FULL PAPER

Molecular identification and risk factor analysis of the first Lumpy skin disease outbreak in cattle in Mongolia

Myagmarsuren ODONCHIMEG[1], Dashzevge ERDENECHIMEG[1]*,
Adiyasuren TUVSHINBAYAR[2], Munkhtuul TSOGTGEREL[3],
Enkhbold BAZARRAGCHAA[4], Ankhanbaatar ULAANKHUU[4], Tsend SELENGE[5],
Dalantai MUNKHGEREL[1], Ariunbold MUNKHTSETSEG[1], Adilbish ALTANCHIMEG[1],
Raadan ODBILEG[1], Gurdorj SOYOLMAA[1], Yondonjamts ENKHMANDAKH[1],
Enkhbaatar BATMAGNAI[1], Sengee SUGAR[6], Takashi KIMURA[7],
Chihiro SUGIMOTO[8], Norikazu ISODA[9], Basan BATSUKH[2], Yoshihiro SAKODA[9]*

[1]Institute of Veterinary Medicine, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia
[2]General Authority for Veterinary Services, Ministry of Food, Agriculture, and Light Industry, Ulaanbaatar, Mongolia
[3]School of Veterinary Medicine, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia
[4]Division of Transboundary and Infectious Diseases Diagnosis and Surveillance, State Central Veterinary Laboratory, Ulaanbaatar, Mongolia
[5]Livestock Commercialization Project, Ulaanbaatar, Mongolia
[6]Malchin Mongol LLC., Ulaanbaatar, Mongolia
[7]Laboratory of Comparative Pathology, Department of Clinical Sciences, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[8]Project for Strengthening the Practical Capacity of Public and Private Veterinarians, Japan International Cooperation Agency, Ulaanbaatar, Mongolia
[9]Laboratory of Microbiology, Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan

ABSTRACT. Lumpy skin disease (LSD) is a transboundary viral infectious disease in cattle caused by a *Capripoxvirus*. LSD has been recently introduced in some Asian countries. However, in Mongolia, no report of LSD is publicly available. We clinically examined LSD symptoms in 1,034 cattle from 4 soum (district) in Dornod province in Mongolia. Sixty-one cattle of them were confirmed with symptoms of LSD and then viral *P32* gene was detected by a PCR. The overall prevalence of LSD in cattle was 5.9%. Females odds ratios (OR)=2.27 than males, adults (>2.5-years-old, OR=3.68) than young (1–2.5-years-old) and calves (<1-year-old) were at higher risks for LSD cases in Mongolia, while locations near the tube well and pond water are major risk areas for viral transmission due to density of insects often is high. For virus isolation, skin nodule tissue samples of 4 cattle located in four distinct soums were used for viral propagation using the MDBK cell line. Internal terminal repeat region and *RPO30* gene of 4 Mongolian isolates were amplified and sequenced. In the phylogenetic trees, Mongolian LSDVs (2021) were clustered together with the Chinese (2020) and Vietnamese isolates (2020). This is the first report alarming the LSD outbreak in Mongolia that was confirmed by our study. The newly isolated viruses would be a useful base for developing diagnostic tools and inactivated vaccine technology. A large-scale study of LSD is next priority for establishing successful control strategy of further disease outbreak.

KEYWORDS: lumpy skin disease, molecular identification, Mongolia, outbreak, risk factor

Lumpy skin disease (LSD) is a viral disease in cattle caused by *Capripoxvirus*, a member of the family *Poxviridae* [29]. The clinical symptoms of LSD include fever, nodules up to 5 cm in diameter that primarily appear on the head, neck, abdomen, inguinal area, udder, perineum, genitalia, and finally cover all over the body [5]. A morbidity is often documented in 5% and 45% cattle, while mortality

rate sometimes reaches 10% [3, 32]. Notably, LSD is the one of the transboundary diseases which is listed in the World Organization for Animal Health (OIE). The most significant mode of transmission has been suggested to be via the mechanical transmission by various blood-feeding vectors, such as mosquitoes, flies, and ticks [24, 33]. Thus, movement of virus-infected animals should be restricted to reduce the risks of transmission [18]. After the first report of LSD in Zambia in 1929, LSD virus (LSDV) fast spread in not only cattle and also in buffalo in several Africa, the Middle East, and European countries [30, 34]. In some Asian countries, LSD outbreaks have been documented in India [14], Bangladesh [13], Vietnam [27], Thailand [4], and China [21].

More recently, LSDV has been firstly reported in Russia, in 2015 [25] and in China, in 2019 [21] which are neighboring countries to Mongolia. However, no report is publicly available in Mongolia, except Mongolian the State Central Veterinary Laboratory (SCVL) confirmed a few cases of LSD in cattle in Dornod province in Mongolia, August, 2021, thereby Mongolian the General Authority for Veterinary Services (GAVS) reported LSD cases to the OIE (https://wahis.woah.org/#/dashboards/country-or-disease-dashboard). Therefore, it is urgently needed to investigate LSD outbreak in Mongolia, especially in Dornod province for current prevalence and potential risk factors of LSD. In the present study, we have conducted a cross-sectional study to disclose outbreak of LSD in Mongolian cattle populations in Dornod province in Mongolia.

