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ORIGINAL ARTICLE

Serological and molecular prevalence of lumpy skin disease virus in Korean water deer, native and dairy cattle in Korea

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Lumpy skin disease (LSD) is a severe cross-border infectious disease that causes fever, skin and visceral nodules in cattle. LSD is caused by the lumpy skin disease virus (LSDV), a dsDNA virus that belongs to the genus Capripoxvirus. Although LSD has been found only in Southern Africa traditionally, in the last decade it is spreading very quickly through the Middle East and into Eastern Europe and China. It usually affects cattle and water buffalos being transmitted by blood-feeding insects. As it causes a huge economic impact, LSD is a notifiable disease by World Organisation for Animal Health, and managed as the legal infectious disease class I in Korea. Therefore, the purpose of this study was to confirm the existence of LSDV antigens or antibodies in Korean livestock. We collected 1,200 blood samples from cattle (Korean native and dairy cattle) and Korean water deer in 4 major provinces of the country, then tested the existence of LSDV antigen and antibody. None (0.0%) of the 1,200 blood samples were positive for both antigen and antibody of LSDV. To the best of our knowledge, this is the first study that examines the prevalence of LSDV in Korea. Our study aims to report the LSDV occurrence situation obtained by surveillance in Korea and provide information that may help prevention of LSD epidemics.

Key Words: Dairy cattle, Korean native cattle, Korean water deer, Lumpy skin disease virus, Prevalence

INTRODUCTION

Lumpy skin disease (LSD) is a severe cross-border infectious disease that causes fever, skin and visceral nodules in cattle (OIE, 2021). LSD usually causes devastating economic losses; therefore, it is put on the notifiable disease by OIE and managed as the legal infectious disease class I in Korea. LSD is caused by the Lumpy skin disease virus (LSDV), a dsDNA virus that belongs to the genus Capripoxvirus. It is genetically very similar to the other two Capripoxvirus species sheepox virus and goatpox virus (Abutarbush and Tuppurainen, 2018). LSDV has 156 open reading frames (ORFs) out of 150 kbp genome, of which the central region is related to viral replication and morphogenesis, while the outer region determines the antigenicity and host range (Namazi and Khodakaram Tafti, 2021).

Naturally, LSDV can affect a domestic cow and water buffaloes, besides a wild deer was reported to exhibit skin nodules in the outbreak in Ranch, India (Kumar et al, 2021). LSDV has an incubation period of 2 to 5 weeks. LSDV-infected animals usually show fever, poor body condition, reduced feed and water intake, decreased milk production, lymph node enlargement, and skin nodules. The number of lesions varies from mild to severe, in which lesions are covered all over the body (Prozesky and Barnard, 1982). It occasionally causes death, due to secondary bacterial infection (OIE, 2021). In addition, necrotic plaques may appear at the mucous on the oral and nasal cavity, causing purulent or

mucopurulent nasal discharge and excessive salivation. Ulcerative lesions may appear at the cornea of one or both eyes, leading to restricted vision, and even blindness. In severe cases, the characteristic lesions may appear on the surface of mostly all organs including the gastrointestinal and respiratory tract (Prozesky and Barnard, 1982).

LSDV is known as commonly transmitted by blood-feeding insects, such as stable flies (*Stomoxys calcitrans*), mosquitoes (*Aedes aegypti*), and hard ticks (*Rhipicephalus* and *Amblyomma* species) showing a wide distribution in Korea (Magori-Cohen et al, 2012; Kahana-Sutin et al, 2017; Sprygin et al, 2019).

Traditionally, it was found in southern Africa. However, LSDV advanced north and spread through the Middle East and Eastern Europe into Russia in the last decade. From then on, LSDV has been detected in China (2019), Taiwan, and Nepal (2020), stretching the affected area. The transmission of LSDV is associated with an increase of the vector activity according to global warming (Namazi and Khodakaram Tafti, 2021). Considering these situations, it is not assured that Korea is safe from the virus and maintain the LSDV-free status anymore. Therefore, the purpose of this study was to confirm the existence of LSDV antigens or antibodies in Korean livestock and wild animals.

