

First Molecular Characterization of Bovine Papular Stomatitis Virus and Meta-Analysis of Parapoxviruses in Turkey

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ABSTRACT

Bovine papular stomatitis virus (BPSV) belongs to the genus Parapoxvirus of the family Poxviridae. Orf virus (ORFV), Pseudocowpox virus (PCPV), BPSV and Parapoxvirus of red deer in New Zealand (PVNZ) are also among the members of the genus Parapoxvirus. The BPSV affects ruminants of all age groups as well as frequently being transmitted to humans as a zoonotic disease. In this study, the BPSV infection affecting calves was identified and genetically investigated. A meta-analysis of all Parapoxvirus studies in Turkey was also conducted in this study. Polymerase chain reaction was performed using a set of pan-parapoxvirus primers for the partial B2L gene of the BPSV, resulted in approximately 590-bp PCR product. A phylogenetic tree based on partial B2L sequences of parapoxvirus strains was constructed and analyzed and compared with the GenBank reference sequences. As a result of molecular and phylogenetic analysis, Turkish BPSV strains are different from the Asian and American BPSV strains at both nucleotide and amino acid levels. Turkish strains constitute a unique cluster together with European strains. The sequences of the BPSV strains showed a 95.6-99.6% nucleotide similarity with the GenBank BPSV strains, in addition to an 83.6-84.7 % similarity with Pseudocowpox virus strains, and an 82.1-83.6% similarity with Orf virus strains. It is found that geographical differences are of importance for the molecular, epidemiological investigation of BPSV. As seen in the meta-analysis, bovine papular stomatitis virus of the genus Parapoxvirus has never been studied at the molecular level in Turkey. Thus, the present study carries out the molecular characterization of the BPSV for the first time in Turkey.

Keywords: Bovine Papular Stomatitis Virus; Molecular Characterization; Turkey.

INTRODUCTION

Currently, there are four different members of the genus parapoxvirus that cause disease, namely the Orf virus (ORFV), Pseudocowpox virus (PCPV), bovine papular stomatitis virus (BPSV), and parapoxvirus of red deer in New Zealand (PVNZ) (1). Previously, parapoxviruses were classified based on the natural host range (cattle or small ruminant), clinical symptoms (oral mucosa or udder), and serology (2). However, these data were not enough to make definite classification. Therefore, it is considered important

to perform genetic analyses, i.e. genomic characterization (3).

Bovine papular stomatitis virus (BPSV) belongs to the genus parapoxvirus of the family Poxviridae. The clinical characteristics include papular and erosive lesions (painful reddish papules, ulcers, and proliferative scabs) on the udders, lips, and oral mucosa of cattle as well as the zoonotic characteristics. In young cattle, the BPSV infection develops sometimes in the mouth. It manifests itself in this area (hard palate and oral mucosa) with gingivitis and “horseshoe-shaped”

Table 1. Studies of Parapoxviruses of ruminants and humans in Turkey

Study year	Regions of Turkey	Parapoxvirus genus	Species	Accession Numbers	References
2013	–	Orf virus	Human	JQ936990	Karakas et al., 2013
2013	Gaziantep	Orf virus	Human	KC776922	Midilli et al., 2013
2014	Muğla	PCPV	Cattle & Human	KF554010	Oguzoglu et al. 2014
2016	Antalya	Orf virus	Goat	KP869116	Akkutay-Yoldar et al., 2016
2017	Konya	Orf virus	Goat	KX781310	Sevik, 2017
2017	Isparta	Orf virus	Cattle	MG731226	Sevik, 2019
2020	–	Orf virus/Vaccine	Sheep	–	Gulyaz et al., 2020
2017	Ankara	Orf virus	Human	MF997468	Not published
2013	–	Orf virus	Goat	KC491193	Not published
1987	–	Orf virus	Sheep	KF714241	Not published
2010	–	Orf virus	Goat	KF714240	Not published
2019	Aydın	Orf virus	Goat	MK645801	Not published
2017	Erzurum	BPSV	Cattle	MH160406	Present study
2017	Erzurum	BPSV	Cattle	MH160407	Present study

PSPV: Pseudo cow poxvirus, BPSV: Bovine popular stomatitis virus

lesions (4). For this reason, it is not possible to diagnose the infection by judging at the shape of the lesion.

