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Short Communication

The Detection and Molecular Characterization of Lumpy Skin Disease Virus, Northeast Turkey

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ABSTRACT

Lumpy skin disease (LSD), which belongs to the Capripoxvirus genus, is an infection that has caused serious economic losses in the leather industry. The first reported outbreak of LSD occurred in Africa, 1929. Shortly thereafter, it spread to Asia, Europe, and the Middle East. The virus produces nodules that arise primarily on the skin of infected cattle, buffalo, and wild ruminants. The aim of the present study was to perform a pathological evaluation and molecular characterization of LSD by using specimens collected from cattle that had been killed by the disease in Erzurum, Turkey. Skin nodules from four different cattle were collected from two different regions in the central part of Erzurum. These samples were analyzed using polymerase chain reaction (PCR) and histopathological methods. Virological test results revealed that the virus is a member of the Capripoxvirus genus. Therefore, the present study provides epidemiological data about the LSD infection in Erzurum and contributes further information about the infection's current status.

Key words: Lumpy Skin Disease, Cattle, Molecular Characterization, Turkey

INTRODUCTION

The lumpy skin disease (LSD) virus is a member of the genus Capripoxvirus, which belongs to the family *Poxviridae* and the subfamily *Chordopoxviridae*. Capripoxvirus has a complex symmetry and 150-kb double-stranded DNA (Tuppurainen and Oura, 2012). Sheep pox virus (SPPV) and goat pox virus (GTPV), which affects small ruminants, belong to this same genus. LSD shows 97% genetic homology with SPPV and GTPV (Gelaye *et al.,* 2015).

While SPPV and GTPV are usually host specific, in some cases, their presence has been reported in either species (Babiuk *et al*., 2008). In spite of its genetic similarities to these two viruses, LSD has only been reported to affect cattle and buffalos (OIE, 2010).

Arthropods serve as vectors for the transmission of the virus. In addition to the breed, general health, and nutritional status of the animals, season and climate are two important factors influencing the transmission. While flies, ticks, and mosquitoes play a vital role in the transportation of the virus, direct transmission from cattle to cattle is also possible (OIE, 2010). Epidemiological studies have reported that *Aedes aegypti* mosquitoes (Chihota *et al*., 2001), *Stomoxys calcitrans* flies (Yeruham *et al.,* 1995), and *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* ticks (Lubinga *et al.,* 2014) are carriers of the LSD virus in endemic areas, as well.

In 1929, the first case of LSD virus was reported in Africa. This was followed by reports of the virus in the Middle East (Oman, Yemen, Israel, Kuwait, and Bahrain). The first report of the Capripoxvirus genus in Turkey occurred in Kahramanmaraş. The disease was then detected in several other cities, such as Hatay, Adana, Batman, Hakkari, Malatya, Adiyaman, and Osmaniye. Efforts to control, surveillance and eradication to the disease still continue in Turkey (Gürçay *et al.,* 2015; OIE, 2015; Uyar *et al.,* 2015).

The Capripoxvirus decreases the yield of meat and milk in sheep and goats and causes serious damage to wool and leather. LSD infections lead to high economic loss by causing of skin lesions, lowering milk yields, and abortions, weight loss, and infertility (Babiuk *et al.,* 2008).

The aim of this study was to detect the LSD virus in the skin lesions in northeast of Turkey, which dead cattle that had been clinically diagnosed with the disease, characterize the virus at a molecular level, and investigate its pathological findings.

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MATERIALS AND METHODS

In the villages of Çiftlik and Soğucak in Erzurum, Turkey, nodular skin samples were obtained from four dead cattle that had been clinical diagnosed with LSD. Virological and pathological examinations were then conducted on these samples.

Viral DNA isolation and polymerase chain reaction

A commercial kit (GF-1 Viral Nucleic Acid Extraction Kit, Vivantis, Malaysia) was used for the extraction of the DNA samples. The kit procedure was performed according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed on isolated nucleic acids, and Lamien *et al.* (2011) protocol was used for PCR primers and their optimization.