MATERIALS AND METHODS

Ethics statement

All samples were randomly collected from the study animals after obtaining consent from the local animal clinics and the provincial Wildlife Rescue and Conservation Centers. This study was approved by the Institutional Committee of Graduate Studies and Research at Jeonbuk National University, Korea (IACUC decision no.: JBNU 2022-051).

Sample collection and preparation

Between March 2021 and February 2022, we collected 1,200 blood samples (691 from Korean native cattle, 419 from dairy cattle, and 90 from Korean water deer) from 4 provinces in Korea. In Gyeonggi, a total of 325 samples (193 from Korean native cattle, 107 from dairy cattle, and 25 from Korean water deer) were collected and from Gangwon, a total of 313 samples (162 from Korean native cattle, 131 from dairy cattle, 20 from Korean water deer) were collected. In Jeonbuk, a total of 269 samples (151 from Korean native cattle, 73 from dairy cattle, and 45 from Korean water deer) were collected and from Gyeongbuk, a total of 293 samples (185 from Korean native cattle and 108 from dairy cattle) were collected. Entire blood samples were divided and put into both ethylenediaminetetraacetic acid (EDTA) tube and Serum-separating tube for molecular detection of the viral DNA and antibodies, respectively. For sera, blood samples were centrifuged at 4℃, 3,000 rpm for 15 min, the supernatants were transferred to 1.5 ml microtube and decomplemented by heating at 56℃ water bath for 30 min.

Serology

Antibodies against the Lumpy skin disease virus were detected using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (ID Screen® Capripox Double Antigen Multi-species, ID vet® Co., Montpellier, France) according to the manufacturer's instructions. Briefly, the samples and the controls are distributed in the wells, forming an antigen-antibody complex. The plates are then washed and the conjugate (purified LSDV antigen labeled with peroxidase HRP) is added to wells. After the elimination of the excess conjugate, the reaction is revealed by a tetramethylbenzidine (TMB) solution. In the presence of antibodies in the sample, a blue color appears which becomes yellow after blocking. However, in the absence of antibodies in the samples, no staining appears. The reading is performed at

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450 nm. The results of the test are defined based on the calculated S/P ratio. If the S/P ratio is higher than 30%, the tested sample is defined as positive in the presence of antibodies against LSDV.

Quantification of LSDV DNA in blood samples

Viral DNA from blood samples was extracted using DNeasy® Blood & Tissue Kits (Qiagen Co., Hilden, Germany) according to the manufacturer's instructions. The DNA was used as a template in the real-time qPCR. Detection of the GPCR gene of the Lumpy skin disease virus by the real-time qPCR was performed using commercial Kit (ViroReal® Kit Lumpy Skin Disease Virus, Ingenetix GmbH Co., Austria) according to manufacturer's instructions. The real-time qPCR reaction reagent consisted of 10 μL of DNA Reaction Mix (2×), 1 μL of LSDV Mix, 1 μL of CR Assay Mix and 5 μL of the template DNA and was adjusted to a final volume of 20 μL with nuclease-free water. The PCR conditions had an incubation with uracil-DNA glycosylase at 50℃ for 2 min, DNA polymerase activation at 95℃ for 20 s, followed by 45 cycles of denaturation at 95℃ for 5 s, annealing at 60℃ for 1 min. The real-time PCR amplification was performed on a 7500 Fast Real-Time PCR System (Applied Biosystems, USA), and PCR data were analyzed using the 7500 Software v.2.0.5 (Applied Biosystems, USA).

Statistical analysis

Chi-square tests were used to determine the significance of differences in prevalence among regions and among animal species. Pearson's correlation was used to compare the results from ELISA and real-time qPCR assays. *P* values of <0.05 were significant using SPSS 17.0 (SPSS INC., Chicago, Illinois, USA).

RESULTS AND DISCUSSION

A total of 691 Korean native cattle, 419 dairy cattle and 90 Korean water deer were tested negative for antigen and antibody to LSDV (Fig. 1). This study investigated the molecular prevalence of the GPCR gene of LSDV, and seroprevalence from Korean native cows, dairy cattle, and Korean water deer. Although our investigation was conducted in only four provinces of the whole country, since these regions include border regions where LSDV is likely to be introduced, it can be said that the investigation in these regions is a sufficiently meaningful result.