The BPSV affects ruminants of all age groups as well as frequently being transmitted to humans (5). In people with close contact with the infected animals, the BPSV infection is generally associated with nodules and pustules on the hands (6). There are many human-related and non-human-related studies concerning parapoxvirus in the world (1,6-10).

It is possible to analyze the genetic structure of viruses thanks to the advances in technology. With these analyses, it is possible to distinguish types and discover new species. For example, the discovery of the Seal poxvirus (11) and Sea Lion poxvirus-1 (12) and their classification in the genus parapoxvirus was realized by these analyses. So far polymerase chain reaction (PCR), sequencing and phylogenetic analyses have been used for revealing the types of parapoxviruses (13). These methods have facilitated studying the molecular characteristics of parapoxviruses in cattle and small ruminant and to differentiate from infections caused by other pathogens (papillomaviruses, other poxviruses, FMD, etc.).

This study is directed to perform a meta-analysis of the parapoxvirus studies (14-20) in Turkey (Table 1). There are a limited number of molecular characterization studies about parapoxviruses in Turkey (14-20). Several studies report that ORFV and PCPV circulate in the ruminant population (14,

18, 19). Furthermore, zoonotic ORFV has been reported in some studies in Turkey (16, 17) (Table 1). However there has not been a report on BPSV in the cattle population in Turkey. The second aim of this study is to identify and undertake a genetic characterization of parapoxviruses in calves in Turkey.

MATERIAL AND METHOD

Samples

The present report describes BPSV infection of two cases of calves in the province of Erzurum, located in the eastern Turkey. Both calves were 3 months old and weighed around 60-80 kg. In the clinical examination of the calves, mild to moderate fever, salivation, reddish papules on the muzzle, inside the nostrils, lips, on the hard palate and in the oral cavity were detected. The lesions in the mouth were observed on all mucosal surfaces except the dorsum of the tongue and were most common inside the lips. No other lesions were detected in any other part of their bodies.

DNA Extraction and PCR Amplification

Materials from these calves were used for molecular characterization of the virus (Figure 1). Oral swabs taken from calves were used for viral DNA is extraction using High Pure viral nucleic acid kit (Roche, Germany) according manufacturer's instructions. Polymerase chain reaction was