MATERIALS AND METHODS

Sample collection

Skin nodule tissue samples of cattle were collected according to GAVS guidelines (A/224) and a scientific committee approval (21/05/01) of the Institute of Veterinary Medicine, Mongolia. In addition, herders were questioned by a questionnaire including general knowledge of LSD, possible contact with viral transmission, activating blood-sucking insect, water source and so on. In the cross sectional study, cattle in Chuluunkhoroot, Dashbalbar, Gurvanzagal, and Bulgan soums (district) in Dornod province were involved from 19th of September until 2nd of October, 2021 (Fig. 1). If presence of nodules on skin of head, neck, and any part of the body, scar on the udder, nasal mucosa and oedema were considered as clinical symptoms of LSD in each cattle, skin nodule tissue samples of each cattle suspected with LSD were collected for further laboratory examination. Skin nodule tissue samples were stored in −20°C
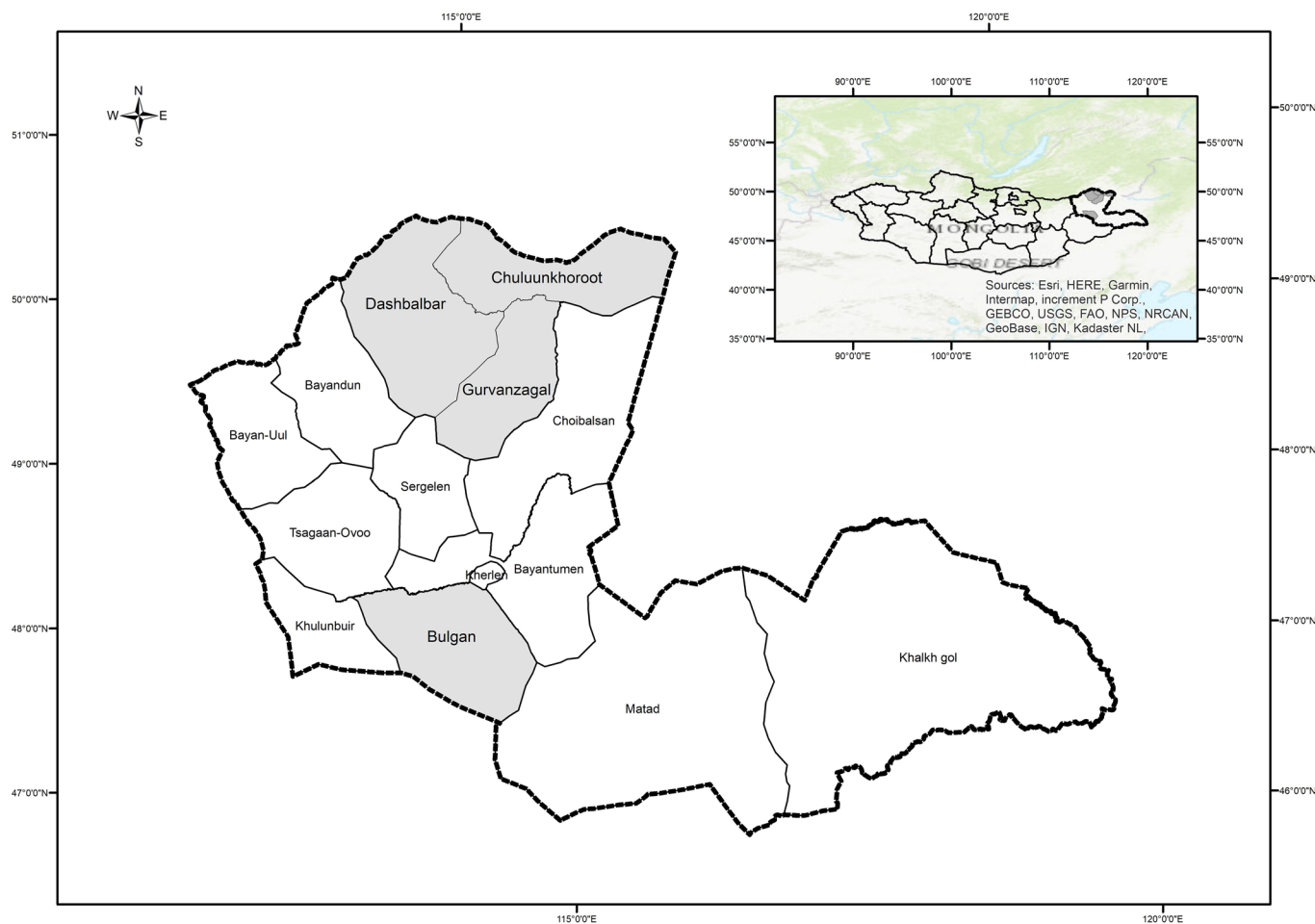

Fig. 1. Map of Dornod province, Mongolia. Gray highlighted areas indicate the sampling areas in this study.

until use for DNA extraction or virus isolation, otherwise a few of tissue samples was preserved in 10% neutral buffered formalin for pathological examination.

DNA extraction and PCR detection of virus

DNA was extracted from skin nodule tissue samples using an Instant Virus RNA/DNA Kit (Roboscreen GmbH, Leipzig, Germany) according to the manufacturer's instruction. PCR detection of *Capripoxvirus P32* gene was followed as previously described [16]. Briefly, a 18 µL of milli-Q water containing 1 µM each of forward (5′-TCCGAGCTCTTTCCTGATTTTTCTTACTAT-3′) and reverse (5′-TATGGTACCTAAATTATATACGTAAATAAC-3′) primers was added into a PCR tube containing powdered DNA polymerase, dNTPs, $MgCl_2$, KCl, Tris-HCl, and stabilizer and tracking dye (Bioneer, Daejeon, Korea), and finally 2 µL of the extracted DNA was added in the tube. PCR was performed by an initial denaturation at 95°C for 2 min, following 34 cycles at 95°C for 45 sec, 50°C for 50 sec, and 72°C for 1 min, and the final extension at 72°C for 2 min. A 5 µL of PCR product was run in 1.5% agarose gel and then stained with 0.05% ethidium bromide (Sigma- Aldrich, St. Louis, MO, USA). The expected size was 192 bp to be considered as positive as amplicons for LSDV *P32* gene.

Virus isolation

All laboratory activities were conducted in Biosafety level-3 laboratory of SCVL, Mongolia. Skin nodule tissue samples of at least a cattle from each soum was used for virus isolation. Briefly, tissue samples were homogenized and then followed 3 cycles of freezing and thawing processes. After centrifugation at 1,500 rpm for 10 min, a supernatant was retrieved and filtrated. Resultant skin suspensions were inoculated in Madin-Darby bovine kidney (MDBK) cell line and then incubated at 37°C in an atmosphere 5% CO_2 for at least 6 days.

DNA sequencing and phylogenetic analysis

Amplification of an inverted terminal repeat (ITR) region and *RPO30* gene were performed using forward GTGGAAGCCAATTAAGTAGA-3′) and reverse (5′-GTAAGAGGGACATTAGTTCT-3′), and outer forward (5′-CAGCTGTTTGTTTACATTTGATTTTT-3′), inner forward (5′- TTTGAACACATTTTATTCCAAAAAG-3′), inner reverse (5′-TCGTATAGAAACAAGCCTTTAATAGA-3′), and outer reverse (5′-AACCTACATGCATAAACAGAAGC-3′) primers, respectively, as previously described [12, 26]. The amplicons of the ITR region and *RPO30* gene were extracted from 1.5% agarose gel using a Qiaquick Gel Extraction kit (Qiagen, Redwood, CA, USA). Purified amplicons were sequenced using a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, CA, USA). Nucleotide sequences of LSDV, sheep pox and goat pox viruses were retrieved from GenBank. Phylogenetic trees were reconstructed by a MEGA 7 software using Neighbor-Joining method [20].

