74	62,74	85,31	82,5	82,5	82,5	97,99	100	-																
10 HQ530153	60,59	60,9	85,71	81,97	81,97	81,97	97,65	99,2	99,2	-															
11 KU198929	60,59	60,59	85,71	81,97	81,97	81,97	97,65	99,2	99,2	100	-														
12 KT071671	60,59	60,59	85,71	81,97	81,97	81,97	97,65	99,2	99,2	100	100	-													
13 JX969001	63,52	63,52	81,85	79,34	79,34	79,34	96,59	97,31	97,31	96,97	96,97	96,97	-												
14 KJ647287	60,47	60,47	82,84	80,39	80,39	80,39	97,3	98	98	96,97	97,67	97,67	98,68	-											
15 KJ647285	61,27	61,27	82,32	80,94	80,94	80,94	96,94	97,65	97,65	97,31	97,31	97,31	99,02	99,02	-										
16 LC040886	63,41	63,41	82,84	80,39	81,38	81,38	95,88	96,62	96,62	96,28	96,28	96,28	98,69	98,01	98,35	-									
17 LC000638	63,41	63,41	81,9	79,9	79,9	79,9	96,59	97,31	97,31	96,98	96,98	96,98	99,35	98,69	99,02	98,69	-								
18 AB770484	70,06	70,06	76,45	81,21	81,21	81,21	84,65	85,68	85,68	84,28	84,28	84,28	86,61	86,64	86,16	88,38	87,52	-							
19 AB770485	70,06	70,06	76,45	81,21	81,21	81,21	84,65	85,68	85,68	84,28	84,28	84,28	86,61	86,64	86,16	88,38	87,52	100	-						
20 KJ647289	65,39	65,39	78,08	78,58	78,58	78,58	79,28	80,45	80,45	81	81	81	80,89	82,02	80,39	81,91	81,91	89,34	89,34	-					
21 KP757872	64,88	64,88	76,77	78,97	78,97	78,97	78,01	79,22	79,22	79,78	79,78	79,78	79,66	80,03	80,28	80,72	80,72	89,34	89,34	99,35	-				
22 JQ063064	64,88	64,88	76,77	78,97	78,97	78,97	78,01	79,22	79,22	79,78	79,78	79,78	79,66	80,03	80,28	80,72	80,72	89,34	89,34	99,35	100	-			
23 NC002161	67,87	67,87	78,01	81,6	81,6	81,6	80,56	80,62	80,62	81,16	81,16	81,16	82,7	82,75	82,22	84,61	83,68	92,44	92,44	93,43	93,46	93,46	-		
24 AF178654	67,87	67,87	78,01	81,6	81,6	81,6	80,56	80,62	80,62	81,16	81,16	81,16	82,7	82,75	82,22	84,61	83,68	92,44	92,44	93,43	93,46	93,46	100	-	
25 AF178655	67,77	67,77	79,6	80,6	80,6	80,6	81	82,12	82,12	82,65	82,65	82,65	83,12	83,17	82,65	84,1	84,1	92,03	92,03	94,6	94,62	94,62	99.02	99.02	-