Sequence reaction and phylogenetic analysis

After PCR, two LSD virus positive DNA samples were chosen for sequencing which performed at the Pendik Veterinary Control Institute in Istanbul, Turkey. The sequencing raw data was compared with reference sequences obtained from GenBank. The virus was then analyzed using bioinformatics software (Figure 1).

Pathological examination

Skin samples were fixed in a 10% neutral formalin solution and embedded in paraffin blocks after a routine protocol. Sections of 5-µm thickness were made from these blocks and dehydrated using an alcohol series. Sections were then stained with hematoxylin-eosin and examined using a light microscope.

RESULTS

Virological and pathological findings substantiated the presence of the virus in the samples. Diagnosis of LSD was then confirmed by 591 bp PCR amplicons that had been obtained from the samples. Sequencing and phylogenetic analysis of the samples showed that both strains belonged to the Capripoxvirus genus (Figure 1).

The macroscopic examination detected 1-5 cm nodular lesions all over the body, primarily on the legs, neck, back, and perineum. Where histopathological analyses showed edema, hyperemia, acanthosis (Figure 2), hyperkeratosis, and severe hydropic degeneration in the epidermis, it showed edema, vasculitis, mononuclear cell infiltrations and inclusion bodies in the dermis (Figure 3).

DISCUSSION

The high economic losses caused by LSD are due to abortions, weight loss, infertility, lowered milk yields and skin lesions (Babiuk *et al.,* 2008). LSD also causes significant economic loss in the leather industry. The infection was first detected in Africa and then spread to Asia, Europe, and the Middle East. The first case of LSD in Turkey was reported in 2013 (Gurcay *et al.,* 2015).

In this study, virological and pathological examinations detected the presence of the LSD virus in the skin lesions of dead cattle that had been diagnosed with the disease. Clinical diagnosis was confirmed with 591-bp PCR amplicons that had been obtained from the samples. This is the first study to both report the virus in Erzurum and to diagnose the disease at a molecular level.

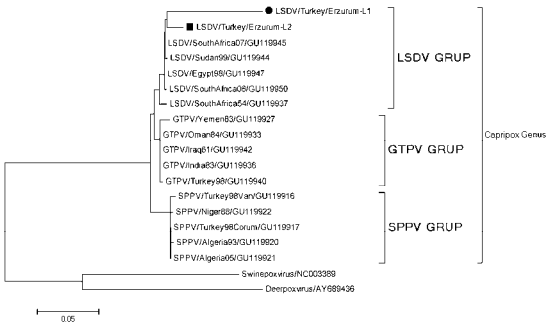

Fig. 1: The phylogenetic analysis of Capripoxviruses with reference strains obtained from GenBank.

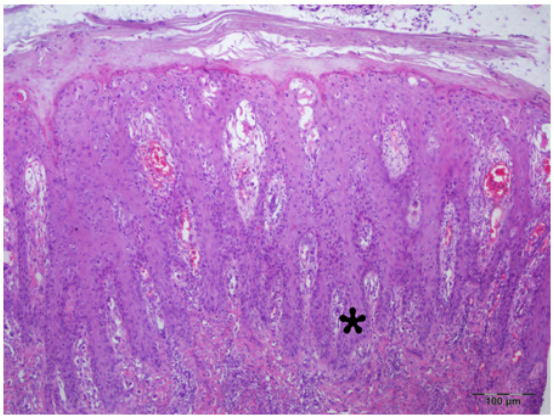

Fig. 2: Acanthosis in the epidermis (*). Bar 100 µm, hematoxylin-eosin stain.

