Association of two clusters of Orf virus isolates in outbreaks of infection in goat in the Central Anatolian region of Turkey

Murat Şevik¹

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Abstract Orf virus (ORFV) is the etiological agent of contagious pustular dermatitis and can cause skin disease in sheep and goats. In this study, two outbreaks of ORFV infection in goats in the Central Anatolian region of Turkey were investigated. Samples were collected from 1- to 4-month-old kids ($n = 9$) in two different flocks in the Aksaray and Konya Provinces during the months of March and May 2016. The presence of ORFV in suspected samples was confirmed by PCR using primers specific to envelope gene (B2L). The analysis of the B2L gene sequences revealed that the nucleotide homology between the two isolates in the present study was 100%, whereas the similarity with Parapoxvirus isolates from different regions ranged from 83.6 to 99%. Phylogenetic analysis of the B2L gene revealed that there are two main clusters of ORFV isolates which were responsible for past outbreaks in Turkey. The information presented here will provide an insight into genetic diversity of field isolates of ORFV circulating in the Central Anatolian region of Turkey.

Keywords Orf virus · Goat · B2L gene · Turkey

Contagious pustular dermatitis (orf), also known as contagious ecthyma, is a viral skin disease that occurs in sheep, goats and wild ruminants such as deers and musks. Young sheep and goats (1- to 6-month-old) are more susceptible to infection than adults. The disease also has a zoonotic potential especially people who work with animals (e.g. farmers, animal caretakers, veterinarians, butchers) [14].

The causative agent, orf virus (ORFV), is an enveloped, oval DNA virus within the genus *Parapoxvirus* in the family *Poxviridae*. Other members of the *Parapoxvirus* genus are bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV), squirrel parapoxvirus (SPPV) and parapoxvirus of red deer in New Zealand [5]. The envelope gene (B2L) of the ORFV encodes a highly immunogenic envelope protein that induces a strong antibody response, and this gene has been widely used for molecular detection and genetic characterisation of ORFV [17].

Infection with ORFV has been associated with proliferative and self-limiting lesions on the gums, lips, oral mucosa, nostrils and muzzles. Sometimes lesions can be seen on feet, teats and eyelids. Occasionally, lesions can also extend into the internal organs. Although high morbidity, mortality rate is usually low (rarely exceeds 1%), but it can reach up to 90% in lambs when secondary infections occur. The disease spreads through direct and indirect contacts [7, 13].

ORFV infection has a worldwide distribution and it has serious economic impact due to decreases in production caused by malnutrition in suckling lambs and kids, death of young animals and inability to graze [2, 8, 12, 13, 15]. Outbreaks of orf have been reported in Turkey; however, molecular characterisation of ORFV and investigation of genetic variations in the local ORFV strains in Turkey have been performed in few studies [1, 11, 16]. Therefore, the aims of this study were to identify the causative agent in orf-suspected goats and genetic characterisation of local ORFVs circulating in the Central Anatolian region of Turkey.

This study was conducted during the months of March and May 2016 in the Aksaray and Konya Provinces in the Central Anatolian region of Turkey. In this study, two outbreaks of ORFV infection in Saanen goats were

✉ Murat Şevik
dr_muratank@hotmail.com

¹ Department of Molecular Microbiology, Veterinary Control Institute, Meram, 42080 Konya, Turkey

investigated. The first outbreak occurred in March 2016 in a small Saanen goat farm with 22 goats including 6 kids maintained in the Aksaray Province (37°40'N, 33°04'E). Two of the affected 1 month old kids died due to inanition. The morbidity and the mortality rates were 40.9% (9/22) and 9% (2/22), respectively.

The second outbreak occurred in May 2016 in another Saanen goat farm with 55 goats including 23 kids maintained in the Konya Province (37°52'N, 32°31'E). The farm owner reported that he bought five goats from a farm in Aksaray Province, and clinical signs were observed 3–5 days after they were housed. In this farm, morbidity rate was 41.8% (23/55) but there was no mortality.