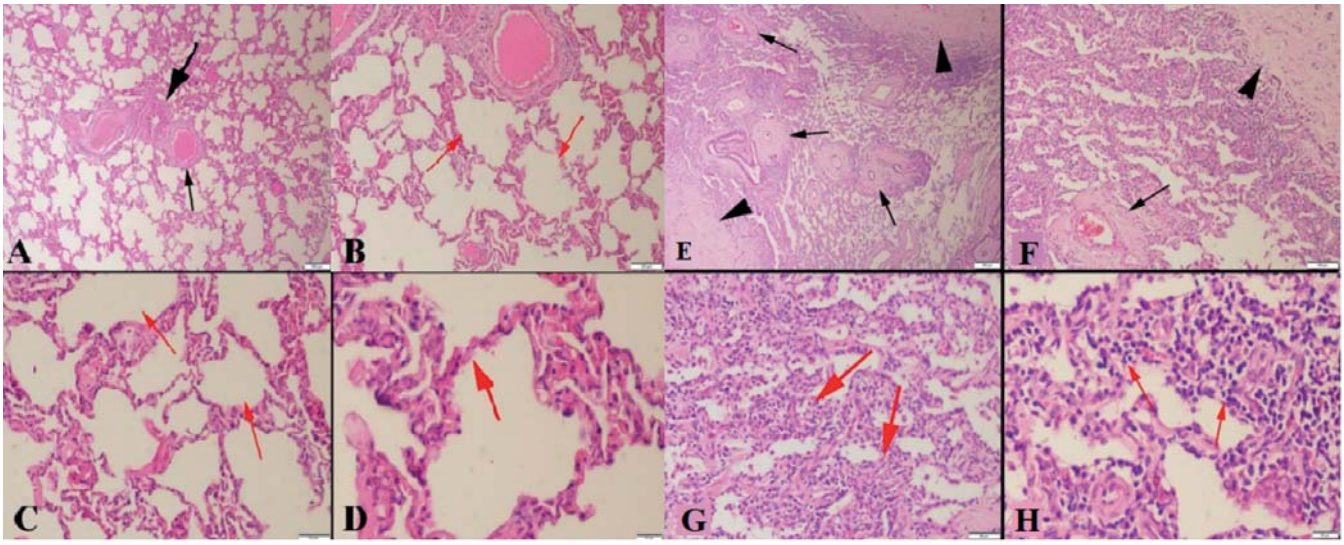

Figure 5 - The histological view of a normal looking lung (A), lung veins by small scale magnification (black thin cursor) and black (thick cursor) normal microscopic view of a bronchiole, Bar= 200µm. (B), view of alveoles (red thin cursors), Bar= 100µm. (C), close view of alveoles (red thin cursors), Bar= 50µm. (D), view of alveole septal tissue (red thick cursor), Bar= 20µm, Hemotoxylin Eosine (HE). Histopathological view of a lung with pneumonia (E), increase in veining (black thin cursors) and common ligament growth (black cursor heads), Bar= 200µm. (F), close view of veining and ligament increase, Bar= 100µm. (G), septal tissue thickening (red thick cursors), Bar= 50µm. (H), inflammatory cell infiltration (red thin cursors), Bar= 20µm.

Antigenic analysis

Antigenic analysis was carried out by the DIF method for the 380 nasal and conjunctival swab samples taken from 190 animals. Of them, BPIV3 antigen was found in six nasal swab, while BPIV3 could not be detected in any of the conjunctival swab samples (Table 2).

Histopathological results

The animal number 163, whose nasal swab sample showed the presence of BPIV3 by RT-PCR and DIF tests and was vaccinated against other respiratory infection pathogens (Bovine Herpesvirus-1 (BHV-1), Bovine Viral Diarrhea Virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV), Pasteurella, etc.) died. After conducting necropsy, the obtained lung tissue samples underwent a histopathological examination, using a healthy animal's lung as a control. No macroscopic or microscopic histopathologic finding was visible in the lung used as control, while macroscopic samples of BPIV3 infected lesions showed severe venous hyperemia and partly necrotic areas. Other findings, such as an increase in epithelial cell proliferation along with septal tissue thickness and inflammatory cell infiltration, were also noted. Since the animal undergoing histopathological examination was vaccinated against other agents causing respiratory tract infection, it was suggested that the lung's pathological findings were caused by BPIV3.

Owing to the fact that as the infected lung areas were placed in formaldehyde following the necropsy, the antigenic or molecular diagnosis could not be performed on such tissues.

DISCUSSION

The Bovine respiratory disease complex (BRDC) is one of the commonly observed multifactorial bovine health problems around the world caused by several viral pathogens like BPIV3, BHV-1, BCoV (Bovine Coronovirus), BRSV, BAV (Bovine Adenovirus), BVDV as well as bacterial ones like *Pasteurella multocida, Mannheimia haemolytica, Histophilus somni*[12,15]. Based on this, the winter and autumn months are generally preferred as the BPIV3 sampling period due to its proceeding subclinical manifestations. There are several predisposing factors associated with disease aggravation like frequent infections, weight loss, decreased carcass quality, inadequate prophylaxis, increased veterinary expenditures, decreased fertility, as well as fatal pneumonia caused by co-infection with other pathogens causing extensive economic losses[10]. While a serological and epidemiological study conducted by Tiwari et al.[37] in 2016 performed on livestock and dairy animals revealed that BPIV3 is endemic all over the world, a seropositivity rate of 18–94.6% was also detected in various Turkish serological studies[1,13,16,24,29,41,42,43]