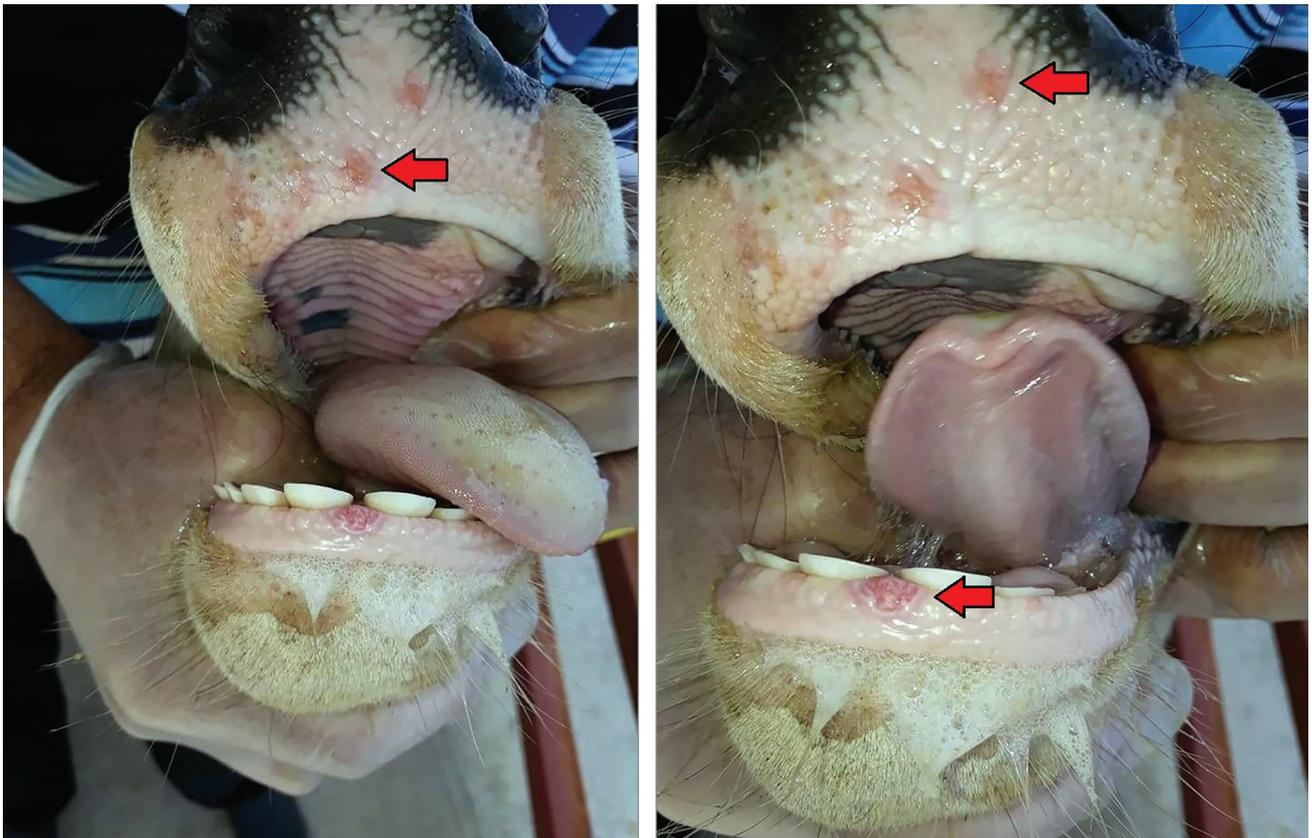


Figure 1. Macroscopic lesion on a calf with papular lesions on lips and nasal planum/merma.

performed using a set of pan-parapoxvirus primers (these primers recognize all parapoxviruses) (13) for the partial B2L gene of the BPSV. The test resulted an approximately 590-bp PCR product. The PCR products were purified with the Gene Jet PCR Purification Kit (Thermo Scientific, USA). Sequencing was performed using the ABI PRISM 310 Genetic Analyzer (Applied Biosystem, CA, USA.) with primers for PCR.

Molecular Analysis (Sequences Analysis)

The sequencing of PCR amplicons were carried out using the ABI PRISM 310 Genetic Analyzer (Applied Biosystem, CA, USA). The sequences of the extracted DNA were compared with other parapoxvirus reference strains, available in the GenBank Database (<http://www.ncbi.nlm.nih.gov>). Nucleotide and amino acid alignment comparisons were made with the Clustal W method, using the Bioedit program (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) (21). Phylogenetic analyses were performed by the maximum-likelihood (ML) method using MEGA6 (22).

RESULTS

PCR Results

All swab samples taken from the lesions were determined as positive by PCR. The sequence analyses of the positive amplicons resulted in similar results to that of BPSV strains in the GenBank. The sequences were called “BPSV/TR-Erz-BPSV-2017-1” and “BPSV/TR-Erz-BPSV-2017-2” (Figure 2) and submitted to the GenBank database under accession number MH160406 and MH160407, respectively.

Molecular Analysis

Sequencing results of the BPSV study strains showed nucleotide similarities with GenBank BPSV strains by 95.6-99.6%, Pseudocowpox virus strains by 83.6-84.7%, and Orf virus strains by 82.1-83.6%. In addition, a phylogenetic tree based on the partial B2L sequences of the parapoxvirus strains was constructed (Figure 2).

In this study the Turkish BPSV strains were found to be clustered together with several European BPSV iso-



Figure 2. Phylogenetic analysis based on the papapoxviruses partial B2L gene nucleotide sequences. The B2L partial sequences of 33 Parapoxvirus strains available from GenBank are denoted by Parapoxvirus genus name, followed by the accession number, strain ID and country.

Diamond (◆) shapes show Turkish orf virus strains,
 square (■) shape shows Turkish PCPV strain and round
 (●) shape shows BPSV strains detected in our study.

