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EMERGENCE OF THE FIRST LUMPY SKIN DISEASE IN 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, as well as associated clinical manifestations of the disease, diagnostic methods, and control measures taken to combat further spread of the pathogen. Initially, LSD was reported in a cattle farm located 49 km from the 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 3557 registered 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.

Key words: control measures, diagnosis, epidemiological data, Kazakhstan, lumpy skin disease.

1. Introduction

Lumpy skin disease virus (LSDV) belongs to the Capripoxvirus genus of the Poxviridae family. It 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 [1]. The World Organization for Animal Health classifies Lumpy Skin Disease (LSD) as a notifiable disease due to its significant economic impact [2].

LSDV was first discovered in Zambia, where it was recorded in 1929. Subsequently, LSDV has become endemic across almost the entire African continent and in the Middle East, Turkey, and Azerbaijan, and is continuing to spread north, posing a threat to Europe and the Central Asia region. In 2015, LSD outbreaks were documented in Greece [3], from where it spread to the Balkans. Similarly, in 2015, the disease was clinically confirmed in North Caucasus in Russia, where it became epidemic and spread throughout the country [4,5]. In 2016, LSD re-emerged in several regions of Southern Russia, including Astrakhan oblast bordering

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 Statistics Bureau of the Agro-Industrial Complex of the Ministry of Agriculture of the Republic of Kazakhstan, the total cattle population in the country is estimated to be about 7.161 million heads, which are mostly local breeds (87.1%); the remaining are hybrids and exotic breeds. The livestock system practised 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 amounted to 20,000 tons. In rural areas, cattle are the primary source of income and are mainly kept for milk and meat production. The commercial smallholding dairy and beef farms are mostly market-oriented and located around urban areas practising intensive management.

2. Materials and Methods

2.1. 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 the Ministry of Education and Science of the Republic of Kazakhstan (permit number: 1205/106).

2.2. Outbreak in Kazakhstan

An incursion of the previously exotic LSD in Kazakhstan was first recorded in July 2016, in Makash village in Atyrau oblast (Figure 1), located 49 km from the borderline with Astrakhan oblast in 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 7 July 2016, the owner of a small cattle farm practising mixed dairy and beef management reported that several animals at the holding were showing previously unseen clinical signs. The unusual behaviour of the cattle within the herd was combined with multiple skin lesions similar to those reported by Davies [1] and Weiss [6]; 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 extensive haemorrhage of the uterus. Moreover, skin lesions in the form of multiple convex indurations were visible in aborted foetuses, similar to those described by [7]. The Office International des Epizooties (OIE) World Animal Health Information System (WAHIS) was notified as soon as LSD suspicion was confirmed by positive laboratory results on 22 July

2016.