This study aimed to diagnose BPIV3 by RT-PCR technique using nasal and conjunctival swabs along with blood samples of 190 non-vaccinated animals of different races, ages, and genders showing respiratory infection. Simultaneously, DIF for nasal and conjunctival swab samples of the same animals was also conducted for antigen-based diagnosis. A histopathological examination was also carried out for lung tissue samples following the necropsy of an animal vaccinated against other viral or bacterial pathogens (BRSV, BHV-1, BVDV, Pasteurella, etc.), causing respiratory infections for determining the effects of the virus on tissues.

Our study was not successful in conducting the virus isolation procedure while performing cell culture plating. The same was also substantiated by Noori et al.[26] in their 2014 study that was conducted on the virus isolation technique from cell culture passages. In their study, there was only one case of virus isolation out of 25 positive samples detected in DIF and PCR analysis. This suggested that viruses, due to their fragile structure, could hardly adapt to cell lines. Thus cell culture studies are not good in diagnosing BPIV3 infections.

Several studies on BPIV3 molecular detection have been conducted worldwide[15,17,18,23,27,39,44]. In this study, a variable prevalence, between 0% and 21.6%, has been reported. Similarly, a Turkish study by Hacioglu[14] in 2011 revealed a positivity rate

of 1.41% (1/71) in the nasal swab samples by RT-PCR technique, while Timurkan et al.[36] in 2019 as well as Toker and Yesilbag[38] in 2021, reported a positivity rate of 1.94% (3/155) and 0.51% (1/193) in nasal swab and lung tissue samples, respectively. Comakli et al.[7], in their study on teat tissue samples, detected BPIV3 viral genome at a rate of 21.67% (26/120) by qRT-PCR technique.

This difference in prevalence values was due to several variables like the specificity (different primers) and sensitivity (qPCR) of the applied molecular diagnosis technique, the sample's viral load (nasal swab, lung tissue sample, tracheal sample), the animal's state being seropositive or convalescent as well as the fragility of the agent.

163 NS strain obtained in this study was located in BPIV3 genotype C and was detected in close genetic relationship with some other isolates discovered in China, Turkey, Japan, the USA, and South Korea. A comparative study with other isolates by Albayrak et al.[1] in 2019 revealed that it was 99.68% similar to the isolate (MH357343) found in the Samsun study, while Timurkan et al.[36] in 2019 described a similarity of 97.99% to the one found in the Erzurum study. In accordance with this information, our study supported the fact that BPIV3 genotype C was in circulation in various cattle populations around Turkey. This study is the first one that has attempted both antigen-based and molecular detection along with genetic evaluation of BPIV3 in south of Turkey.

The strain obtained in our study showed similarities to the isolates (HQ530153, KU198929, KT071671) discovered by Zhu et al.[44] and Wen et al.[40] in China at a rate of 99.2%, as well as the isolates (LC000638, LC040886) obtained in a Japanese study by Konishi et al[19] in 2014 at the rates of 96.62% and 97.31%, respectively. It was also similar to the virus (JX969001) isolated by Oem et al.[28] in South Korea at a rate of 97.31% and also to the strains (KJ647285, KJ647287, KJ647289) described by Neill et al.[25] in the USA at the rates of 97.65%, 98%, and 80.45%, respectively. On the other hand, it had a 79.22% genetic similarity with the strain (KP757872) isolated by Sobhy et al.[33] in Egypt, while the strain's genetic similarity obtained in our study to SF-4 was accepted as the reference strain (AF178655) and was designated as 82.12% Bailley[4].