These two farms had no history of vaccination against ORFV infection. In both outbreaks, especially young goats (1- to 4-month-old) were affected. Infected animals had fever around 40 °C. Lesions on the lips, ear, hoof, skin and around the mouth were observed in infected animals. Samples of scabs were collected from 1-to 4-month-old infected kids ($n = 9$) from two farms, and they were mechanically homogenised in 200 µl phosphate buffered saline using TissueRuptor (Qiagen, Valencia, CA). Scab homogenates were used for extraction. Viral DNA was extracted from homogenates using a commercial nucleic acid extraction kit (QIAamp Cador Pathogen Mini Kit, Qiagen, Valencia, CA) following the manufacturer's instructions. PCR was performed by using primers PPP-1 (5' GTCGTCCACGATGAGCAGCT) and PPP-4 (5' TACGTGGGAAGCGCCTCGCT) based on B2L gene of virus which amplifies a 594 bp product [10]. Amplification of the B2L gene was performed as described by Inoshima et al. [10]. PCR products treated with ExoSAP-IT (ThermoFisher, Austin, USA) and sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on an ABI 3500 DNA Analyzer (Applied Biosystems, USA).

Nucleotide and amino acid sequences were analysed using the Chromas Pro software (Version 1.7.5, Technolysium Ltd.). Phylogenetic tree was constructed for the B2L gene of ORFV with additional sequences from GenBank. Neighbour-joining (NJ) tree was constructed with the programme MEGA software version 6, based on the evolutionary distances between different sequences calculated by Kimura two-parameter model. The confidence of the neighbour-joining tree was assessed by bootstrapping, using 1000 replicates, and only values above 50% are reported. The B2L gene nucleotide sequences reported in this paper are available in the GenBank under accession numbers KX781309 and KX781310.

The expected 594 bp PCR products were obtained from genomic DNA extracted from all scab samples ($n = 9$). The two representatives ORFV isolates (each from different outbreak) in this study and 22 Parapoxvirus

strains (including BPSV and PCPV strains) downloaded from GenBank were aligned and subjected to phylogenetic analysis. Analysis of the B2L gene sequences revealed that the sequence identity at the nucleotide level between the two isolates in the present study was 100%, whereas the similarity with Parapoxvirus isolates from different regions ranged from 83.6 to 99%. A 100% level of identity was observed between the deduced amino acid sequences of the two isolates in the present study, whereas the similarity with other Parapoxvirus isolates ranged from 81.8 to 99.4%. The two isolates in the present study shared 97.2–99% and 96.4–99.4% sequence identity at the nucleotide and amino acid level, respectively when compared with previously characterised Turkish isolates. The highest nucleotide sequence homology (99%) was observed with a previous Turkish isolate (Turkey Caprine-1/2013). However, two isolates presented here displayed two amino acid substitutions (A134R and V309A) when compared with Turkey Caprine-1/2013.

A phylogenetic tree based on B2L gene sequences revealed that Turkish ORFV isolates grouped into two main clusters (Fig. 1). Cluster I included Turkish isolates and foreign isolates from Brazil, China, Finland, India, Korea, and Taiwan; whereas one of the previous Turkish isolate (Turkey ORF3-2007) grouped with Egypt, Ethiopia and Israel ORFV isolates in a different cluster.

Contagious ecthyma is endemic in Turkey, and animals are vaccinated with a live attenuated vaccine in regions where the disease is seen. Immunization with live attenuated vaccine is a powerful tool to prevent and control the disease [21]. However, outbreaks of orf have also been reported in vaccinated animals [4]. Central Anatolian region is one of the most important regions for sheep and goat breeding. Increases in farm size and movement of infected animals into disease-free areas increase the risk of the transmission of infectious agents such as ORFV. In this study, two outbreaks of ORFV infection in goats in the Central Anatolian region of Turkey were investigated. In these outbreaks, morbidity was found to be around 41% (40.9% and 41.8%) and mortality was low (9%). However, it has been reported that mortality rate can reach up to 90%, depending on host resistance and mixed infections with other viruses and bacteria [9].