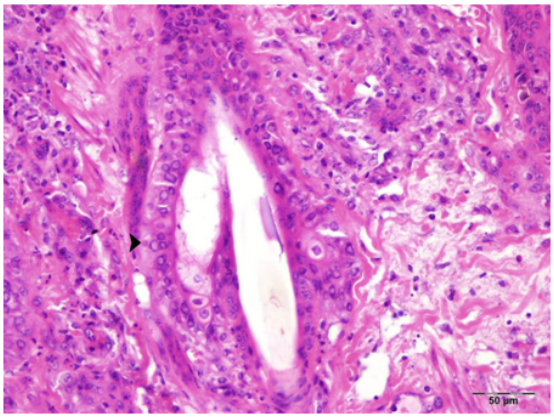

Fig. 3: Intracytoplasmic eosinophilic inclusion bodies in epithelial cells (arrowhead). Bar 50 µm, hematoxylin-eosin stain.

In areas where the disease is epidemic, high morbidity rates suggest that cattle-to-cattle transmission of the disease is effective as flies and ticks. Wet and warm weather also enables the virus to spread to susceptible hosts (Tuppurainen and Oura, 2012).

According to the climatic situation, existence of the disease in the southern eastern Anatolia and Mediterranean region of Turkey have demonstrated that it is parallel to current literature (Coeltzer, 2004). However, in an area like Erzurum, where the climate is cold and dry,

leads to the question how moved to the area concerning the transmission of the Capripoxviruses. It is therefore likely that animal movement, transportation of animal feed, and the temperature differences between the seasons in Erzurum can facilitate the disease's transmission.

A previous study reported that the disease's severity depended on its dosage and the age and breed of its hosts (Ayelet *et al.,* 2013). In that study in a comparison of LSD in two different breeds that indigenous zebu breeds had higher rates of morbidity, younger animals were more susceptible to the disease and that the female gender was at a higher risk for the development of the infection. However, in our study comparisons were limited by the fact that samples were only taken from two locations. Therefore, larger-scale studies that contain detailed evaluation of epidemiological data should be made.

Microscopically, the present study reported edema, hyperemia, vasculitis, acanthosis, hydropic degeneration, and mononuclear cell infiltration. The macroscopic and microscopic findings from the skin lesions were also compatible with the results of previous studies (Ahmed and Dessouki, 2013; Coetzer, 2004; El-Neweshy *et al.,* 2013; Vorster and Mapham, 2008; Uyar *et al.,* 2015). This study, and many others, has also detected the presence of different sized intracytoplasmic eosinophilic inclusion bodies in LSD samples (Ali *et al.,* 1990; Brenner *et al.,* 2006; Uyar *et al.,* 2015).

As with other viral diseases, vaccination is the primary mode of protection against LSD. Because of LSD had been detected as a emerging infection, the vaccine is not available in our country.

The first approach to protecting animals from the LSD virus should be use sheep pox and goat pox vaccines. There are sheep pox and goat pox vaccines in our country. These vaccines can be used to control of LSD disease.

Kitching (2003) reported that a single vaccine can be used for all capripoxvirus infections because of theirs antigenic proximity. However, in Galeya *et al.* (2015) retrospective study of 13 Capripoxvirus outbreaks where GTPV and LSDV agents had been detected, it was discovered that the strains belonged to different clusters after a local vaccine strain was used during phylogenetic analysis. But outbreak of the disease continued. Based on their findings, Galeya *et al.* (2015) advocated that local vaccines should be developed and that host-specific vaccines should be used to control future outbreaks. Galeya *et al.* (2015) also advised that disease strains should be compared with available vaccines (goat pox and sheep pox) in epidemic regions, and if possible, isolated to develop a vaccine specific to LSD.

The present study has shown that the city of Erzurum is an endemic area for the LSD virus. Study findings shows that with a mild temperature climate regions can be suitable area for the LSD virus infection. Vaccination protocols for cattle breeding should therefore be taken into consideration in mild temperatures areas as warm climates, which are affected by LSD virus infections.

REFERENCES

Ali AA, EH Attia, A Selim and YM Abdul-Hamid, 1990. Clinical and pathological studies on lumpy skin disease in Egypt. Vet Rec, 127: 549-550.

