MAKU J. Health Sci. Inst. 2022, 10(2): 116-122.
doi: 10.24998/maeusabed.1094449

Mehmet Akif Ersoy University
Journal of Health Sciences Institute
http://dergipark.gov.tr/maeusabed

Research Article / Araştırma Makalesi

Comparing the Reference Strains and Turkey Isolates of Bovine Parainfluenza Virus 3 (BPIV3) Detected Around Western Mediterranean Region with Its Amino Acid and Nucleotide Positions

Batı Akdeniz Bölgesinde Tespit Edilen Bovine Parainfluenza Virus 3 (BPIV3) İzolatının Referenz Suş ve Türkiye İzolatları ile Aminoasit ve Nükleotit Pozisyonlarının Karşılaştırılması

Ali KÜÇÜK[1*], Yakup YILDIRIM[2]

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

Abstract: BPIV 3, one of the major viral pathogens of respiratory system disease complex in cattle, is a viral agent generally appearing during autumn and winter months in Northern Hemisphere and causing upper respiratory tract infections. Even though the isolates of this virus are in close antigenic relations, it is classified within three different genotypes as BPIV3 Genotype A, BPIV3 Genotype B and BPIV3 Genotype C. In this research, we aimed to compare the strains of BPIV 3 strain circulating around Western Mediterranean region of Turkey and isolated from different regions of this country and the reference strain of BPIV 3, Shipping Fever (SF-4) with amino acid and nucleotide positions. In the study, previously detected BUR/BPIV 3 isolate with M gene region partially analyzed was used. Phylogenetic researches carried out partially on M gene region in Turkey, different BPIV 3 isolates recorded in gene bank and amino acid and nucleotide positions of BPIV 3 strain detected by ourselves were compared. The changes in Turkey strains of BPIV 3 and nucleotide and amino acid positions of the reference strain were revealed. As a result, detecting base and codon differentiations caused by point mutations among BPIV 3 isolates and correspondingly the appearing amino acid changes was considered crucial in terms of revealing the immunization power of the strain to be used in vaccine production and providing the standardization of BPIV 3 molecular detection.

Keywords: Cattle, Bovine ParainfluenzaVirus3, Amino acids and Nucleotide Positions.

Öz: Sığırlarda solunum sistemi hastalıkları kompleksinin major viral patojenlerden biri olan Bovine Parainfluenza Virus 3 (BPIV3) Kuzey Yarımküre'de genellikle sonbahar ve kış aylarında ortaya çıkan ve üst solunum yolu enfeksiyonlarına yol açan viral bir etkendir. Bu virusun izolatları yakın antijenik ilişki içerisinde olsalar da BPIV3GenotypeA, BPIV3GenotypeB ve BPIV3GenotypeC olarak üç farklı genotipte sınıflandırılmıştır. Bu araştırmada Batı Akdeniz bölgesinde sirkülasyon halinde olan BPIV3 suşunun Türkiye'nin farklı bölgelerinden izole edilmiş suşlar ve BPIV3'ün referenz suşu Shipping Fever (SF-4) ile aminoasit ve nükleotit pozisyonlarının karşılaştırılması amaçlanmıştır. Çalışmada daha önceki araştırmalarımızda tespit ettiğimiz ve M gen bölgesi parsiyel olarak analiz edilmiş BUR/BPIV3 izolatı kullanıldı. SF-4 ile Türkiye'de parsiyel olarak M gen bölgesi üzerine filogenetik araştırmaları ve Genbank'a kayıtları yapılmış farklı BPIV3 izolatları ve bizim tespit ettiğimiz BPIV3 suşunun aminoasit ve nükleotit pozisyonları karşılaştırıldı. BPIV3'ün Türkiye suşları ve referenz suşun nükleotit ve aminoasit pozisyonlarındaki değişimleri ortaya konuldu. Sonuç olarak BPIV3 izolatları arasında noktasal mutasyonlarla meydana gelen baz ve kodon farklılaşmalarının buna bağlı olarak da ortaya çıkan aminoasit değişimlerinin belirlenmesinin aşı üretiminde kullanılacak suşun immunizasyon gücünün ortaya koyulması ve BPIV3'ün moleküler tespitinin standardizasyonunun sağlanması açısından önemli olduğu kanaatine varıldı.