Several previous studies conducted on BPIV3 antigen-based detection by DIF test reported a prevalence rate between 9.8% and 41.5%[2,5,6,8,11,17,22,31]. Our study also observed a positivity rate lower than the other studies, which might have occurred due to conscious breeding in the sampling areas, improved veterinary services, common vaccination applications, and better coping strategies with infections. Based on this, it was suggested that both mucosal and systemic immunity play a crucial role in maintaining a lower positivity rate as mucosal immunity prevents the infection and decreases the dispersion, while systemic immunity allows an infection to have lesser manifestations[9,21]. As the sampled animals had strong mucosal immunity, the presence of seropositivity, as well as maternal antibodies against the agent, clearly depicted the interplay among several other biological agents resulting in a lower prevalence rate.

In our study, the diagnosis was clear in animals whose nasal swab samples were obtained for BPIV3 detection through molecular and antigen-based analyses. The presence of clinical symptoms in animals without the detection of BPIV3 viral genome confirmed the presence of a respiratory system infection either caused by other viral, bacterial, or parasitic pathogens or due to secondary infections occurring after BPIV3 infection. The disease did not contain the agent as either the agent or the animals were in a convalescence period since one or two weeks had passed after the appearance of severe symptoms. On the other hand, the agent's detection could not be performed on conjunctival swab samples as it showed a greater affinity toward respiratory tract cells, where N-acetyl-neuraminic acid (NANA) receptor molecules such as BPIV3 pneumocytes, bronchial and tracheal epithelial cells were abundant[21]. Additionally, due to the absence, or an extremely shorter viremia period, the absence of virus detection in blood samples was considered a normal occurrence.

CONCLUSIONS

To conclude, due to a higher positivity rate observed in the DIF test as compared to the RT-PCR during the laboratory diagnosis of BPIV3 infection, more studies involving a greater number of samples are required to determine and compare the specificity and sensitivity of the above-mentioned methods in diagnosing BPIV3 infections. On the other hand we believe that the DIF method is practical and reliable for virologic detection of BPIV3 from nasal swap samples, it is a cheaper and faster diagnostic method compared to molecular methods and virus isolation studies. For an accurate virological diagnosis during the acute phase, nasal swab samples should be collected properly and must be brought quickly to the diagnostic laboratories in a suitable transport medium under the cold chain distribution process. Additionally, it should also be considered that nasal swab samples should always be collected in the acute phase of the disease and from the animals showing respiratory infection symptoms for an effective virological and molecular diagnosis. On the other hand, we believe that genetic differences between BPIV3 isolates are important to determine the strain to be used for vaccine production. Therefore, phylogenetic studies should be performed that include more and different gene regions.

Declaration

Availability of data and material All data and material are available.

Code availability Not applicable for that section

Author's contribution YY and AK conceived and designed research. AK and YY conducted experiments. YY and AK contributed new reagents or analytical tools. YY and AK analyzed data. YY and AK wrote the manuscript. All authors read and approved the manuscript.

Funding: This research was supported by Burdur Mehmet Akif Ersoy University Research Fund (Project No: 0476-DR-17) as a PhD thesis on "Investigation of Parainfluenza Virus Type 3 (BPIV3) in Cattle Using Direct Immunfluorescence (DIF) Test and Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) Technique".

Ethics Approval The sampling was conducted according to permission from local ethical committee for animal experiments (MAKU-HADYEK 318/2017).

Consent to Participate Consent of participation in this study was obtained.

Consent of Publication All authors agree to publish this manuscript.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this article.