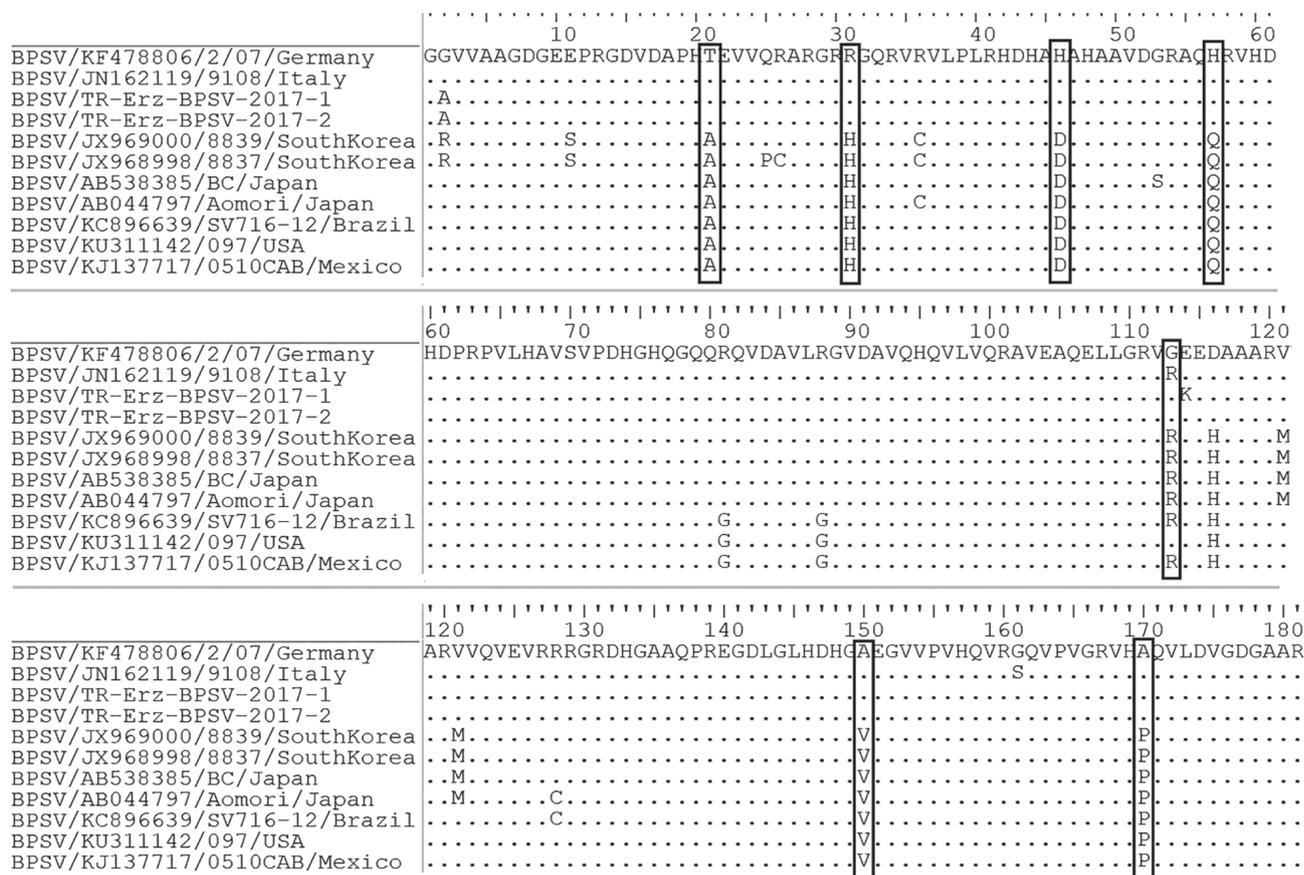


Figure 3. Molecular characterization of BPSV based on internal region of B2L gene. Alignment of amino acid sequences from internal region of B2L gene among Turkish BPSV and several reference BPSV strains.

lates. The Turkish strains were phylogenetically closer to the European isolates (Italy and Germany) (Figure 2). The same pattern was observed when the amino acid sequences of the B2L gene region were compared. As it can be seen in Figure 3, Turkish strains with the amino acid residues at 21 (T→A), 31 (R→H), 46 (H→D), 57 (H→Q), 116 (D→H), 150 (A→V) and 170 (A→P) were same as the European strains.

These residues were different in Asian and American strains. However, Turkish strains with the amino acid residues 2 (G→A) and 114 (E→K) were found to be different from European strains (Fig. 3). When both phylogenetical analysis and amino acid residues were judged it became clear that BPSV strains were clustered in 3 different subgroups: European and Turkey, Asian and American. As a result of the meta-analysis, no previous BPSV data was found in Turkey so far; therefore, it was not possible to perform a phylogenetic evaluation among Turkish strains.

DISCUSSION AND CONCLUSIONS

BPSV infection in cattle is generally neglected worldwide, due to the low morbidity rates. In Turkey, despite the increased incidence of parapoxvirus disease outbreaks, there hasn't been any attempt to further investigate the disease. Parapoxvirus infections are generally considered a different disease from other important vesicular diseases (i.e., foot and mouth disease, bovine viral diarrhea – mucosal disease) in ruminants. The parapoxvirus infection occurrences and distribution in cattle and humans have been reported in several studies (8, 23) in various countries around the world. Several case reports highlighted the presence and widespread distribution of the parapoxvirus infections in cattle (24). The findings of the present study have similar contributions in this regard. BPSV is also found to be present in Turkey, similar to findings all around the globe.