Histopathological examination

Skin nodule tissue samples were fixed by a 10% neutral buffered formalin, and then embedded in paraffin. Paraffin sections were stained with a conventional hematoxylin and eosin staining. Necrosis, inflammatory cell infiltration, and inclusion bodies were observed under a light microscopy (×100 magnification).

Statistical analysis

Age of cattle was categorized as calf ≤1 year; young 1–2.5 years; adult 2.5–4 years. All herders involved in questionnaire for further analysis of the risk factors of LSD. All the epidemiological data was double entered and cleaned in Microsoft Office Excel 2010 (Microsoft Co., Redmond, Washington, USA). Univariable and multivariable analysis were performed using SPSS version 26 (IBM, Chicago, IL, USA). Categorical variables were compared using Fisher's exact test and the χ^2 test. A statically significant difference was considered when the *P*-values were <0.05. Adjusted odds ratios (OR) and the 95% confidence intervals (CIs) for LSD risk factors were analyzed using a univariate and multivariable logistic regression.

RESULTS

Clinical diagnosis

A total 1,034 cattle were physically examined for symptoms of LSD including presence of nodules on skin of head, neck, and any part of the body, scar on the udder, nasal mucosa and oedema. Sixty-one cattle presented at least a clinical sign (Fig. 2). Skin nodule tissue were sampled from the 61 cattle for further PCR detection and histopathological examination of LSD.

PCR detection of virus P32 gene

All collected skin nodules samples of 61 cattle were confirmed as a positive for LSDV using a PCR targeting on partial *P32* gene (192 bp). The prevalence of LSD by soums were 6.8% in Chuluunkhoroot, 5.6% in Gurvanzagal, 9.8% in Dashbalbar, and 2.3% in Bulgan of Dornod province (Table 1). The overall prevalence of LSD was 5.9%.

Risk factors associated with the occurrence of LSD

Univariate logistic regression showed that adjusted OR of having the disease in animals in Dashbalbar soum was 4.84 (CI: 1.44– 13.95) times higher than that in animals in Bulgan soum, while no statistically significant difference of risks of LSD was calculated between Bulgan, Gurvanzagal, and Chuluunkhoroot soums (Table 1). Female (OR=2.27, CI: 1.06–4.83) and adult (OR=3.68, CI:
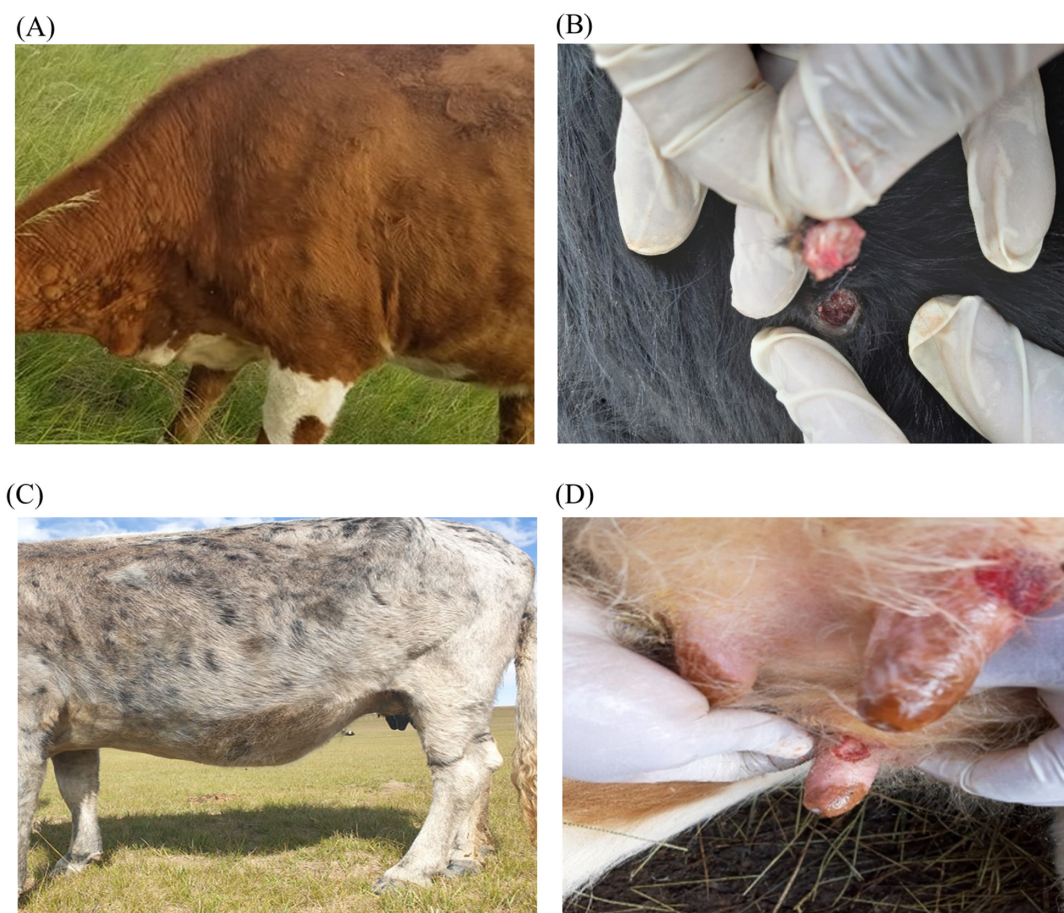
Fig. 2. Clinical presentation of lumpy skin disease (LSD) in cattle, Dornod province of Mongolia, 2021. (**A**) Nodular lesions of LSD affected cow. (**B**) Characteristic 'inverted conical zone' of necrosis and so-called a sit fasts lesion. (**C**) Oedema. (**D**) Lumps and scar on the udder.