In this study, first outbreak was observed in the Aksaray Province in March 2016, and second outbreak was in the Konya Province in May 2016. According to farmer's report, second outbreak was occurred when purchased animals, from a farm in the Aksaray Province, introduced into farm. It is possible that some of the purchased animals were persistently infected when introduced into new farm. The field isolates from these two outbreaks shared 100% sequence identity at the nucleotide and amino acid level. It

Fig. 1 Phylogenetic tree constructed based on nucleotide sequences of the B2L gene (513 bp) showing the genetic relationships between ORFVs of Turkey and other virus isolates. Other sequences are from GenBank. The sequences obtained in this study are marked with *round black spot* and previous Turkish isolates are marked with *black triangle*

seems that same strain of ORFV was responsible for two outbreaks.

It is usually thought that clinical lesions of ORFV infection are found only on the lips. However, in this study clinical lesions on the lip, hoof, ear and skin were observed. Contagious ecthyma's symptoms can look like many other diseases such as sheep and goat pox, peste des petits ruminants, foot and mouth disease, ulcerative dermatosis and dermatophylosis. Therefore, it is difficult to differentiate ORFV infection from these diseases based on clinical symptoms [19, 20]. The conventional methods of diagnosis that depend on histopathology and virus isolation are time and labour consuming. With the development of molecular biology, PCR technique has been proven the most reliable method for the diagnosis of ORFV infection [10]. Therefore, in this study I used PCR method for the rapid and specific detection of ORFV infection. Furthermore, only PCR and genomic analyses can distinguish ORFV from other Parapoxvirus species [17].

In the ORFV genome, ORFV011, 059, 109, 110 and 132 genes have roles in the pathogenesis of ORFV infection. The ORFV011 gene encodes a conserved and highly immunogenic envelope protein (B2L). Sequence and phylogenetic analyses based on the B2L gene are widely used to figure out the evolutionary relationship among ORFV isolates from different geographic origins [17]. Therefore, field isolates were characterised by sequencing the 594 bp fragment within the B2L gene.

Nucleotide and amino acid sequences demonstrated that Konya/2016-ORFV1 and Aksaray/2016-ORFV2 isolates

obtained from two outbreaks in this study were closest to the Turkey Caprine-1/2013 (KP869116) isolate obtained from the Antalya Province in Turkey in 2013. However, two isolates in the present study displayed two amino acid substitutions (A134R and V309A) when compared with Turkey Caprine-1/2013. These amino acid substitutions may have occurred because of host immune response. It has been reported that poxviruses are able to evade the immune system through their genetic mechanisms that increase genetic variation [3]. Furthermore, comparative sequence analysis of the B2L gene of two isolates in this study revealed high amino acid identity with other ORFV isolates from different countries; they had 96.4%–99.4% identity at the amino acid level (data not shown). This may be due to the DNA polymerase proof reading activity [6].

The phylogenetic tree based on B2L gene sequences suggests that there were two main clusters of ORFV isolates infecting sheep and goats in Turkey. All of the Turkish isolates except isolate Turkey ORF3/2007 (KC491191) were in cluster I with Brazil, China, Finland, India, Korea, and Taiwan isolates. The phylogenetic analysis may indicate the hypothetical migration of the viral strains, but it is difficult to determine the precise route by which the identified ORFV variants were introduced [18].

Because of its low severity and self-limiting character, the disease is rarely reported. However, due to its zoonotic potential and impact on animal health, more attention should be given to ORFV infection. Understanding the molecular epidemiology of an infectious disease improves

the effectiveness of eradication and control programs. This study may provide an insight to the molecular epidemiology of ORFV among goat population in Central Anatolian region of Turkey. Further studies are needed to improve understanding circulation of the ORFV strains in Turkey.

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