Recently, the spread of LSDV is menacing. During the last decade, the virus has been distributed across Africa into Europe and Asia. Especially, LSDV is detected in China (2019), Taiwan and Nepal (2020), which are all close to Korea. If the virus is transmitted into Korea, an amount of loss is expected like the aforementioned

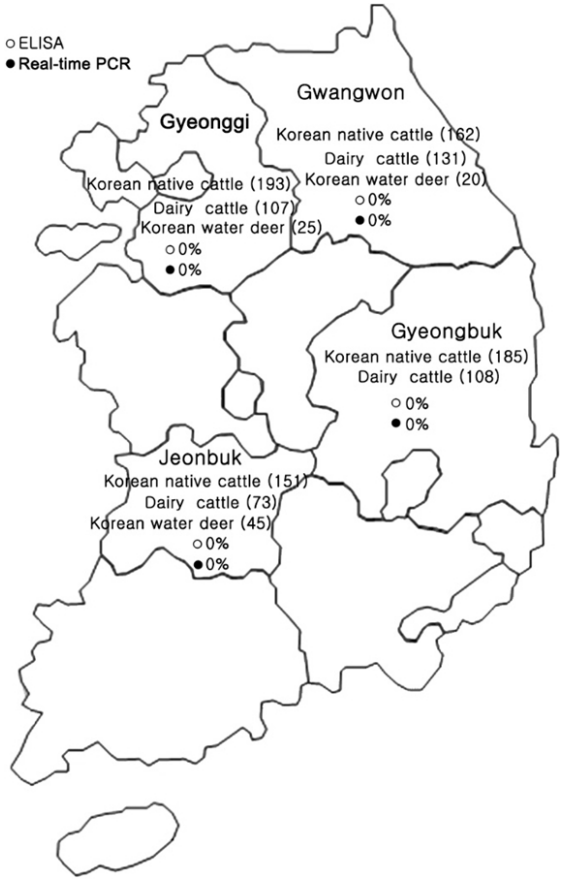

Fig. 1. Prevalence of lumpy skin disease virus (LSDV) antigen and antibody in Korean native cattle, dairy cattle, and Korean water deer. None (0.0%) of the 1,200 blood samples were positive for both antigen and antibody of LSDV.

countries (Limon et al, 2020). The major potential risk factor is a vector, Stable flies (*Stomoxys cadlcitrans*). It is very common blood-feeding insect in Korea. Stable flies can aviate up to 29 km in a condition of laboratory, and 3 km in the open air. They may introduce LSDV in Korea, coming from Taiwan harbor into Jeju-island through the ship travel. In addition, they may also leave Chinese border and within a few days or weeks reach South Korea and introduce LSDV. Besides, due to global warming, insects go active as early as April, which may affect as a great risk of LSDV transmission (Cook, 2020). Fortunately, the antigen of- and antibody to LSDV in water deer we investigated were all negative. If the traffic volume increases as the Covid-19 situation improves, the possibility of an overseas malignant livestock disease that has not occurred in Korea will increase. Therefore, it is considered that LSDV monitoring, especially in Korean water deer as in this study should be continued.

The reason why water deer (*Hydroptes inermis*) was selected as the target for LSDV test in this study is that the deer species represents the most typical clinical skin lesion of LSD i.e., skin nodules in the outbreaks in India, and accordingly, Korean water deer was thought to exhibit clinical symptoms that can be distinguished visually. In addition, the Korean water deer was thought to play an important role in LSDV infection as an active farm-neighborhood peri-domestic wildlife.

Lastly, there are several commercially available vaccines to prevent LSDV. They are usually homogenous or heterogenous inactivated vaccines, and their efficacy is generally good (Tuppurainen et al, 2021). However, using any of them may lead to disqualification of LSDV-free status so, it is mostly recommended not to use them before the outbreak of the disease (OIE, 2021). Therefore, strict and effective biosecurity is the only way to prevent LSDV, ensure the welfare of domestic cattle and wild animals, and protect the sustainability of livestock industry.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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