Table 1. Risk factors associated with lumpy skin disease in cattle from the Dornod province in Mongolia following a univariable logistic regression analysis

Variables	Category	Total number	Positive number	Negative number	Positive rate (%)	OR	95% CI	*P*-value
Soum (District)	Chuluunkhoroot	192	13	179	6.8	2.97	0.95–9.31	0.080
	Gurvanzagal	532	30	502	5.6	2.45	0.85–7.05	0.131
	Dashbalbar	142	14	128	9.8	4.84	1.44–13.95	0.010
	Bulgan	168	4	164	2.3	Ref		
Sex	Female	778	53	725	7.3	2.26	1.06–4.83	0.040
	Male	256	8	248	3.2	Ref		
Age	Adult	552	52	500	10.4	3.67	1.72–7.85	<0.001
	Young	191	1	190	0.5	0.18	0.02–1.50	0.155
	Calf	291	8	283	2.8	Ref		
Water source	Pond	459	32	427	7.5	4.59	1.60–13.13	0.003
	Tube well	326	25	301	8.3	5.08	1.74–14.81	0.001
	River	249	4	245	1.6	Ref		
Introduction of new animal	Yes	0	0					
	No	1,034	61	973	5.9	Ref		

CI, confidence interval; OR, odds ratio; Ref, reference.

1.72–7.86) animals were at higher risk of contracting the disease than males, young and calves, respectively. The univariable analysis showed that locations near tube well and pond water, were potentially risk areas for disease transmission. There was no significant effect of introducing new animal in a population for disease transmission.

Since the univariable analysis showed *P*-values <0.2 for all variables, except introducing new animal, multivariable analysis was performed using four variables including soum, sex, age, and locations near water sources. In the multivariable model, females (OR=2.40, CI: 1.12–5.16) showed a significantly higher risk of LSD occurrence as compared to males (Table 2). On the contrary, adult animals (OR=0.05, CI: 0.01–0.37) and locations near tube well (OR=0.16, CI: 0.06–0.48) and pond (OR=0.19, CI: 0.07–0.54) had a significantly lower risk of contracting the disease when compared to that of their counterparts. There were not specific factors of LSD in different soums. LSD prevalence in females and adults was higher than that observed in males and young and calves, respectively, in the four areas, whereas positive ratios of LSD by water source were varied among the four areas (Supplementary Table 1).

On the other hand, questionnaire of potential symptoms of LSD and risk factors was completed from a total 53 owners of cattle involved in the present study. Most herders (n=46, or 86.8%) had no idea why their cattle had developed LSD. The remaining herders believed reasons that infected cattle were mixed with uninfected ones in grazing and water drinking areas (n=4, 7.5%) or that the disease was mainly spread by insects (n=3, 5.6%).

Isolation and phylogenetic analysis of Mongolian LSDV

Four days after viral inoculation of the MDBK cell line, specific cytopathic effect (CPE) was observed clearly in cell culture. Four virus isolates (each isolate from each soum) showed with high titre ($10^{4.8}$–$10^{5.5}$ TCID$_{50}$/mL). DNA was extracted from these isolates and then the ITR region and *RPO30* gene were amplified successfully. The nucleotide sequences of the Mongolian isolates were deposited into the GenBank under accession numbers OL692420, OL692421, OM802504, and OM802505 (the ITR region) and OM897116, OM897117, OM897118, and OM897119 (*RPO30* gene). The phylogenetic analysis of the ITR region and *RPO30* gene of the LSDV revealed that these LSDVs were closely related to viruses isolated in China (MN598007 and MW355944) in 2019 and 2020, respectively, and Vietnamese isolates (MZ577076) in 2020 (Fig. 3A and 3B).

Histopathology

Skin tissues were collected from cattle with LSD symptoms. Histopathological examination showed that there were widespread necrosis involving cutis and sub-cutis, with infiltration of macrophages, lymphocytes, and a few neutrophils (Fig. 4). These inflammatory cells appeared to infiltrate the surrounding area of central amorphous necrosis. Some cells with vacuolar degeneration contained intracytoplasmic eosinophilic inclusion bodies, indicating poxvirus infection, although their cell types were difficult to identify.

DISCUSSION

Lumpy skin disease is the one of the global threats impacting cattle industries. Once LSDV emerged in non-endemic area, it rapidly spread neighboring regions through hematophagous arthropod vectors, but density of insect in the area is a major important risk factor [7, 22]. Although few cases of LSDV has been confirmed in Dornod province in Mongolia by SCVL before our study, disease outbreak has not been well recorded. Therefore, the present cross-sectional study of clinical LSD infection was urgently conducted in Dornod province. In our study, although, no death was recorded during sampling period (September-October, 2021), the clinical prevalence of LSD in cattle was ~6% (61/1,034) that leads a huge economic losses including restrictions on international trade of live animals and losses animal products such as milk, meat, hide, and so on. Diagnosis of LSD is mainly based on clinical signs, but some virus-infected cattle do not present any apparent clinical symptoms [9, 30, 31]. For instance, one third of the infected cattle were not presenting clinical symptoms, despite being viremia [31]. Therefore, more extensive epidemiolocal studies using laboratory diagnoses of LSD are required such as nucleic acid amplification test (NAAT) and virus isolation. In this study, we only focused the affected cattle with clinical symptoms of LSD but further investigation should be conducted using seroprevalence of LSD, NAATs and

Table 2. Risk factors associated with lumpy skin disease in cattle from the Dornod province in Mongolia following a multivariable logistic regression analysis

Variables	Level	Estimates	SEM	OR	95% CI	*P*-value
Intercept		4.739	1.335			0.000
Soum (District)	Chuluunkhoroot	0.241	0.828	1.27	0.25–6.44	0.771
	Dashbalbar	0.319	0.874	1.37	0.24–7.62	0.715
	Gurvanzagal	1.039	0.839	2.82	0.54–14.62	0.215
	Bulgan (Ref)					
Sex	Female	0.877	0.390	2.40	1.11–5.16	0.025
	Male (Ref)					
Age	Adult	−2.979	1.016	0.05	0.01–0.37	0.003
	Young	−1.553	1.067	0.21	0.02–1.71	0.146
	Calf (Ref)					
Water source	Tube well	−1.822	0.549	0.16	0.05–0.47	0.001
	Pond	−1.679	0.540	0.18	0.06–0.53	0.002
	River (Ref)					

CI, confidence interval; OR, odds ratio.