References

1. Albayrak H., Yazıcı Z., Ozan E., Tamer C., Abdelwahed A., Wehner S., Ulrich K., Weidmann M. (2019). Characterisation of the first bovine parainfluenza virus 3 isolate detected in cattle in Turkey. Vet Sci 6, 1-6. https://doi.org/10.3390/vetsci6020056
2. Alkan F., Bilge Da alp S., Ye ilba K., O uzo lu T.Ç., Akça Y., Burgu I. (2000). Virological and serological studies on the role of PI-3 virus, BRSV, BVDV, BHV-1 on respiratory infectious of cattle. I. The detection of etiological agents by direct immunofluorescence technique. Detsch Tieraezt Wochensch, 107, 193-195.
3. Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., (1990). Basic local alignment search tool, Journal of Molecular Biology. 215, 403-410.
4. Bailley J.E., McAuliffe J.M., Skiadopoulos M.H., Collins P.L., Murphy B.R. (2000). Sequence determination and molecular analysis of two strains of bovine parainfluenza virus type 3 that are attenuated for primates. Virus Gen, 20: 173-182. https://doi.org/10.1023/a:1008130917204
5. Çeriba ı A.O., Özkaraca M., Çeriba ı S., Özer H. (2014). Histopathologic, immunoperoxidase and immunofluorescent immunofluorescent examinations on natural cattle pneumonia originated from parainfluenza type 3, respiratory syncytial virus, adenovirus type 3 and herpesvirus type 1. Rev Med Vet, 165:201-212.
6. Çeriba ı S., Özkaraca M., Özer H., Çeriba ı A. (2012). Prevalence of parainfluenza type 3 virus antigens in pneumonic caprine lungs by direct immunofluorescence and immunoperoxidase techniques. Small Rum Res, 108: 127-132. https://doi.org/10.1016/j.smallrumres.2012.07.033
7. Çomaklı S., Özdemir S. (2019). Comparative evaluation of the immune responses in cattle mammary tissues naturally infected with bovine parainfluenza virus type 3 and bovine alphaherpesvirus-1. Pathogens, 8: 26. https://doi.org/10.3390/pathogens8010026
8. Delgado I.D., Valle M.B., Barrero N.R., Miranda L.S., Elvira S.M., Carrasco A.C. (2005). Use of direct immunofluorescence and immunoperoxidase diagnostic kits for bovine parainfluenza 3 and bovine herpes virus 1 in Cuba. Vet Mex, 36: 295-301.
9. Elenkumaran S. (2013). Bovine Parainfluenza Virus 3. In: Mononegaviruses of Veterinary Importance Volume 1: Pathobiology and Molecular Diagnosis. Ed. Munır M., 118-140, CABI, London,
10. Ellis J.A. (2010). Bovine Parainfluenza-3 Virus. Vet Clin North Am Food Anim Pract, 26: 575-593. https://doi.org/10.1016/j.cvfa.2010.08.002
11. Gençay A., Akça Y. (2004). Direkt immunofloresan ve mikronötralizasyon testleri ile koyunlarda parainfluenza-3 (PI-3) virus enfeksiyonunun ara tırılması. Erciyes Üniv Vet Fak Derg, 1:91-96.
12. Griffin, D., Chengappa, M.M., Kuszak, J., McVey, D.S. (2010). Bacterial pathogens of the bovine respiratory disease complex. Vet Clin North Am Food Anim Pract, 26: 381-394. https://doi.org/ 10.1016/j.cvfa.2010.04.004
13. Gür S. (2018). An investigation of bovine viral diarrhoea virus (BVDV) and bovine parainfluenza type 3 virus (BPI3V) infections in small-scale cattle herds in Afyonkarahisar province. Etlik Vet Mikrobiyol Derg, 29: 51-56. https://doi.org/10.35864/evmd.512991
14. Hacıo lu S. (2011). Sı ırların solunum yollarında bovine parainfluenza 3 (BPIV-3) ve infectious bovine rhinotracheitis (IBR)' in moleküler ve serolojik tanısı. Dissertation, Ankara Üniversitesi
15. Headley, S.A., Okano, W., Balbo, L.C., Marcasso, R.A., Oliveira, T.E., Alfieri, A.F., Filho, L.C.N., Michelazzo, M.Z., Rodrigues, S.C., Baptista, L.A., Saut, S.J.P., Alfieri A.A. (2018). Molecular survey of infectious agents associated with bovine respiratory disease in a beef cattle feedlot in southern Brazil. J Vet Diagn Invest, 30: 249-251. https://doi.org/10.1177/1040638717739945.