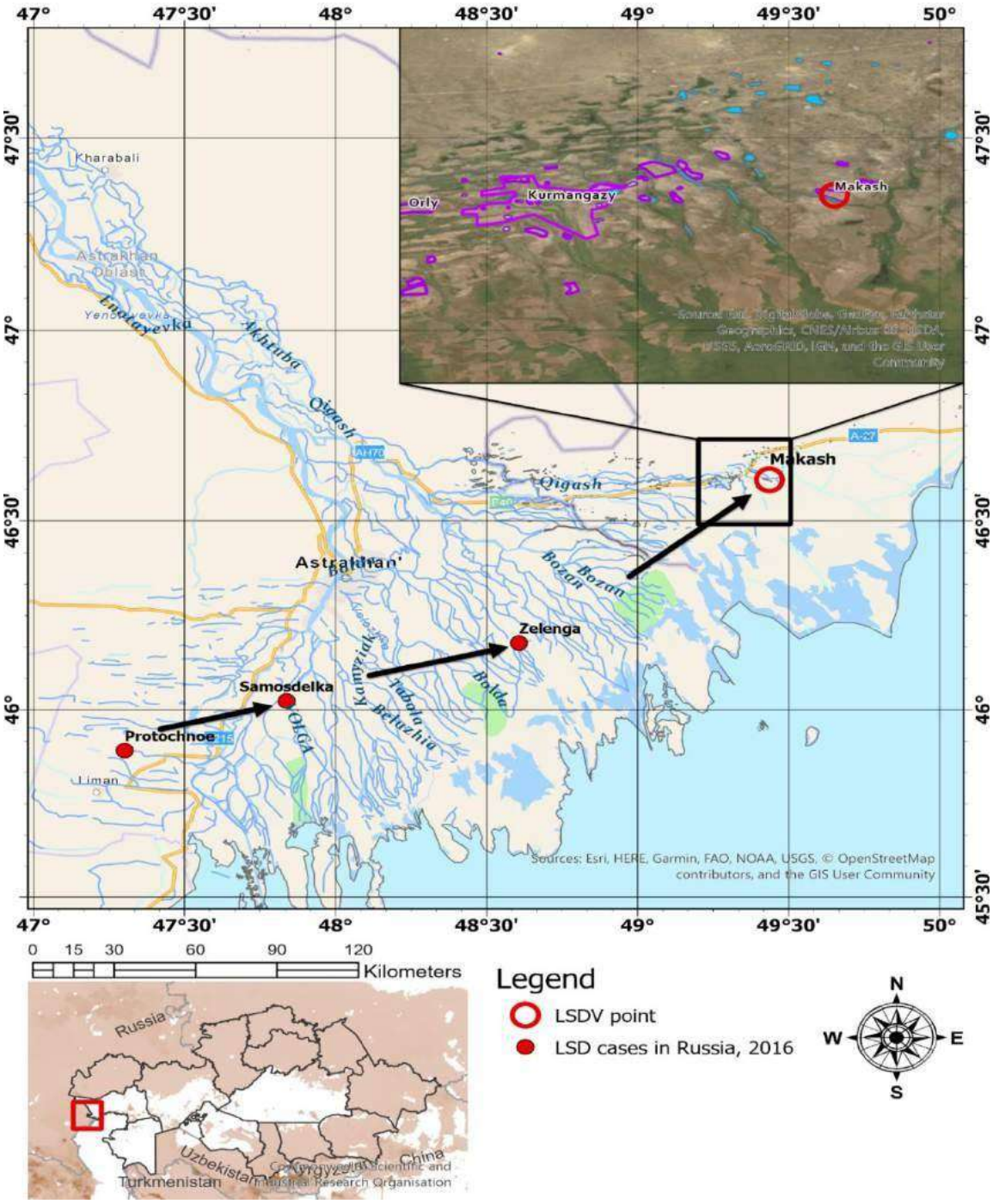

Figure 1. The location of the lumpy skin disease (LSD) outbreak in Atyrau oblast. Areas drawn in pink indicate seasonal communal grazing lands. LSDV, lumpy skin disease virus. The red box is overview map of cattle distribution ((FAO) Food and agriculture organization).