(A)

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        MN642592 LSDV Kubash/KAZ/16
        MW656253 LSDV/280-KZN/RSA/2018
        MN072619 LSDV Kenya
   37   MH893760 LSDV/Russia/Dagestan/2015
        KX683219 LSDV KSGP 0240
        AF325528.1 LSDV NI-2490 isolate Neethling
   41   MW732649 LSDV LSDV/HongKong/2020
        MW355944 LSDV China/GD01/2020
        ▲ OM802505_LSDV_Mongolia_Do-21_4 2021
   33   ▲ OL692420_LSDV_Mongolia_Do-21_1_2021                LSDV
   95   ▲ OL692421_LSDV_Mongolia_Do-21_2_2021
        ▲ OM802504_LSDV-Mongolia_Do-21_3_2021
        MZ577076 LSDV 20L81 Bang-Thanh/VNM/20
        MH646674 LSDV/Russia/Saratov/2017
        KX764643 LSDV SIS-Lumpyvax vaccine
   43   KX764644 LSDV Neethling-Herbivac vaccine
        MN636843 LSD/148/GP/RSA/1997
   53   MT134042 LSDV/Russia/Udmurtiya/2019
        MW435866 LSDV SA-Neethling
        MW656252 LSDV/Haden/RSA/1954
           96 AY077836 Goatpox virus G20-LKV
              KX576657 Goatpox virus strain Gorgan
           KC951854 Goatpox virus
    100    MH381810 Goatpox virus strain AV41               GTPV
           MN072620 Goatpox virus isolate India
     65   MN072621 Goatpox virus isolate Vietnam
           MW020570 Goatpox virus
           MN072629 Sheeppox virus Pendik
     63   MW167071 Sheeppox virus isolate V293             SPPV
           AY077832.1 Sheeppox virus 10700-99
    100 MN072631 Sheeppox virus isolate Turkey
        AY077834.1 Sheeppox virus NISKHI
     65   MW167070 Sheeppox virus isolate V123
    0.005
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(B)

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        ▲ OM897117_LSDV_Mongolia_Do21_2_2021_RPO30
        ▲ OM897116_LSDV_Mongolia_Do21_1_2021_RPO30
        ▲ OM897118_LSDV_Mongolia_Do21_3_2021_RPO30
   42   ▲ OM897119_LSDV_Mongolia_Do21_4_2021_RPO30
        MN598007_LSDV/Xinjiang/2019
        MW355944_LSDV_China/GD01/2020
   45   MW732649_LSDV/HongKong/2020
        MZ577076_LSDV_20L81_Bang-Thanh/VNM/20
        MZ577073_LSDV_20L42_Quyet-Thang/VNM/20
           MW656252_LSDV/Haden/RSA/1954
           MW435866_LSDV_SA-Neethling
           MN636843_LSD-148-GP-RSA-1997
   85   MN636842_LSD-220-2-NW-RSA-1993
           MN636839_LSD-103-GP-RSA-1991                     LSDV
   92   KX764643_LSDV_SIS-Lumpyvax
   46   MT134042_LSDV/Russia/Udmurtiya/2019
   49   AF325528_LSDV_Neethling_2490
           MW631933_LSD complete genome
           MH646674_LSDV/Russia/Saratov/2017
   41   MW656253_LSDV/280-KZN/RSA/2018
           MT643825_LSDV_210LSD-249/BUL/16
           MT130502_LSDV_Neethling-RIBSP
   26   MN642592_LSDV_Kubash/KAZ/16
        MH893760_LSDV/Russia/Dagestan/2015
        KY702007_LSDV_SERBIA/Bujanovac/2016
        MW020570_GTPV_V103
        MN072625_GTPV_Yemen
        MN072624_GTPV_Sudan
        MN072623_GTPV_Oman
   86   MN072621_GTPV_Vietnam                                GTPV
        MN072620_GTPV_India
        1  MN072622_GTPV_Turkey
           66 KX576657_GTPV_Gorgan
           MN072627_SPPV_Saudi Arabia
   65   MG000157_SPPV_Romanian Fenner
        MN072628_SPPV_Nigeria
        MW167071_SPPV_V293                                   SPPV
   99   MW167070_SPPV_V123
        MW020571_SPPV_V104
   87   MN072631_SPPV_Turkey
        MN072630_SPPV_Saudi Arabia
    0.005
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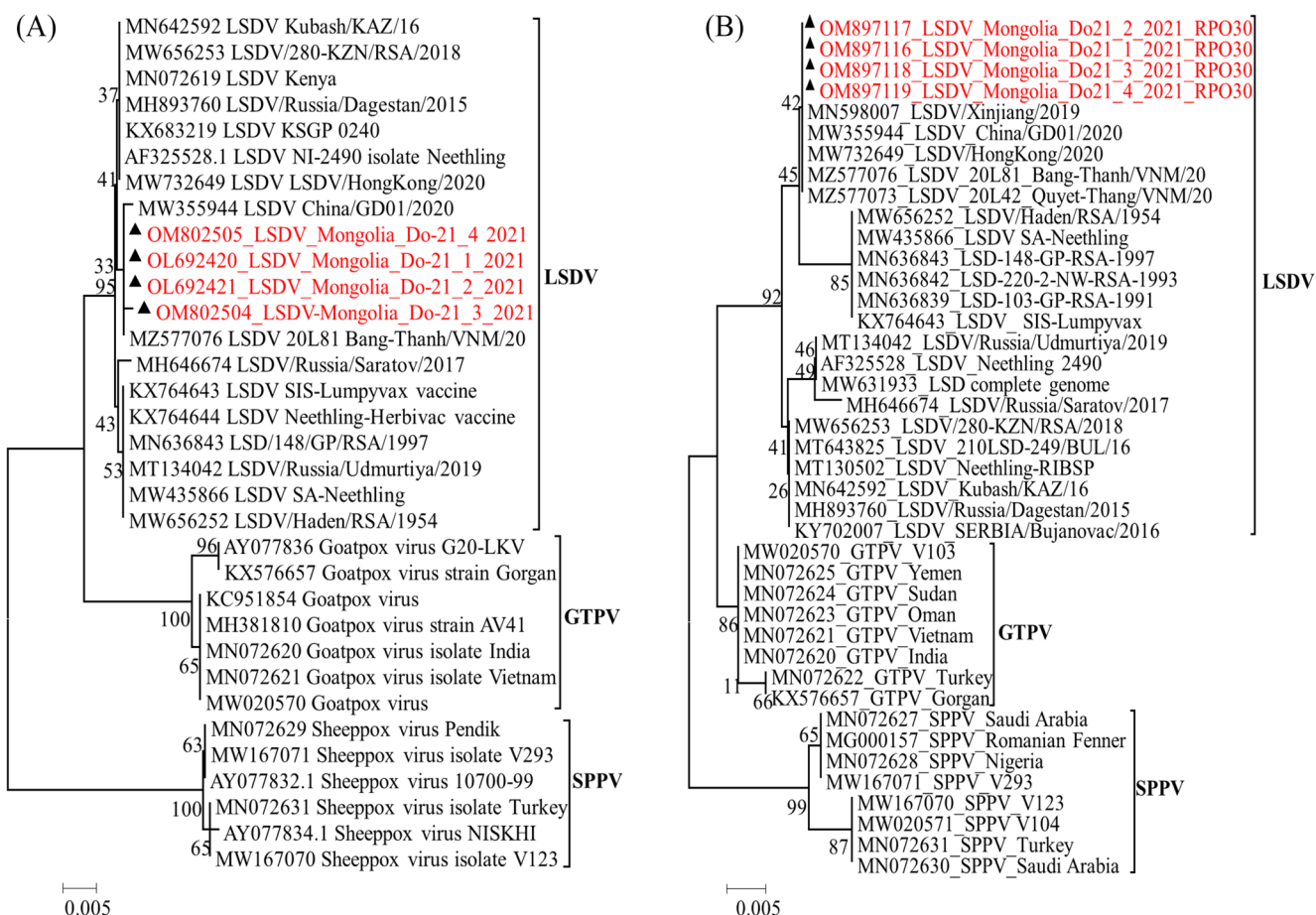
Fig. 3. Phylogenetic analysis of lumpy skin disease virus isolates from the Dornod province in Mongolia based on the partial nucleotide sequences of the inverted terminal repeat region (**A**) and partial *RPO30* gene (**B**). The neighbor-joining method was used to construct a phylogenetic trees using MEGA 7 software.