Anahtar Kelimeler: Sığır, Bovine Parainfluenza Virus 3, Aminoasit ve Nükleotit Pozisyonları.

*Corresponding author : Ali KÜÇÜK e-mail : alikucuk@mehmetakif.edu.tr
Geliş tarihi / Received : 28.03.2022 Kabul tarihi / Accepted: 13.06.2022

Introduction

Bovine Respiratory Disease Complex (BRDC) is one of the multi factorial health problems commonly seen around the world. In its etiology, many pathogens can be found such as BPIV3, bovine herpes virus-1 (BHV-1), bovine corona virus (BCoV), bovine respiratory syncytial virus

To cite this article: KÜÇÜK A, YILDIRIM Y. (2022). Comparing the Reference Strains and Turkey Isolates of Bovine Parainfluenza Virus 3 (BPIV3) Detected Around Western Mediterranean Region with Its Amino Acid and Nucleotide Positions. MAKU J. Health Sci. Inst., 10(2), 116-122.
ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

(BRSV), bovine adenovirus (BAV), bovine viral diarrhea virus (BVDV) as viral and *Pasteurella multicidae, Mannheimia haemolytica, Histophilus somni* as bacterial (Ellis, 2010). Factors such as weight loss depending on decrease in animal welfare due to infections, decrease in carcass quality, increase in veterinary expenses and prophylaxis, decrease in fertility and animal deaths cause breeders to undergo full scale economical losses (Ellis, 2010).

BPIV 3, one of the most important factors creating BRD complex, is found within *Respirovirus* genus in *Paramyxoviridae* family *Orthoparamyxovirinae* subfamily. The virus has linear, non-segmental (-) ss RNA (Rima et al., 2019). It has three genotypes as BPIV3 genotype A, BPIV3 genotype B and BPIV3 genotype C (Spilki, 2016). BPIV isolates might show genetic differences among themselves. During sequence studies, all genomic areas, except P gene region, have been shown to be protected at high levels (Ellis, 2010). The agent with a pleomorphic morphology usually has an icosahedral structure and is surrounded by a lipid-bilayer membrane (Chamber and Takimoto 2011; Maclachlan et al., 2017). The sequence of BPIV 3 genome with a length of 15.4 kb is as N-P-M-F-HN-L respectively towards 3' 5' and it includes 6 gene regions coding 9 proteins (Ellis, 2010). Out of these 9 proteins, N-M-P-F-HN- and L proteins are structural while V-C and D proteins are non-structural and are synthesized from P gene region (Karron and Collins, 2007; Ellis, 2010). M protein is known to play an important role on the morphology of the virus and infection phase (Elenkumaran, 2013).

The agent is primarily transmitted by droplet and nasal secretion. BPIV 3 that enters the respiratory tract initiates the cellular infectivity by being adsorbed into sialic acid receptors located on cell surfaces (Ellis, 2010). The virus causes viral/bacterial secondary or co infections by creating local immune suppressions in bronchus and bronchiole epithelium cells (Ellis, 2010; Arslan and Küllük, 2017). In field infections, the most commonly isolated viral agents together with BPIV 3 are BHV-1 and BRSV while the bacterial ones are *Mannheimia hemolytica* and *Mycoplasma spp.* (Ellis, 2010; Tiwari et al., 2016). During the studies carried out since the detection of the virus, it has been accepted as an endemic respiratory system infection in cattle populations in each region (Spilki, 2016). The infection is mostly seen during winter and autumn months in Northern Hemisphere (Ellis, 2010). BPIV 3 that might cause infections for cattle of all ages is observed more frequently in animals of 2-8 months old generally. The infections caused by the agent itself alone usually progress sub-clinically while dyspnea, cough, high fever and nasal-conjunctival defluxion occurs if other pathogen agents also participate (Yıldırım, 2009; Ellis, 2010).

