

Short Communication

Emergence of the first Lumpy Skin Disease outbreak among livestock in the Republic of Kazakhstan in 2016.

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Abstract

Lumpy skin disease (LSD) is an emerging transboundary viral disease of cattle originating from the African continent. Here we describe the first LSD outbreak reported in the Republic of Kazakhstan, in July 2016. Initially, LSD was reported in a cattle farm located 49 km from Kazakh –Russian border in, Atyrau Oblast in West Kazakhstan. Subsequently, the disease spread to neighbouring farms situated within the same district. Following a preliminary investigation, the local State Veterinary Service declared a strict quarantine according to the State Contingency Plan, along with immediate total stamping out and cattle movement restrictions.

During the outbreak, the number of affected cattle within an epidemiological unit reached 459 cattle out of registered 3557 susceptible cattle with 12.90% morbidity and 0.96%, mortality. This manuscript presents the epidemiological situation, the diagnosis, the control measures including mass vaccination and the stamping out campaign.

Keywords: Control measures; diagnosis; epidemiological data; Kazakhstan; lumpy skin disease

Introduction

Lumpy skin disease virus (LSDV) belongs to the Capripoxvirus genus of the, Poxviridae family, is a highly contagious infectious disease of cattle. It is characterised by fever, skin nodules, enlargement of superficial lymph nodes, salivation, lacrimation and nasal discharge as well as oedema and swellings of the joints and the dewlap (Davies 1991). The World Organization for Animal Health classifies Lumpy Skin Disease (LSD) as a notifiable disease due to its significant economic impact (Tuppurainen and Oura 2012).

LSDV was first discovered in Zambia, where it was recorded in 1929. Subsequently, LSDV has become endemic almost the whole African continent and in the Middle East, Turkey, Azerbaijan continuing to spread to the North posing a threat to Europe and the Central Asian region. In 2015, LSD outbreaks were documented in Greece (Tasioudi et al. 2016) from where it spread to the Balkan region. Similarly, in 2015, the disease was clinically confirmed in North Caucasus of Russia where it become epidemic and spread throughout the country (Zeynalova et al. 2016, Sprygin et al. 2018). In 2016, LSD re-emerged in several regions of Southern Russia, including Astrakhan oblast bordering with Atyrau oblast in West Kazakhstan.

The paper aims to report on the first occurrence of LSD in the Republic of Kazakhstan and to describe the associated clinical features of the disease, diagnostic methods as well as control measures taken to eliminate further dissemination of the pathogen.

According to Statistic Bureau of Agro-industrial complex of Ministry of Agriculture of the Republic of Kazakhstan the total cattle population of the country is estimated to be about 7.161 million heads, mostly local breeds (87.1%); the remaining are hybrids and exotic breeds (data not available). The livestock system practiced in the country is mixed farming, including intensive, small-scale beef and dairy management. Live animals are not exported from the country; meanwhile, the export share of animal products in 2017 accounted for 20 thousand tons. In rural areas, cattle are the primary source of income and mainly kept for milk and meat production. The commercial smallholding dairy and beef farms are mostly market-oriented and located around urban areas practicing intensive management.

Materials and Methods

Animal Ethics

The protocol was approved by the Committee on the Ethics of Animal Experiments of the Research Institute for Biological Safety Problems (RIBSP) of the Science Committee of Ministry of Education and Science of the Republic of Kazakhstan (permit number: 1205/106).