virus isolation for current viral infection in all cattle. During the LSD outbreak in Mongolia, control measures including restriction of affected animal movement and culling of the affected animals were conducted. Also, susceptible cattle were vaccinated using live attenuated sheep pox vaccine (Perego strain, 10 times greater dose recommended for sheep) which is produced by Biocombinat LLC, Mongolia. For vector control measure, repellent chemicals and smoky fire were used to reduce insects attack.

In Dornod province, all animals in each soum shared their pasture and the main water source. Native Mongolian cattle breeds, grazed on the pasture they were raised, are dominant. It is noteworthy that insects were excessively abundant in 2021 as compared to that was in the last few years. This might be explained by the amount of rainfall in Dornod was higher between June and September when compared to that was recorded in this same area for the last few years. During LSD outbreak, average of daily temperatures was 21°C, whereas cases of LSD were no longer occurred when the cold season (October) begun, which is consistent with previous studies [6, 25] period. This indicates that there is risk of the disease spreading in Mongolia during the summer time (average temperature, 22°C), when the vector is abundant, suggesting that an arthropod's role in virus transmission in Mongolia.

Main prevention and control measures for LSD include vaccination, stamping out, quarantine, educating herders and public awareness [23]. In the present study, most of the herders had no knowledge about the disease, its transmission routes and primary prevention method. Thus, until LSD diagnosed by local veterinarians, no isolation of affected animals was implemented that might expand LSD outbreak in Dornod province. However, the disease prevalence is influenced by numerous factors including season, geographical location, pasture types, climatic factors, abundance of the vectors, and animal movement [15, 17].

Risk factors analysis suggests that females were more prone to contract LSD compared to males, and animals within the reproduceable age, between 3–10 years old, were most susceptible. It has been suggested that the yearly pregnancy and calving, as well as the reduced amount of feed supplies compared to their actual requirement influence the susceptibility to the disease [17]. Related to the Mongolian climate and pasture-based herding, Mongolian cattle become pregnant in the fall and give birth in spring. Pregnant cattle face a harsh climate during winter and spring, which require them to spend more energy for fetal development [28]. In Mongolia the milking period is in summer and fall. Calves do not go on pasture together with the cows, and they stay near the herder's house. Thus, young cattle do not share water sources and pasture with adult cattle, and they might be better protected from flies and mosquitoes. These factors may influence to the low prevalence of LSD infection among young cattle. According to the other studies, LSD morbidity in
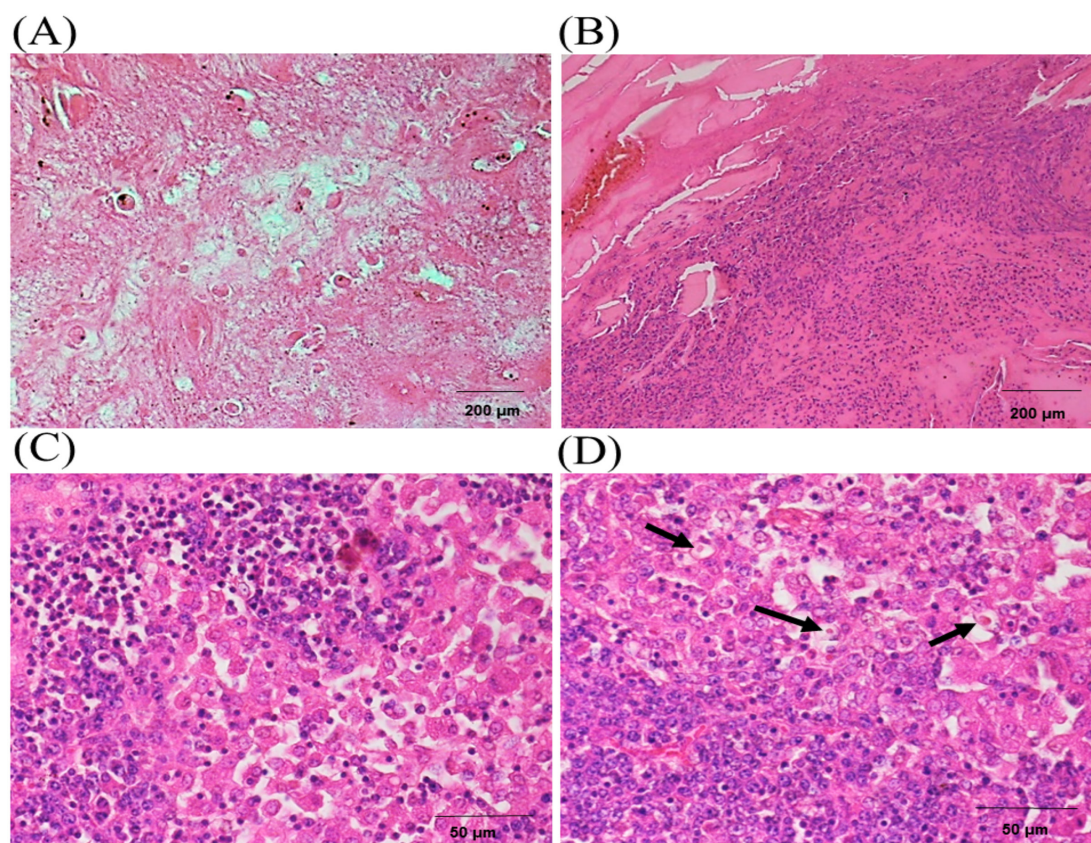
Fig. 4. Histopathological feature of lumpy skin disease in the skin of cattle. **(A)** Widespread necrosis of subcutis. **(B)** Inflammatory cell infiltration around the necrotic lesion. **(C)** Infiltration of inflammatory cells morphologically suggestive of macrophages, lymphocytes, and neutrophils. **(D)** Intracytoplasmic inclusion bodies (arrows) in cells showing ballooning degeneration. Haematoxylin and eosin staining.