BPSV cases in Turkey along with characterization of the

virus are reported for the first time in this study. Although the clinical cases of parapoxviruses (BPSV, Pseudocowpox, and Orf virus) have previously been reported, this study presents in addition the molecular characterization of the BPSV for the first time in Turkey.

The present study suggests that there is a BPSV sub-group/cluster, which we did not encounter in the literature as a second important finding. There are many strains available in the GenBank worldwide. BPSV parapoxviruses are divided into different sub-groups based on partial B2L gene phylogenetic analysis with these strains from Genbank. In the partial sequence of the B2L gene region, obtained with the strains from the Genbank, the BPSV strains were found to have 3 different groups, based on the continent of origin. These groups include European-Turkish group of strains, American strains and Asian group of strains. This can be observed through either phylogenetic analysis or amino acid findings (Figure 2 and Figure 3). In the Figure 3, seven different amino acids (21, 31, 46, 57, 116, 150 and 170) for the Europe-Turkey group were found to differ from the Asian group and American groups. Turkey is geographically a bridge, connecting Asia and Europe. Therefore, in many phylogenetic studies, Turkish strains were either assigned to European strains or to Asia strains (25-28). In this study, we can attribute its close relationship with European strains to the fact that Turkey usually imports meat and livestock (breeder or animal product) from the Europe. This hypothesis needs to be supported by more molecular epidemiological studies.

The third and important contribution of this study was the meta-analysis of parapoxviruses. As far as we know, there were some different molecular characterization studies on parapoxviruses in Turkey (15-18). As a chronologically the first report, Karakas *et al.* (16) identified Orf virus in a human (military soldier), and emphasized that it was beneficial to use B2L gene in molecular characterization studies. In the same year, Midilli *et al.* (17) also identified Orf virus in humans. However, this zoonotic infection was addressed in a different aspect. For the Orf virus, causing a nasocomial infection, hygiene and disease control aspects were addressed and the ways of transmission of the virus were investigated. Moreover, Oğuzoğlu *et al.* (18) reported for the first time another member of parapoxviruses, which is a pseudocowpoxvirus. This study also addressed zoonotic features. As implied in the name of the disease (Milker's Nodes), the disease was

identified in a person milking cows. Akkutay-Yoldar *et al.* (14) reported an Orf virus case in Antalya, in Saanen and Aleppo goats.

In a study conducted by Sevik (19) in the central Anatolia, phylogenetic analysis of the B2L gene showed that Orf viruses formed two different clusters. He stated that this provides important information on the genetic diversity of parapoxviruses. In another study, Sevik (20) investigated the Orf virus in cattle in Aegean, Central Anatolian and Mediterranean regions in Turkey. As a result, he found that cattle were unusually infected with ORFV and showed that ORFV crossed the host species barrier, from goats to cattle. Gülyaz *et al.* (15) performed a molecular study of an Orf virus obtained in goats as well as conducting a cross-protection study. He concluded in his study that a bivalent vaccine containing lamb and the kid isolates should be prepared for effective immunity against Orf infection. As a result of all these meta-analyses, it can be emphasized firstly that parapoxviruses do not show a species specific barrier; secondly parapoxviruses have a zoonotic potential; thirdly that at least a bivalent vaccine should be used for effective vaccination; and lastly that it would be appropriate to use the B2L gene region for diagnosis and molecular studies of parapoxviruses.

In this report, the control of the outbreak was carried out with biosafety and hygienic measures. Infected calves were separated from other healthy newborns by a different physical compartment. For those who are responsible for the care of calves, the use of gloves and other biosafety clothing is a requirement to prevent the spread of the infection (29).

Parapoxvirus infections are generally considered an important disease that must be distinguished from other vesicular diseases in ruminants, such as infection by foot-and-mouth disease virus, lumpy skin disease, bovine papilloma virus and bovine viral diarrhea-mucosal disease (30, 31). For this reason, it is of importance to diagnose such diseases that affect the skin with a very similar characteristic. Further studies are needed to evaluate the prevalence of infection and to determine the risk factors for both humans and animals.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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