Outbreak in Kazakhstan

An incursion of a previously exotic LSD in Kazakhstan was first recorded in July 2016, in Makash village, of Atyrau Oblast (Figure 1), located 49 km from the borderline with the Astrakhan oblast of the Russian Federation. At that time, Russian veterinary authorities had already reported seven outbreaks of LSD in border farms located near the Kigash River Delta, which serves as a natural border with common pastures on both sides of the river. On 7th of July 2016, the owner a small cattle farm practising mixed dairy and beef management reported several animals at the holding were showing previously unseen clinical signs. The unusual behaviour of cattle within the herd was combined with multiple skin lesions, similar to those reported by Davies (1991) and Weiss (1968), fever, nasal discharge,

superficial lymphadenitis, anorexia, emaciation lameness and reluctance to move, feed and drink (Figure 2). Several animals within the herd demonstrated high fever followed by abortion and death. The post mortem investigation revealed an extensive haemorrhage of the uterus. Moreover, skin lesions in the form of multiple convex indurations were visible in aborted foetuses similar to that described by (Rouby and Aboulsoud 2016). The OIE World Animal Health Information System (WAHIS) was notified as soon as LSD suspicion was confirmed by positive laboratory results on 22nd of July 2016.

Blood samples and skin lesions were collected for testing to the Virology section of the BSL-3 laboratory of the RIBSP and to OIE Reference Laboratory, All-Russian Research Institute for Animal Health (ARRIAH). An eradication program was enacted according to the State Contingency Plan (Directive N 339-2) after field samples provided positive results using conventional PCR.

Until 21 July 2016, in the Kurmangazy District, among the officially registered 3557 cattle, the number of affected cattle reached 459 with morbidity and mortality rates accounting for 12.90% and 0.96% respectively. The case fatality rate was 7.41% (OIE 2016). Kazakhstan veterinary services carried out a total stamping out measure at this first affected farm.