calves were low, which might be related to separate pasture from adult cattle and keeping at homestead where there is a low number of insect vectors [2], which is similar to the conditions of this study. The Bulgan soum has the most rivers, while prevalence of LSD was lowest (2.3%). As compared to Bulgan soum, other 3 soums (Chuluunkhoroot, Gurvanzagal, and Dashbalbar) have more ponds and tube wells, formed everywhere due to heavy rainy area, but and these 3 soums have fewer rivers. Having high morbidity rates in these 3 soums (5.6–9.8%) suggest that water resources such as ponds and tube wells are associated with a higher risk of contracting the disease than river water. It implies that the significant difference in LSD prevalence between soums could be at least in part attributed to water source availability. Previous studies suggest that the sharing watering points of ponds and tube wells allow contact and intermingling of different herds, which is leading the risk of exposure and enhance the virus transmission through the speculated mechanical vectors such as fly and mosquitoes [8, 19]. Also, contamination of the pasture and water could be considerable risk factor in communal grazing and watering point utilization despite the fact that contagious transmission is considered to be an inefficient transmission route [11].

LSDV infects MDBK cell line as our established 4 isolates showed strong cytopathic properties on this cell line. Sequencing analysis of the ITR region and *RPO30* gene of our isolates revealed that Mongolian isolates shared 100% identity to Chinese, Vietnamese, Russia, and Kazakhstan isolates, indicating DNA fragments are highly conserved among these isolates. However, Mongolian isolates were clustered together with Chinese and Vietnamese isolates in phylogenetic trees, suggesting that Mongolian isolates are originated from Chinese isolates due to China is a neighboring country to Mongolia. It is noteworthy that any international live animal trade was completely prohibited between Mongolia and China during COVID-19 pandemic and up to date.

Histopathological examination of skin samples showed widespread necrosis involving cutis and sub-cutis, with infiltration of macrophages, lymphocytes, and a few neutrophils, as well as some cells with vacuolar degeneration containing intracytoplasmic eosinophilic inclusion bodies, indicating poxvirus infection. Overall, lesions observed in this study were usually associated with only acute-phase infections which consistent with a previous report [1]. Main characteristics of *Capripoxvirus* infection are the CPE and the intracytoplasmic inclusion bodies [10].

In summary, we investigated the first outbreak of LSD and risk factors, which are important knowledge and factors to consider when developing a strategy to combat further LSD outbreaks in Mongolian native cattle. Furthermore, our LSDV isolates are critical for the development of diagnostic kits and viable LSD vaccine candidates in Mongolia. LSD outbreaks occurred during the summer, which could indicate the role of a blood-sucking insect in LSDV transmission. To prevent future LSD outbreaks in Mongolia, it is

critical to investigate the prevalence of LSDV across the country and implement a well-designed vaccination program. To reduce the risk of LSDV transmission in Mongolia, herders should receive proper awareness and education that would reduce virus-infected animal movement between provinces.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