In this study, we aimed to compare BPIV 3 strain we isolated in our previous studies (Accession no: MT949524) (Küçük and Yıldırım, 2022), BPIV 3 strains identified based on M gene region in Turkey and amino acid and nucleotide positions and differences of SF-4 strain. Genetic studies among different BPIV3 isolates indicate that regions except the P gene region have a high level of genomic conservation (Ellis 2010). On the other hand, Horwood et al. (2008) revealed different genotypes of the virus as a result of partial analysis of the M gene region of BPIV3. The M gene region was preferred in our study because of its high genomic conservation and its use in genetic typing.

Materials and Methods

Ethics Statement

This research was conducted after the approval of Animal Testing Local Ethics Council (Approvel Number: HADYEK 318/2017).

Virus

In our study we carried out in field research around Burdur, we used BPIV 3 strain that we isolated. This strain was isolated from the nasal swap sample taken from a 7-month-old male cattle displaying general respiratory system infection symptoms such as high fever, cough, abdominal respiration and bilateral mucopurulent nasal flow for 6 days. The nasal swab sample was centrifuged

To cite this article: KÜÇÜK A, YILDIRIM Y. (2022). Comparing the Reference Strains and Turkey Isolates of Bovine Parainfluenza Virus 3 (BPIV3) Detected Around Western Mediterranean Region with Its Amino Acid and Nucleotide Positions. MAKU J. Health Sci. Inst., 10(2), 116-122.
ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

at 4°C for 20 min and 3000 rpm total RNA extraction from the supernatant was carried out according to Rio et al. (2010) and Sample were treated with the one-step RT-PCR kit (Geneall®HyperScript™ one-step RT-PCR master mix, Korea) and RT-PCR was run under described Maidana et al. (2012). Detection of the virus was performed from this nasal swap sample taken in molecular and antigenic ways and sequence analysis was carried out for genetic characterization.

Nucleotide Sequence

Genetic characterization and comparison of the isolates was performed by using 3963-4273 nucleotide positions of partial M gene region (Mfwd: 5´AGTGATCTAGATGATGATCCA 3´ nt and Mrev: 5´GTTATTGATCCAAT TGCTGT − 3´ nt) (Maidana 2012). The consensus nucleotide sequences were verified using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) (Altschul, 1990). The multiple sequence alignments of the data were performed using the Clustal W algorithm. Nucleotide and amino acid alignments and position detections of BPIV 3 strains used in the study were carried out using MegaX program.

Results

The sequence of the M protein was 97-99 % similar to those of the corresponding regions in the partial genome records of the Turkish local strains and 82 % similar of the reference isolates. On the other hand aminoacid similarity is 99,99-100 % similar with local strain and %99,97 similar with SF-4. Nucleotide and amino acid positions of Burdur and Samsun isolates (Accession No:MH357343) were similar. However, 6 nucleotide positions of our isolate and BPIV 3 strain isolated in Erzurum (Accession No:KY511410) were found different. In addition, the aspartic acid (Asp) observed at 1324th position of our isolate transformed into tyrosine (Tyr) in Erzurum isolate. On the other hand, base differences were seen at 45 positions between SF-4 isolate (Accession No:AF178655) that is considered as the reference strain of BPIV 3 and Burdur and Samsun strains. Besides, arginine (Arg) and threonine (Thr) structures located in 1343rd and 1356th amino acid positions of SF-4 respectively transformed into lysine (Lys) and isoleucine (Leu). Amino acid and nucleotide differences detected between the isolates have been shown in Table 1 and Table 2.

Table 1. Comparison of amino acids according to M gene regions of three selected local (Turkey) strains and SF-4.

	Position of Aminoacids		
Isolates	**1 1 2 4**	**1 3 4 3**	**1 3 5 6**
MH357343	Asp	Lys	Ile
KY511410	Tyr	Lys	Ile
MT949524	Asp	Lys	Ile
AF178655	Asp	Arg	Thr

Discussion

BPIV 3 is among the most important factors that provide a basis for respiratory system disease complex (Karron and Colins, 2007). Commonly seen all around the world, the virus affects young herds even though it causes diseases in animals of all races and ages (Fulton, 2010). Serological, virological and molecular researches carried out in different ways revealed that BPIV 3 might also be seen in various farm animals such as sheep, goats, buffaloes and camels as well as cattle (Elenkumaran, 2013). The disease usually occurs during autumn and winter months, progresses subclinically, but causes fatal broncho pneumonias to appear when other factors participate in the infection (Ellis, 2010). Factors such as decrease in carcass quality depending on the infection, prophylaxis, increase in veterinary service expenses and animal deaths cause economical losses for breeders (Fulton, 2010).