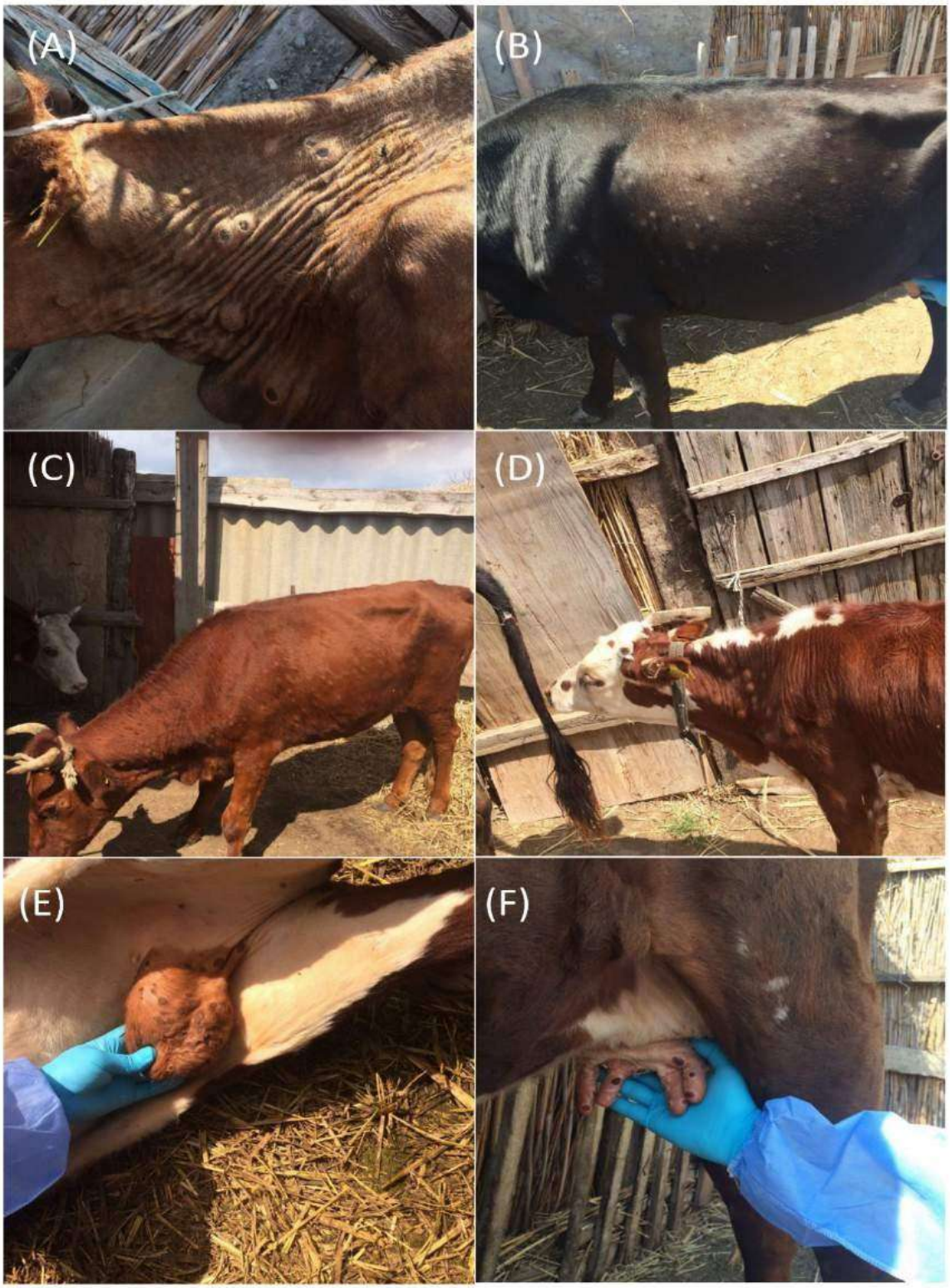

Figure 2. Cattle exhibiting characteristic LSD clinical signs in the outbreak focus area in the Republic of Kazakhstan in 2016. The body surfaces of infected animals exhibited extensive circumscribed and convex skin nodules (A–D) with ulceration of the scrotum and the teats (E,F).

Blood samples and skin lesions were collected for testing by the Virology Section of the BSL-3 Laboratory of the RIBSP and by the OIE Reference Laboratory, All-Russian Research Institute for Animal Health (ARRIAH).

2.3. Control Measures

In the first affected farm, a total stamping out process and 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 vaccinations were conducted in a radius of 30 km. Such significant coverage was explained by the high density of the livestock population and use of common grazing lands to the south and west of the initial focus area. In Kazakhstan, a vaccination campaign was launched immediately after notification was sent to OIE, whereby more than 70000 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 the LSD control and eradication campaign wore personal protective equipment (PPE) when visiting affected farms. Moreover, animal premises (walls, ceilings, and floor) were disinfected using 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 [5,8]. 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 of 12.90% and 0.96%, respectively. The case fatality rate was 7.41% [9]. Kazakhstan veterinary services carried out a total stamping out program at this first affected farm.

2.4. Sample Collection

Samples were collected from 96 cattle of different ages and sexes exhibiting clinical signs characteristic of LSD. In severe cases, elevation in body temperature up to 42 °C was observed, followed by extreme salivation, nasal discharge, and inflammation of mucosa. The body surfaces of infected animals were covered entirely by circumscribed and convex nodules that were firm and

tough when palpated. Animals exhibiting mild symptoms of LSD developed enlargement of superficial lymph nodes, as well as swelling of the limbs and brisket. A total of 74 blood samples, 47 skin lesions, and 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 in the area of the brisket and the neck of the diseased host were collected during the clinical examination of infected animals. Moreover, 21 horn flies (*Hematobia irritants*) and 25 stable flies (*Stomoxys calcitrans*) were caught within livestock premises using a commercial fly catching unit, namely a miniature CDC (Centers for disease control) light trap with UV light (John W. Hock Company, Gainesville, Florida, USA) to investigate a possible insect vector involvement in the transmission of LSD in the field. The light trap was suspended from the ceilings of cattle barns and monitored every two hours for the presence of insects. The insect collection time was designated as follows: 12 h during the night.