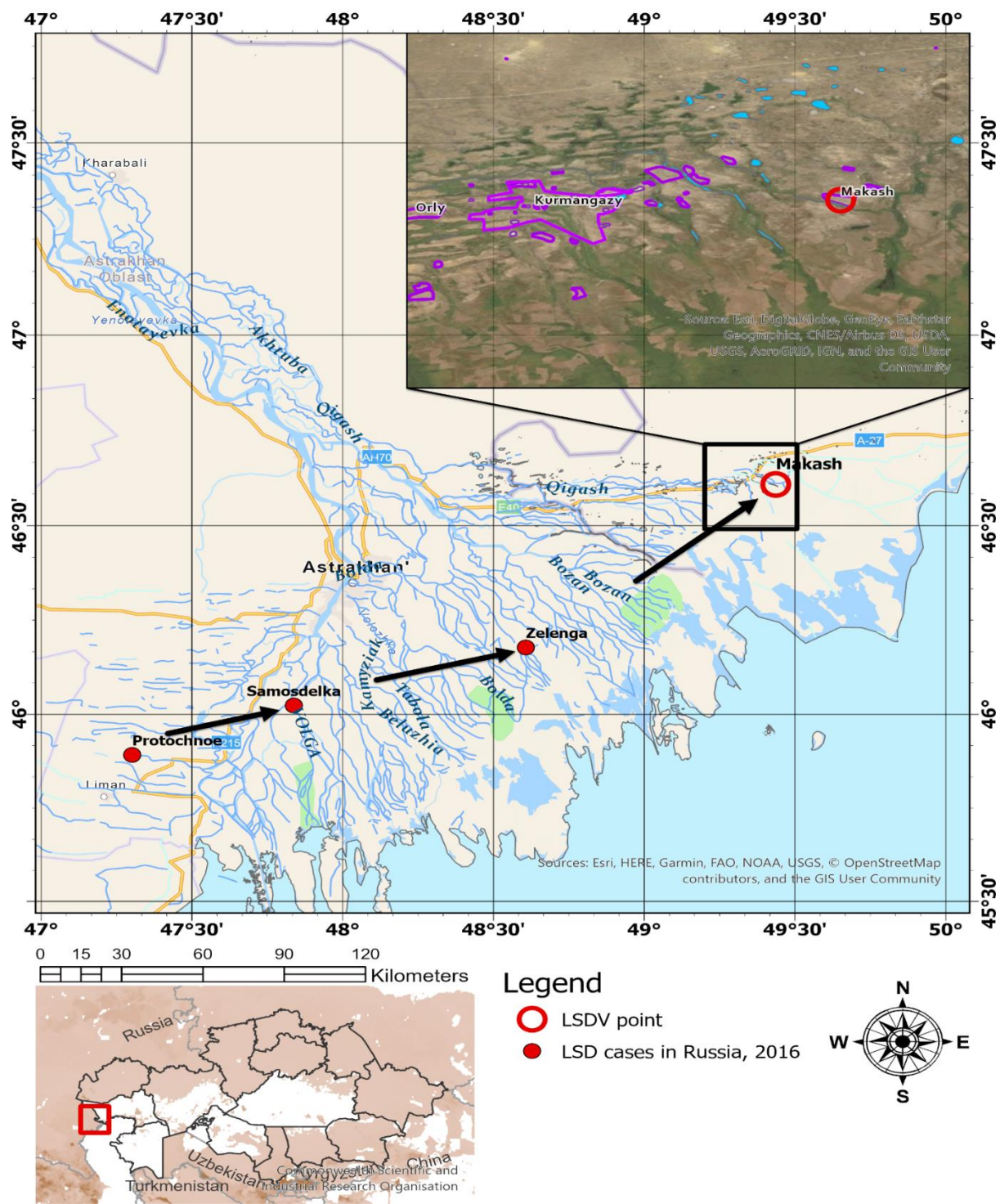


Figure 1. The location of the LSD outbreak in Atyrau oblast. Areas drawn in pink indicates seasonal communal grazing lands.



Figure 2. Cattle exhibiting LSD characteristic clinical signs in the outbreak foci in the Republic of Kazakhstan in 2016. The body surface of infected animals exhibited extensive circumscribed and convex skin nodules (a - d) with ulceration of the scrotum and the teats (e - f).

Sample collection

A total of 74 blood samples, 47 skin lesions, 4 samples of internal organs (2 lymph nodes, 2 lung tissue samples), were taken from diseased and dead animals by official field veterinarians and dispatched to the RIBSP. In addition, 14 hard ticks attached to the diseased host were collected during the clinical examination of infected animals. Moreover, 21 horn flies (*Hematobia irritans*) and 25 stable flies (*Stomoxys calcitrans*) were caught within livestock premises using a commercial fly catching unit “Miniature CDC light trap with UV light” (USA) to investigate a possible insect vector involvement in the transmission of LSD in the field.

Virus isolation

Virus isolation (VI) was conducted according to Standard Operational Procedures of the BSL-3 laboratory of the RIBSP. The tests were carried out as described by OIE (2018). Briefly, 1 ml buffy coat or supernatant were administered on to lamb testes cells in 25 cm² cell culture flasks and allowed to incubate at 37°C for 1 hour. Following incubation cell culture growth media was removed and cell monolayer was rinsed with PBS and overlaid with Glasgow's Minimal Essential Medium containing 0.1% penicillin, 0.2% gentamycin and 2% fetal calf serum (ThermoFisher Scientific). The cell monolayer was examined daily for characteristic cytopathic effect (CPE). In the case no CPE was observed, the cell culture was freeze–thawed three times and second or third blind passages were carried out Cell culture flasks showing CPE were tested with gel-based PCR to confirm that the CPE change was induced by LSDV.

Virus detection by PCR

A PCR assay was performed using the protocol published by Tuppurainen, Venter, and Coetzer (2005).

For DNA extraction, a QIAamp DNA Kit (QIAGEN, USA) was used according to manufacturer's instructions.

For PCR assay, to produce 192 bp of amplified nucleotides reactions the forward 5'-TCC-GAG-CTC-TTT-CCT-GAT-TTT-TCT-TAC-TAT-3' and reverse 5'-TAT-GGT-ACC-TAA-ATT-ATA-TAC-GTA-AAT-AAC-3' primers were used (Ireland and Binopal 1998). The conditions for DNA amplification in a Thermal Cycler (Eppendorf Mastercycler) were as follows: 95°C for 2 min, 95°C for 45 s, 50°C for 50 s, 72°C for 1 min (34 cycles) and 72°C for 2 min. Obtained PCR products were loaded in 1.5% agarose-gel electrophoresis and the results visualized using Bio-imaging systems MiniBIS Pro (Israel).