To cite this article: KÜÇÜK A, YILDIRIM Y. (2022). Comparing the Reference Strains and Turkey Isolates of Bovine Parainfluenza Virus 3 (BPIV3) Detected Around Western Mediterranean Region with Its Amino Acid and Nucleotide Positions. MAKU J. Health Sci. Inst., 10(2), 116-122.
ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

MAKU J. Health Sci. Inst. 2022, 10(2): 116-122.
doi: 10.24998/maeusabed.1094449

Table 2. Comparison of nucleotides according to M gene regions of three selected local (Turkey) strains and SF-4.

Position of Nucleotides

	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	9	9	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Isolates	6	7	8	9	9	9	0	0	1	2	2	2	2	3	5	6	6	6	7	7	7	8	9	0
	9	0	7	9	3	9	8	9	1	1	3	6	8	8	9	2	7	8	2	5	7	6	8	1
MH357343	G	G	T	A	T	T	T	C	A	C	A	A	A	G	A	C	T	T	T	A	C	G	C	A
KY511410	A	T	T	A	T	T	T	C	A	C	A	A	A	G	A	C	T	T	T	A	C	G	C	A
MT949524	G	G	T	A	T	T	T	C	A	C	A	A	A	G	A	C	T	T	T	A	C	G	C	A
AF178655	A	G	C	G	C	C	A	T	G	T	G	T	G	A	G	T	C	C	C	C	T	A	A	G

	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2
Isolates	0	0	1	2	2	3	3	3	3	4	5	6	6	7	9	0	0	1	1	3	5	5	6	6
	4	7	9	5	8	1	1	4	7	9	2	1	4	0	4	0	6	2	8	0	1	4	6	9
MH357343	C	A	C	C	G	G	G	C	T	G	C	A	C	A	C	A	G	A	G	C	A	A	T	T
KY511410	C	G	C	C	A	G	G	T	T	G	C	A	C	G	C	A	G	A	G	C	A	A	T	T
MT949524	C	A	C	C	G	G	G	C	T	G	C	A	C	A	C	A	G	A	G	C	A	A	T	T
AF178655	T	G	T	T	G	A	A	C	C	A	T	C	T	A	T	G	A	T	A	A	G	G	C	C

To cite this article: KÜÇÜK A, YILDIRIM Y. (2022). Comparing the Reference Strains and Turkey Isolates of Bovine Parainfluenza Virus 3 (BPIV3) Detected Around Western Mediterranean Region with Its Amino Acid and Nucleotide Positions. MAKU J. Health Sci. Inst., 10(2), 116-122.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

Limited amount of research is available on phylogenetic analysis of BPIV 3 isolates detected in Turkey. The first phylogenetic analysis of BPIV 3 was performed by Timurkan et al. (2019) (BPIV3/TR/Erz/2014). Soon after this study, Albayrak et al. (2019) carried out the phylogenetic analysis of the strain they isolated in Samsun (Bovine respirovirus 3 isolates Turkey_S1). When viruses containing RNA genomes are compared with DNA based life forms, they display a much higher rate of mutation (Murphy et al., 1999; Lauring et al., 2013). The research conducted showed that viruses with RNA nucleic acid replicated with 10^{-4} -10^{-6} error rate per nucleotide and this number corresponded to a single nucleotide error in almost each cycle (San Juan et al., 2010).

As a result of our study, no difference was seen between nucleotide and amino acid positions of our isolate and BPIV 3 strain detected in Samsun while a difference in 6 nucleotide and in one amino acid position was found between ours and BPIV 3 isolate detected in Erzurum. On the other hand, 45 nucleotide and two amino acid positions were found different between our isolate and BPIV 3 reference strain SF-4. This genetic change between isolates was believed to have been caused by high mutation rates and recombinations occurring in viruses that carry RNA genome.