2.5. Virus Isolation

Virus isolation (VI) was conducted according to the Standard Operational Procedures of the BSL-3 Laboratory of the RIBSP. The tests were carried out as described by OIE [10]. Briefly, 1 mL buffy coat or supernatant was administered on to lamb testes cells in 25 cm^2 cell culture flasks and allowed to incubate at 37 °C for 1 h. Following incubation, cell culture growth media were removed and the cell monolayer was rinsed with PBS and overlaid with Glasgow's minimal essential medium containing 0.1% penicillin, 0.2% gentamycin, and 2% foetal calf serum (ThermoFisher Scientific, Waltham, MA, USA). 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.

2.6. Virus Detection by PCR

A PCR assay was performed using the protocol published by Tuppurainen and Venter [11].

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

For the PCR assay, in order to produce 192 bp of amplified nucleotide 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 [12]. The conditions for DNA amplification in a thermal cycler (Eppendorf Mastercycler, St. Louis, MO, USA) 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 subjected to.5% agarose gel electrophoresis and the results were visualised using a Bio-Imaging Systems MiniBIS Pro system (Jerusalem, Israel).

Complete genome sequencing of the LSDV field strain was performed in collaboration with the Kazakh Scientific Research Veterinary Institute LLP (Almaty, Kazakhstan) and Sciensano, Unit Exotic Viruses and Particular Diseases (Ukkel, Belgium). The LSDV field strain was deposited in GenBank under accession number MN642592 (LSDV isolate Kubash/KAZ/16) [13].

3. Results

3.1. PCR and Virus Isolation

From 7 July until the end of November 2016, three outbreaks were confirmed within Makash village. A total 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 using PCR and VI. Viral DNA was detected in 24 of 74 blood samples, whereas virus isolation revealed an 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 cultures infected from lymph nodes or lungs (Table 1). In addition, LSDV DNA was recovered from 6 out of 14 ticks belonging to the *Dermacentor* genus, 8 out of 21 horn fly samples, and 14 out of 25 stable fly 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 [14].

Type of Sample	PCR (No Positive/No Tested)	Virus Isolation (No Positive/No Tested)	Mean C_T Value
Skin lesions	47/47	47/47	16.7
Blood	24/74	3/74	27.1
Lung	1/2	0/2	11.3
Lymph nodes	2/2	0/2	15.8
Dermacentor	6/14	0/14	16.4
Stomoxys calcitrans	14/25	2/25	24.3
Hematobia irritants	8/21	0/21	22.9

4. Discussion

4.1. Epidemiological Investigation

To date, the source of infection and the mode of transmission of the virus to Kazakhstan remain unclear. This latter 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 unauthorised movements of infected animals in the presence of an insect vector [15]. These assumptions are supported by the presence of the river delta along border, which is thought to be an auspicious habitat for reproduction of the insect vectors. Transmission of LSDV within the herd occurs via aerosols when a sick animal exhales, via direct contact between animals, through contaminated water and feed, or via blood-feeding insects [16,17]. It has been suggested that the spread of LSD into countries such as Iran, Azerbaijan, the Republic of Dagestan, Georgia, and the Russian Federation was associated with direct and indirect animal contact when the farmers were using shared pasture lands between the bordering states [18]. Thus, practising communal grazing and illegal animal trading between transboundary farms can serve as method of LSDV introduction into new areas. Scientists from Azerbaijan have also suggested that human factors could be involved in the mechanical transmission of the pathogen via direct contact with infected animals and their environments, whereby farm workers may transport and spread the virus to healthy herds [4]. In addition, Annandale and Holm [19] reported that cattle insemination with infectious semen can lead to disease development.

Despite the assumptions related to 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*) [20]. This assumption was based on the seasonality of outbreaks of LSD, occurring during hot and wet summer seasons [6,16,21]. In recent studies, LSDV transmission from diseased to susceptible cattle by Stomoxys species have been demonstrated successfully under laboratory conditions [15,22].