Complete genome sequencing of LSDV field strain was performed in collaboration with Kazakh Scientific-Research Veterinary Institute LLP (Kazakhstan) and Sciensano, Unit Exotic Viruses and Particular Diseases (Belgium) and has been deposited in GenBank under accession number MN642592 (LSDV isolate Kubash/KAZ/16) (Mathijs et al. 2020).

Results

PCR and Virus isolation

From 7th July until the end of November 2016, three outbreaks were confirmed within Makash village. A total number of 425 cattle were disposed of in the eradication program. A total of 185 samples were tested by PCR and VI. The presence of viral nucleic acid was laboratory-confirmed in a total of 102 samples, whereas 52 samples tested positive for VI. All skin lesions tested positive by PCR and VI. Viral DNA was detected in 24 of 74 blood samples and whereas virus isolation revealed a LSDV characteristic CPE in 3 out of 74 blood samples. Internal organs were tested positive by PCR, while it was not possible to isolate a live virus in cell culture infected from lymph nodes or lungs (Table 1). In addition, LSDV DNA was recovered from 6 out of 14 ticks, 8 out of 21 horn flies and 14 out of 25

stable flies' samples while live virus was isolated only from 2 out of 25 *Stomoxys calcitrans* samples.

Table 1. Summary of PCR and Virus isolation testing results.

Type of Sample	PCR (no positive/no tested)	% positive	Virus isolation (no positive/no tested)	% positive	Mean C _T value
Skin lesions	47/47	100	47/47	100	16.7
Blood	24/74	32	3/74	22	27.1
Lung	1/2	50	0/2	0	11.3
Lymph nodes	2/2	100	0/2	0	15.8
<i>Ixodidae</i> <i>Dermacentor</i> ticks	6/14	43	0/14	0	16.4
<i>Stomoxys calcitrans</i>	14/25	56	2/25	8	24.3
<i>Hematobia irritans</i>	8/21	38	0/21	0	22.9

Control Measures

In the first affected farm, a total stamping out as well as incineration of carcasses were undertaken to prevent the spread of the disease locally. Quarantine and cattle movement controls were initiated within the Kurmangazy District, as well as strict restrictions on vehicles commuting to and from the affected zones. In addition, ring vaccination were conducted in a radius of 30 km. Such a significant coverage explained by the high density of livestock population and use of common grazing lands on South and West from the initial foci. In Kazakhstan, vaccination campaign was launched immediately after notification was sent to OIE, more than 70.000 cattle in the affected areas and neighboring regions (Makhambet, Isatay, Makat) were vaccinated during the vaccination campaign. A total of one million doses of LSD vaccine (LUMPIVAX®, Neethling-type, Kenya) were purchased before the outbreak and used in cattle against LSD. In Makash, veterinary personnel that were involved in LSD control and eradication campaign wore personal protective equipment (PPE) when visiting affected farms. Moreover, animal premises

(walls, ceilings and floor) were disinfected, utilizing Lysoformin 3000. Farmers were instructed to apply the disinfectants every day.

In response to the LSD outbreak in 2016 on the Russian side of the border, the veterinary authorities culled only those cattle showing typical clinical signs (partial stamping out) and implemented movement restrictions. Susceptible cattle were treated with insect repellents and vaccinated with a heterologous live sheeppox virus vaccine at a dose of 10^{-4} TCID₅₀, produced locally by ARRIAH (Usadov et al. 2018, Sprygin et al. 2018).