16. Kale, M., Öztürk, D., Hasırcıo lu, S., Pehlivano lu, F., Türüto lu, H. (2013). Some viral and bacterial respiratory track infectious of dairy cattle during the summer season. Acta Vet Beograd, 63: 227-236. https://doi.org/10.2298/AVB1303227K
17. Kamdi, B., Singh, R., Singh, V., Singh, S., Kumar, P., Singh, K.P. (2020). Immunofluorescence and molecular diagnosis of bovine respiratory syncytial virus and bovine parainfluenza virus in the naturally infected young cattle and buffaloes from India. Microb Pathog, 145:104165. https://doi.org/10.1016/j.micpath.2020.104165
18. Kishimoto M., Tsuchiaka S., Rahpaya S.S., Hasebe A., Otsu K., Sugimura S., Kobayashi S., Komatsu N., Nagai M., Omatsu T., Naoi Y., Sano K., Okazaki-Terashima S., Oba M., Katayama Y., Sato R., Asai T., Mızutani T. (2017). Development of a one-run real-time PCR detection system for pathogens associated with bovine respiratory disease complex. J Vet Med Sci, 79:517-523. https://doi.org/10.1292/jvms.16-0489
19. Konishi M., Ohkura T., Shimizu M., Akiyama M., Kameyama K., Takeuchi K. (2014). Complete genome sequence of the first isolate of genotype C bovine parainfluenzavirus type 3 in Japan. Genome Announc, 2: e01215-14. https://doi.org/10.1128/genomeA.01215-14
20. Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol, 35: 1547-1549.
21. Maclachlan N.J., Dubovi E.J., Barthold S.W., Swayne D.E., Winton J.R. (2017). Fenner's Veterinary Virology, 5th ed., 327-356, Elsevier Inc, San Diego.
22. Maidana S.S., Lomonaco P.M., Combessies G., Craig M.I., Diodati J., Rodriguez D., Parreno V., Zabal O., Konrad J.L., Crudelli G., Mauroy A., Thiry E., Romera S.A. (2012). Isolation and characterization of bovine parainfluenza virus type 3 from water buffaloes (Bubalus bubalis) in Argentina. BMC Vet Res, 8: 83. https://doi.org/10.1186/1746-6148-8-83.
23. Moore S.J., O'Dea M.A., Perkins N., O'Hara A.J. (2015). Estimation of nasal shedding and seroprevalence of organisms known to be associated with bovine respiratory disease in Australian live export cattle. J Vet Diagn Invest, 27: 6-17. https://doi.org/10.1177/1040638714559741
24. Muftuoglu B., Kurucay H.N., Elhag A.E., Yildirim S., Cicek-Yildiz Y., Tamer C., Ozan E., Sahna K.C., Yildirim Y., Albayrak H., Okur-Gumusova S., Yazici Z. (2021). A Serosurvey for Bovine Respirovirus 3 in Turkish Domestic Ruminants: First Comparison study of A and C genotypes. Vet Med Sci, 7: 1625-1632. https://doi.org/10.1002/vms3.534
25. Neill J.D., Ridpath J.F., Valayudhan B.T. (2015). Identification and genome characterization of genotype B and genotype C bovine parainfluenza type 3 viruses isolated in the United States. BMC Vet Res, 11: 112. https://doi.org/10.1186/s12917-015-0431-8
26. Noori Y.M., ntisar K.S., Nada E.M., Ali Y.H. (2014). Detection and isolation of bovine parainfluenza virus type 3 (PIV-3) in cattle in North Kordofan State, Sudan. JAVS, 15: 70-78.
27. O'Neill R., Mooney J., Connaghan E., Furphy C., Graham D.A. (2015). Patterns of detection of respiratory viruses in nasal swabs from calves in Ireland: a retrospective study. Vet Record, 175: 351. https://doi.org/10.1136/vr.102574
28. Oem J.K., Lee E.Y., Lee K.K., Kim S.H., Lee M.H., Hyun B.H. (2013). Molecular characterization of a Korean bovine parainfluenza virus type 3 isolate. Vet Microbiol, 162: 224-227. https://doi.org/ 10.1016/j.vetmic.2012.10.013
29. Okur Gumusova S., Yazıcı Z., Albayrak H., Cakıroglu D. (2007). Seroprevalence of Bovine Viral Respiratory Diseases. Acta Vet (Beograd), 57: 11-16. https://doi.org/10.2298/AVB0701011O