On the other hand, BPIV3 isolates identified in different geographical regions of Turkey show high phylogenetic similarity. The reason for this is thought to be due to the lack of prophylaxis and biosafety practices, the high prevalence of infection due to the asymptomatic character of the infection, and the dispersal of infected animals to interregional, especially with increased animal movements in some periods.

Fulton et al. (2017) demonstrated the genetic characterization of strains used in the production of BPIV3 modified live vaccines (MLV) and their serological and antigenic relationships with different BPIV3 genotypes that was determined that serum samples from cattle vaccination with MLV vaccines produced with strains in BPIV3genotypeA indicated low antibody levels against BPIV3genotypeC strains that are frequently encountered in the field. Ren et al. (2015) produced six monoclonal antibodies specific for the nucleocapsid (NP) protein of the local strain SD0835 (BPIV3 genotype C) isolate and identified three different antigenic epitopes on the NP using these antibodies. Some monoclonal antibodies were found to be reactive for the BPIV3genotype A and BPIV3genotype C epitopes, while inactive in BPIV3genotype B. In this study, the effect of antigenic variations on immunogenicity among BPIV3 genotypes was revealed**.** Muftuoğlu ve ark. (2021) In the genotype-specific serological study, they carried out in different geographical regions and animal species in Turkey, they found that the BPIV3genotypeC antibody titer was higher than the BPIV3GenotypeA titer all serum samples. They considered the reason for this as a dynamic increase in the prevalence of local virus strains because of geographic isolation and commercial vaccines prepared with BPIV3GenotypeA strain could not provide adequate cross immunization in other BPIV3 genotypes.

In line with this information, it was concluded that genetic differentiation between BPIV 3 strains isolated in different parts of the world may affect vaccine efficacy and standardization of molecular diagnosis of the virus. In addition, it was considered necessary to increase seroprevalence researches on the immune response of reference strains used in vaccine production against isolates in different regions and vaccine development applications using local BPIV 3 isolates might create a more effective immune response.

Conclusion

In this study, BPIV 3 isolate that we detected in a molecular research in Burdur and amino acid and nucleotide changes of the genomic region located between 3963rd-4273rd positions of SF-4,

To cite this article: KÜÇÜK A, YILDIRIM Y. (2022). Comparing the Reference Strains and Turkey Isolates of Bovine Parainfluenza Virus 3 (BPIV3) Detected Around Western Mediterranean Region with Its Amino Acid and Nucleotide Positions. MAKU J. Health Sci. Inst., 10(2), 116-122.

ISSN: 2148-2837/ MAKU J. Health Sci. Inst.

considered as the reference strain of BPIV 3, on M gene regions and other BPIV 3 strains detected in Turkey were compared. Our isolate was found different from these strains when we compared nucleotide and amino acid positions of BPIV 3 strains detected in Turkey and the reference BPIV 3 strain, SF-4.

Even though many studies were carried out on prevalence and seroprevalence of BPIV 3 in Turkey, those performed on its phylogenetic are limited. That's why more molecular and phylogenetic research including different gene regions need to be performed to detect circulating BPIV 3 strains in Turkey and to analyze genetic relations. It is thought that molecular and phylogenetic studies to detect local strains of BPIV3 and reveal their genetic differences with other strains will lay the groundwork for genotype-specific vaccine production or diagnostic techniques that may be needed in the future.

References

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. Veterinary Science 6, 56.

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.

Arslan, H.H., Küllük, E., 2017. Sığırlarda parainfluenza-3 virus enfeksiyonu. Türkiye Klinikleri Journal Veterinary Science İntern Med-Special Topics 3, 173-177.

Chamber, R., Takimoto, T., 2011. Encyclopedia of Life Science (ELS). In: Takimota T (eds). Parainfluenza Viruses. Chichester: John Wiley & Sons Ltd, Corpus ID: 84495941, p: 1-8.

Elenkumaran, S., 2013. Mononegaviruses of Veterinary Importance: Pathobiology and Molecular Diagnosis. In: Munir M (eds). Bovine Parainfluenza Virus 3. Crayton: CPI Group, ISBN**:** 7817-8064-1799, p. 117-140.

Ellis, J.A., 2010., Bovine Parainfluenza-3 Virus. Veterinary Clinics of Food Animal Practice 26, 575–593.