Ayelet G, Y Abate, T Sisay, H Nigussie, E Gelaye, S Jemberie and K Asmare, 2013. Lumpy skin disease: preliminary vaccine efficacy assessment and overview on outbreak impact in dairy cattle at Debre Zeit, central Ethiopia. Antiviral Res, 98: 261-265.

Ahmed AM and AA Dessouki, 2013. Abattoir-based survey and histopathological findings of lumpy skin disease in cattle at Ismailia abattoir. Int J Biosci Biochem Bioinforma, 3: 372-375.

Babiuk S, TR Bowden, DB Boyle, DB Wallace and RP Kitching, 2008. Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. Transbound. Emerg. Dis, 55: 263-272

Brenner J, M Haimovitz, E Oron, Y Stram, O Fridgut, V Bumbarov, L Kuznetzova, Z Oved, A Waserman, S Garazzi, S Perl, D Lahav, N Edery and H Yadin, 2006. Lumpy skin disease (LSD) in a large dairy herd in Israel. Isr J Vet Med, 61: 73-77.

Chihota CM, LF Rennie, RP Kitching and PS Mellor, 2001. Mechanical transmission of lumpy skin disease virus by Aedes aegypti (Diptera: Culicidae). Epidemiol Infect, 126: 317-21.

Coetzer JAW, 2004. Lumpy skin disease. In: Coetzer JAW, Tustin RC (Editors). Infectious Diseases of Live-stock. Cape Town: Oxford University Press, 1268-1276.

El-Neweshy MS, TM El-Shemey and SA Youssef, 2013. Pathologic and immunohistochemical findings of natural lumpy skin disease in Egyptian Cattle. Pak Vet J, 33: 60-64.

Gelaye E, A Belay, G Ayelet, S Jenberie, M Yami, A Loitsch, E Tuppurainen, R Grabherr, A Diallo and CE Lamien, 2015. Capripox disease in Ethiopia: Genetic differences between field isolates and vaccine strain, and implications for vaccination failure. Antiviral Res, 119: 28-35.

Gurcay M, A Sait, A Parmaksız and A Kilic, 2015. Türkiye'de Lumpy Skin Disease Virus Enfeksiyonunun Klinik Bulgular ve PCR Yöntemi İle Saptanması. Kafkas Univ Vet Fak Derg, 21: 417-420.

Kitching RP, 2003. Vaccines for lumpy skin disease, sheep pox and goat pox. Dev Biol (Basel), 114: 161-7.

Lamien CE, C Le Goff, R Silber, DB Wallace, V Gulyaz, E Tuppurainen, H Madani, P Caufour, T Adam, M El Harrak, AG Luckins, E Albina and Diallo A, 2011. Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus. Vet Microbiol, 21, 149: 30-9.

Lubinga JC, SJ Clift, ES Tuppurainen, WH Stoltsz, S Babiuk, JA Coetzer and EH Venter, 2014. Demonstration of lumpy skin disease virus infection in *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* ticks using immunohistochemistry. Ticks Tick Borne Dis, 5: 113-20.

The World Organisation for Animal Health (OIE): Lumpy skin disease, Turkey. http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=14106, Accessed: 07.05.2015.

The World Organisation for Animal Health (OIE), 2010. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.4.14, OIE, Paris. Accessed: 07.05.2015.

Tuppurainen ESM and CAL Oura, 2012. Lumpy Skin Disease: An emerging threat to Europe, the Middle East and Asia. Transbound Emerg Dis, 59: 40-48.

Uyar A, Z Yener, S Yıldırım and OF Keles, 2015. Holştayn Bir İnekte Lumpy Skin Disease (Nodüler Ekzantem) Olgusu. FÜ Sağ Bil Vet Derg, 29: 49-53.

Vorster JH and PH Mapham, 2008. Lumpy skin disease. Jaargang, 10: 16-21.

Yeruham I, O Nir, Y Braverman, M Davidson, H Grinstein, M Haymovitch and O Zamir, 1995. Spread of lumpy skin disease in Israeli dairy herds. Vet Rec, 22, 137: 91-93.