Discussion

Epidemiological investigation

To date, the source of infection and the mode of transmission of the virus to Kazakhstan remain unclear. This issue is especially urgent for transboundary infections. Most researchers believe that spread of the causative agent of LSD outside the epizootic focus region to a new area happens due to unauthorized movements of infected animals in the presence of insect vector (Issimov et al. 2020). These assumptions could be supported by the presence of river delta along border, which is thought to be auspicious habitat for reproduction of the insect vectors. Transmission of LSDV within the herd occurs by aerosols when a sick animal exhales, via direct contact between animals, through contaminated water and feed or by blood-feeding insects, (Carn and Kitching 1995, Kitching and Taylor 1985). It has been suggested that the spread of LSD into countries such as Iran, Azerbaijan, Republic of Dagestan, Georgia and Russia Federation was associated with the direct and indirect animal contacts when the farmers were using shared pasture lands between the bordering states (Mishchenko 2015). Thus, practicing communal grazing and illegal animal trading between transboundary farms can serve as source of LSDV introduction into new area. Azerbaijan scientist have suggested that also that human factors can be involved role in mechanical transmission of the pathogen via direct contact with

infected animals and their environment, farm workers may transport and spread virus to healthy herd (Zeynalova et al. 2016). In addition, Annandale et al. (2014) reported that cattle insemination with infectious semen lead to disease development.

Despite assumption in the transmission of LSD mentioned above, it is generally accepted that a variety of blood-feeding insects play a significant role in LSDV transmission by acting as mechanical vectors. According to the epizootic investigation outcomes of LSD outbreaks in Egypt, it was considered highly likely that the pathogen was transferred by stable flies (*Stomoxys calcitrans*) (Salib and Osman 2011). This assumption was based on the seasonality of outbreaks of LSD, occurring during hot and wet summer seasons (Weiss 1968, Carn and Kitching 1995, Magori-Cohen et al. 2012). In recent studies, LSDV transmission from diseased to susceptible cattle by *Stomoxys* species have been demonstrated successfully under laboratory condition (Sohier et al. 2019, Issimov et al. 2020).

A mathematical model of synoptic system used in recent study to calculate air long-distance dispersal (LDD) of LSDV in Israel revealed that LDD transmission by air is a feasible way of dissemination of vector borne diseases in the Middle East and should be taken into consideration when evaluating risk for new outbreaks (Klausner, Fattal, and Klement 2017). In other studies, mathematical modelling revealed that under natural conditions the blood-feeding insects range rarely exceeds 5 km (Gubbins et al. 2018). Moreover, wind has a direct impact on insect distribution (Yeruham et al. 1995). Such a significant coverage range and vector capability of stable flies to carry pathogen may lead to LSDV escape from the initial outbreak foci and rapid dissemination over neighbouring farms. In relatively recent clinical experiments, the potential of ticks as a mechanical vector has been successfully demonstrated. Ticks in different molting stages have carried LSDV following feeding to repletion on artificially infected animals (Tuppurainen et al. 2011,

Tuppurainen et al. 2013). In addition, LSDV has been detected in the saliva of mature ticks making them capable of virus transmission (Lubinga et al. 2013).

In the Kazakhstani scenario of disease development, LSD was recorded mostly among emaciated animals, lactating cows, and calves. During the current LSD epidemic in Kazakhstan, the morbidity and mortality rates constituted 12.90% and 0.96% respectively. Due to rapid response of State Veterinary Service in combination of strict quarantine, stamping out and mass vaccination campaign allowed to limit LSD outbreak within the initial foci.

Conclusion

Given the fact that there is a significant density of livestock in the West Kazakhstan oblast and unauthorized trade in animals occurs, it is likely that LSD will continue to spread, leading to serious social and economic consequences for the whole country and posing a real threat to animal husbandry of developing countries of the Central Asia.

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Conflict of interest

The author declare that they have no conflict of interest.

Data availability: The data obtained during this study are openly available in KNB Data Repository [doi:10.5063/F1ZG6QM0](https://doi.org/10.5063/F1ZG6QM0).

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