Fulton, R.W., 2010. Bovine respiratory disease research (1983–2009). Animal Health Research Reviews 10, 131–139.

Fulton, R.W., Neill, J.D., Saliki, J.T., Landis, C., Burge, L.J., Payton, M.E., 2017. Genomic and antigenic characterization of bovine parainfluenza-3 viruses in the United States including modified live virus vaccine (MLV) strains and field strains from cattle. Virus Res, 235, 77-81.

Horwood, P.F., Gravel, J.L., Mahony, T.J., 2008. Identification of two distinct bovine parainfluenza virus type 3 genotypes. J. Gen. Virol, 89, 1643–1648.

Karron, R.A., Collins, P.L., 2007. Fields Virology 5th Edition. In: Knipe DM, Howley PM (Eds). Parainfluenza viruses. Philadelphia: Williams & Wilkins, ISNB: 9780-7817-60607, p. 1497–1526.

Küçük, A., Yıldırım, Y., 2022. Antigenic and histopathologic evaluation, with molecular characterization and identification of BPIV3 in cattle with respiratory system infections. Large Anim. Rev, 28, 123-130.

Lauring, A.S., Frydam, H., Andino, R., 2013. The role of mutational robustness in RNA virus evolution. Nature Reviews Microbiology 11, 327-336.

Maclachlan, N.J., Dubovi, E.J., Barthold, S.W., Swayne, D.E., Winton, J.R., 2017. Fenner's Veterinary Virology 5th edition. In: Maclachlan NJ, Dubovi EJ (Eds). Paramyxoviridae and Pneumoviridae. San Diego: Elsevier Inc, ISBN: 9780-1280-09468, p:327-356.

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.

Muftuoglu B, Kuruçay HN, Elhag AE, Yıldırım S, Çiçek Yıldız Y, Tamer C, Ozan E, Sahna KC, Yıldırım Y, Albayrak H, Gümüşova Okur S, Yazıcı Z (2021): A serosurvey for bovine respirovirus 3 in Turkish domestic ruminants: The first comparison study of A and C genotypes. *Vet Med Sci.* 7:1625– 1632.

Murphy, F.A., Gibbs, E.P., Horzinek, M.C., Studdert, M.J., 1999. Veterinary Virology 3rd Edition, California: Academic press, ISNB: 9780-0805-52033, p: 411-429.

Newcomer, B.W., Neill, J.D., Galik, P.K., Riddell, K.P., Zhang, Y., Passler, T., Velayudhan, B.T., Walz, P.H. , 2017. Serologic survey for antibodies against three genotypes of bovine parainfluenza 3 virus

in unvaccinated ungulates in Alabama. American Journal of Veterinary Research, 78, 239– 243.

Ren, J.L., Zhu, Y.M., Zhou, Y.H., Lv, C., Yan, H., Ma, L., Shi, H.F., Xue, F., 2015. Identification of three antigen epitopes on the nucleocapsid protein of the genotype C of bovine parainfluenza virus type 3. Vet Microbiol. 178,61-69.

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. Journal of General Virology 100, 1593-1594.

Rio, D.C., Ares Jr, M., Hannon, G.J., Nilsen T.W., 2010. Purification of RNA Using TRIzol (TRI Reagent). Cold Spring Harb Protoc. PMID: 20516177.

Sanjuán, R., Nebot, M.R., Chirico, N., Mansky, L.M., Belshaw, R., 2010. Viral mutation rates.Journal of Virology 84, 9733–9748.

Spilki, F.R., 2016. Mononegaviruses of Veterinary Importance Volume 2: Molecular Epidemiology and Control. In: Munir M (Eds). Bovine Parainfluenza virus 3. Crayton: CPI Group, ISNB: 9781780644172, p: 98-105.

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. Journal of Veterinary Research 63, 167-173.

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 Journal of Veterinary Medicine 6, 23-27.

Yıldırım, Y., Yılmaz, V., Faraji, A., 2009. Seroprevalance of viral respiratory system infections in cattle in the border provinces of North-East Turkey. Kafkas Universitesi Veteriner Fakültesi Dergisi 15, 601-609.