30. Rima B., Balkema-Buschmann A., Dundon W.G., Duprex P., Easton A., Fouchier R., Kurath G., Lamb R., Lee B., Rota P., Wang L., ICTV Report Consortium (2019). ICTV Virus Taxonomy Profile: Paramyxoviridae. J Gen Virol, 100: 1593-1594. https://doi.org/10.1099/jgv.0.001328
31. Saeed I.K., Yahia H.A., Khalid M.T., Nada E.M., Yasir M.N., Baraa A.M., Osama I.M., Salma B.E., Fahad A.A. (2016). Parainfluenza virus 3 infection in cattle and small ruminants in Sudan. JAVAR, 3: 236-241. https://doi.org/10.5455/javar.2016.c160
32. Sharma S.N., Adlakha S.C. (2009). Textbook of Veterinary Virology. International Book Distributing Company, Delhi
33. Sobhy N.M., Mor S.K., Bastawecy I.M., Fakhry H.M., Youssef C.R.B., Goyal S.M. (2017). Surveillance, isolation and complete genome sequence of bovine parainfluenza virus type 3 in Egyptian cattle. Int J Vet Sci, 5: 8-13. https://doi.org/10.1016/j.ijvsm.2017.02.004
34. Spilki F.R. (2016). Bovine Parainfluenza Virus Type 3. In: Mononegaviruses of Veterinary Importance Volume 2: Molecular Epidemiology and Control, Ed. Munır M., CABI, London.
35. Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Mol Biol Evol, 9: 678-687.
36. Timurkan M.O., Aydın H., Sait A. (2019). Identification and molecular characterisation of bovine parainfluenza virus-3 and bovine respiratory syncytial virus - first report from Turkey. J Vet Res, 63: 167-173. https://doi.org/10.2478/jvetres-2019-0022
37. Tiwari K., Cornish C., Gamble B., Thomas D., Sharma R.N. (2016). Seroprevalence of bovine parainfluenza virus type 3 (bPI-3V) in ruminants from Grenada. Open J Vet Med, 6: 23-27. https://doi.org/10.4236/ojvm.2016.62004
38. Toker E.B., Ye ilba K. (2021). Molecular characterization and comparison of diagnostic methods for bovine respiratory viruses (BPIV-3, BRSV, BVDV, and BoHV-1) in field samples in northwestern Turkey. Trop Anim Health Prod, 53: 79. https://doi.org/10.1007/s11250-020-02489-y
39. Veljovic L., Knezevic A., Milic N., Krnjaic D., Mikovic R., Zoric A., Markovic M., Milicevic V., Stamenkovic M., Stanojevic M., Maksimovic-Zoric J., Petrovic T., Nisavic J. (2016). Isolation and molecular detection of bovine parainfluenza virus type 3 in cattle in Serbia. ACTA Vet, 66: 509-519. https://doi.org/10.1515/acve-2016-0044
40. Wen Y.J., Shi X.C., Wang F.X., Wang W., Zhang S.Q., Li G., Song N., Chen L.Z., Cheng S.P., Wu H. (2012). Phylogenetic analysis of the bovine parainfluenza virus type 3 from cattle herds revealing the existence of a genotype A straining China. Virus Genes, 45: 542-547. https://doi.org/10.1007/s11262-012-0810-1
41. Yavru S., Simsek A., Yapıcı O., Kale M. (2005). Serological evaluation of viral infections in bovine respiratory tract, Acta Vet (Beogard), 2, 219-226. https://doi.org/10.2298/AVB0503219Y
42. Yazıcı Z., Gumusova S., Tamer C., Muftuoglu B., Ozan E., Arslan S., Bas O., Elhag A.E., Albayrak H. (2019). The first serological report for genotype C bovine parainfluenza 3 virus in ruminant species of mid-northen Turkey: Traces from the past. Trop Biomed, 36: 803-809. PMID: 33597501
43. Yıldırım Y., Yılmaz V., Faraji A. (2009). Seroprevalance of viral respiratory system infections in cattle in the border provinces of North-East Turkey. Kafkas Univ Vet Fak Derg, 15: 601-609. https://doi.org/10.9775/kvfd.2009.078-A
44. Zhu Y.M., Shi H.F., Gao Y.R., Xin J.Q., Liu N.H., Xiang W.H., Ren X.G., Feng J.K., Zhao L.P., Xue F. (2011). Isolation and genetic characterization of bovine parainfluenza virus type 3 from cattle in China. Vet Microbiol, 149: 446-451. https://doi.org/10.1016/j.vetmic.2010.11.011.