A mathematical model of a synoptic system used in a recent study to calculate aerial 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 [23]. In other studies, mathematical modelling revealed that under natural conditions, the blood-feeding insects' range rarely exceeds 5 km [24]. Moreover, wind has a direct impact on insect distribution [25]. Such a significant coverage range and vector capability of stable flies to carry pathogens may lead to LSDV escape from the initial outbreak focus area 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 [26,27]. In addition, LSDV has been detected in the saliva of mature ticks, making them

capable of virus transmission [28].

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 were 12.90% and 0.96%, respectively. Due to the rapid response by the State Veterinary Service, in combination with strict quarantine, stamping out practices, and a mass vaccination campaign, the LSD outbreak was limited within the initial focus area.

5. Conclusions

Given the fact that there is a significant density of livestock in the West Kazakhstan oblast and unauthorised trade of 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 in developing countries of the Central Asia.

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2016 ЖЫЛЫ ҚАЗАҚСТАНДА НОДУЛЯРЛЫҚ ДЕРМАТИТТІҢ АЛҒАШҚЫ АУРУЫНЫҢ ПАЙДА БОЛУЫ

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Аңдатпа. Ірі қара малдың кесек тері ауруы - Африка континентінен шыққан ірі қара малдың трансшекаралық вирустық ауруы. Мұнда біз Қазақстан Республикасында 2016 жылдың шілдесінде тіркелген бірінші LSD індетін, сондай-ақ аурудың клиникалық көріністерін, диагностикалық әдістерді және қоздырғыштың одан әрі таралуымен күресу үшін қабылданған бақылау шараларын сипаттаймыз. Бастапқыда LSD Батыс Қазақстандағы Атырау облысындағы Қазақстан-Ресей шекарасынан 49 шақырым жерде орналасқан мал фермасында тіркелген. Артынша ауру сол ауданға қарасты көрші шаруашылықтарға да тараған. Алдын ала тергеуден кейін жергілікті мемлекеттік ветеринария қызметі төтенше жағдайлардың алдын алу жоспарына сәйкес қатаң карантин жариялады, сонымен қатар малдың қозғалысына шектеу қойылды. Эпидемиологиялық бөлімшедегі ауруға шалдыққан ірі қара малдың саны тіркелген 3557 бас ірі қара малдың 459-ға жетіп, аурушаңдық 12,90% және өлім-жітім 0,96% құрады. Бұл қолжазба эпидемиологиялық жағдайды; диагноз; жаппай вакцинациялауды қоса алғанда, бақылау шаралары; және ауруды жою науқанын көрсетеді.

Түйін сөздер: бақылау шаралары, диагноз, эпидемиологиялық деректер, Қазақстан, сүйелді дерматит.

ПОЯВЛЕНИЕ ПЕРВОГО ЗАБОЛЕВАНИЯ НОДУЛЯРНОГО ДЕРМАТИТА В КАЗАХСТАНЕ В 2016 ГОДУ

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Аннотация. Нодулярный дерматит (НД) — это новая трансграничная вирусная болезнь крупного рогатого скота, происходящая из африканского континента. Здесь мы описываем первую вспышку ЗУД, зарегистрированную в Республике Казахстан в июле 2016 г., а также связанные с ней клинические проявления заболевания, методы диагностики и меры борьбы с дальнейшим распространением возбудителя. Первоначально заражение НД было зарегистрировано на животноводческой ферме, расположенной в 49 км от

казахстанско-российской границы в Атырауской области в Западном Казахстане. Впоследствии болезнь распространилась на соседние хозяйства, расположенные в пределах одного района. После предварительного расследования местная государственная ветеринарная служба объявила строгий карантин в соответствии с Государственным планом действий в чрезвычайных ситуациях, а также немедленный полный убой и ограничения на передвижение скота. Во время вспышки количество пораженного крупного рогатого скота в пределах эпидемиологической единицы достигло 459 голов из 3557 зарегистрированных восприимчивых голов крупного рогатого скота с заболеваемостью 12,90% и смертностью 0,96%. В данной работе представлена эпидемиологическая ситуация; диагноз; меры борьбы, включая массовую вакцинацию; и кампания по искоренению очага болезни.

Ключевые слова: меры контроля, диагностика, эпидемиологические данные, Казахстан, нодулярный дерматит.