REFERENCES

1. Abdallah FM, El Damaty HM, Kotb GF. 2018. Sporadic cases of lumpy skin disease among cattle in Sharkia province, Egypt: Genetic characterization of lumpy skin disease virus isolates and pathological findings. *Vet World* **11**: 1150–1158. [Medline] [CrossRef]
2. Abera Z, Degefu H, Gari G, Kidane M. 2015. Sero-prevalence of lumpy skin disease in selected districts of West Wollega zone, Ethiopia. *BMC Vet Res* **11**: 135. [Medline] [CrossRef]
3. Al-Salihi KA. 2014. Lumpy Skin disease: Review of literature. *Mirror Res Vet Sci Anim* **3**: 6–23.
4. Arjkumpa O, Suwannaboon M, Boonrod M, Punyawan I, Liangchaisiri S, Laobannue P, Lapchareonwong C, Sansri C, Kuatako N, Panyasomboonying P, Uttarak P, Buamithup N, Sansamur C, Punyapornwithaya V. 2022. The first Lumpy skin disease outbreak in Thailand (2021): Epidemiological features and spatio-temporal analysis. *Front Vet Sci* **8**: 799065. [Medline] [CrossRef]
5. Beard PM. 2016. Lumpy skin disease: a direct threat to Europe. *Vet Rec* **178**: 557–558. [Medline] [CrossRef]
6. Carn VM, Kitching RP. 1995. An investigation of possible routes of transmission of lumpy skin disease virus (Neethling). *Epidemiol Infect* **114**: 219–226. [Medline] [CrossRef]
7. Chihota CM, Rennie LF, Kitching RP, Mellor PS. 2003. Attempted mechanical transmission of lumpy skin disease virus by biting insects. *Med Vet Entomol* **17**: 294–300. [Medline] [CrossRef]
8. Chihota CM, Rennie LF, Kitching RP, Mellor PS. 2001. Mechanical transmission of lumpy skin disease virus by Aedes aegypti (Diptera: Culicidae). *Epidemiol Infect* **126**: 317–321. [Medline] [CrossRef]
9. Davies FG. 1991. Lumpy skin disease, an African capripox virus disease of cattle. *Br Vet J* **147**: 489–503. [Medline] [CrossRef]
10. El-Neweshy MS, El-Shemey TM, Youssef SA. 2013. Pathologic and immunohistochemical findings of natural lumpy skin disease in egyptian cattle. *Pak Vet J* **33**: 60–64.
11. Gari G, Waret-Szkuta A, Grosbois V, Jacquiet P, Roger F. 2010. Risk factors associated with observed clinical lumpy skin disease in Ethiopia. *Epidemiol Infect* **138**: 1657–1666. [Medline] [CrossRef]
12. Gelaye E, Belay A, Ayelet G, Jenberie S, Yami M, Loitsch A, Tuppurainen E, Grabherr R, Diallo A, Lamien CE. 2015. Capripox disease in Ethiopia: Genetic differences between field isolates and vaccine strain, and implications for vaccination failure. *Antiviral Res* **119**: 28–35. [Medline] [CrossRef]
13. Giasuddin M, Yousuf M, Hasan M, Rahman M, Hassan M, Ali M. 2020. Isolation and molecular identification of Lumpy Skin Disease (LSD) virus from infected cattle in Bangladesh. *Bangladesh J Livest Res* **26**: 15–20. [CrossRef]
14. Gupta T, Patial V, Bali D, Angaria S, Sharma M, Chahota R. 2020. A review: Lumpy skin disease and its emergence in India. *Vet Res Commun* **44**: 111–118. [Medline] [CrossRef]
15. Hasib FMY, Islam MS, Das T, Rana EA, Uddin MH, Bayzid M, Nath C, Hossain MA, Masuduzzaman M, Das S, Alim MA. 2021. Lumpy skin disease outbreak in cattle population of Chattogram, Bangladesh. *Vet Med Sci* **7**: 1616–1624. [Medline] [CrossRef]
16. Ireland DC, Binepal YS. 1998. Improved detection of capripoxvirus in biopsy samples by PCR. *J Virol Methods* **74**: 1–7. [Medline] [CrossRef]
17. Kasem S, Saleh M, Qasim I, Hashim O, Alkarar A, Abu-Obeida A, Gaafer A, Hussien R, Al-Sahaf A, Al-Doweriej A, Bayoumi F, Hodhood A, Abdelatif M. 2018. Outbreak investigation and molecular diagnosis of Lumpy skin disease among livestock in Saudi Arabia 2016. *Transbound Emerg Dis* **65**: e494–e500. [Medline] [CrossRef]
18. Kiplagat SK, Kitala PM, Onono JO, Beard PM, Lyons NA. 2020. Risk factors for outbreaks of Lumpy skin disease and the economic impact in cattle farms of Nakuru county, Kenya. *Front Vet Sci* **7**: 259. [Medline] [CrossRef]
19. Kitching RP, Mellor PS. 1986. Insect transmission of capripoxvirus. *Res Vet Sci* **40**: 255–258. [Medline] [CrossRef]
20. Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* **33**: 1870–1874. [Medline] [CrossRef]
21. Lu G, Xie J, Luo J, Shao R, Jia K, Li S. 2021. Lumpy skin disease outbreaks in China, since 3 August 2019. *Transbound Emerg Dis* **68**: 216–219. [Medline] [CrossRef]
22. Machado G, Korennoy F, Alvarez J, Picasso-Risso C, Perez A, VanderWaal K. 2019. Mapping changes in the spatiotemporal distribution of lumpy skin disease virus. *Transbound Emerg Dis* **66**: 2045–2057. [Medline] [CrossRef]
23. Roche X, Rozstalnyy A, TagoPacheco D, Kamate A, Pittiglio C, Alcrudo DB, Bisht K, Karki S, Kayamori J, Larfaoui F, Raizmen E, VonDobschuetz S, Dhingra MS and Sumption K. 2020. Introduction and spread of lumpy skin disease in South, East and Southeast Asia, FAO, Rome. https://www.fao.org/documents/card/en/c/cb1892en/ [accessed on May 22, 2022].
24. Sohier C, Haegeman A, Mostin L, De Leeuw I, Campe WV, De Vleeschauwer A, Tuppurainen ESM, van den Berg T, De Regge N, De Clercq K. 2019. Experimental evidence of mechanical lumpy skin disease virus transmission by Stomoxys calcitrans biting flies and Haematopota spp. horseflies. *Sci Rep* **9**: 20076. [Medline] [CrossRef]
25. Sprygin A, Artyuchova E, Babin Y, Prutnikov P, Kostrova E, Byadovskaya O, Kononov A. 2018. Epidemiological characterization of lumpy skin disease outbreaks in Russia in 2016. *Transbound Emerg Dis* **65**: 1514–1521. [Medline] [CrossRef]
26. Stram Y, Kuznetzova L, Friedgut O, Gelman B, Yadin H, Rubinstein-Guini M. 2008. The use of lumpy skin disease virus genome termini for detection and phylogenetic analysis. *J Virol Methods* **151**: 225–229. [Medline] [CrossRef]
27. Tran HTT, Truong AD, Dang AK, Ly DV, Nguyen CT, Chu NT, Hoang TV, Nguyen HT, Nguyen VT, Dang HV. 2021. Lumpy skin disease outbreaks

in vietnam, 2020. *Transbound Emerg Dis* **68**: 977–980. [Medline] [CrossRef]

28. Tserensonom S, Enkh-Oyun T, Javzandolgor T. 2007. Physiology and Biochemical Parameters of Mongolian Livestock Animals, Ulaanbaatar, Mongolia (in Mongolian).

29. Tulman ER, Afonso CL, Lu Z, Zsak L, Kutish GF, Rock DL. 2001. Genome of lumpy skin disease virus. *J Virol* **75**: 7122–7130. [Medline] [CrossRef]

30. Tuppurainen ESM, Oura CAL. 2012. Review: lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. *Transbound Emerg Dis* **59**: 40–48. [Medline] [CrossRef]

31. Tuppurainen ESM, Venter EH, Coetzer JAW. 2005. The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. *Onderstepoort J Vet Res* **72**: 153–164. [Medline] [CrossRef]

32. Tuppurainen ESM, Venter EH, Shisler JL, Gari G, Mekonnen GA, Juleff N, Lyons NA, De Clercq K, Upton C, Bowden TR, Babiuk S, Babiuk LA. 2017. Review: Capripoxvirus diseases: current status and opportunities for control. *Transbound Emerg Dis* **64**: 729–745. [Medline] [CrossRef]

33. Tuppurainen ESM, Lubinga JC, Stoltsz WH, Troskie M, Carpenter ST, Coetzer JAW, Venter EH, Oura CAL. 2013. Evidence of vertical transmission of lumpy skin disease virus in Rhipicephalus decoloratus ticks. *Ticks Tick Borne Dis* **4**: 329–333. [Medline] [CrossRef]

34. Woods JA. 1988. Lumpy skin disease-a review. *Trop Anim Health Prod* **20**: 11–17. [Medline] [CrossRef]