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RISKS OF OCCURRENCE AND SPREAD OF LUMPY SKIN DISEASE VIRUS IN THE REPUBLIC OF KAZAKHSTAN

ANNOTATION

The data of veterinary reporting of the Department of veterinary medicine of the Ministry of Agriculture of the Republic of Kazakhstan, RSE on REM at the Republican veterinary laboratory, RSE on REM at the National reference center for veterinary medicine were studied and analyzed. Monitoring studies on lumpy skin disease were carried out for an objective assessment of the epizootic situation in the context of regions, districts, rural districts where there are cattle susceptible to the disease. The main factors contributing to the emergence and spread of lumpy skin disease virus in the country have been identified. At the same time, there is a very high probability that the virus will penetrate deep into the country with animals imported from abroad, in particular from the Russian Federation, which may be carriers of the infection or latent patients. Evidence of this is the registration of new epizootic foci of diseases in cattle on its territory. The effect of vaccination on the formation of immunity in animals was studied by serological examination of their blood sera in ELISA. As a result, an assessment of the reactivity of animals to the introduction of the vaccine was given and some of the reasons hindering the formation of immunity in animals were clarified. It is proposed to immunize animals with vaccines registered in the Republic of Kazakhstan and (or) the member states of the Eurasian Economic Union, which have passed the mandatory certification procedure at the OIE Reference centers and are produced at biofactory that meet GMP requirements.

Key words: *Lumpy skin disease, livestock importation, immunity, diagnostics.*

Introduction. Lumpy skin disease (skin tubercle, nodular exanthema, skin nodular rash, patchwork skin disease) is a viral transmissible highly contagious transboundary zoonotic disease of cattle caused by a DNA-containing virus of the Poxviride family. The disease is accompanied by fever, swelling

of the subcutaneous connective tissue and organs, the formation of skin nodules, damage to the eyes, mucous membrane of the respiratory and digestive tracts [1, 2].

Lumpy skin disease of cattle (LSD) by the decision of the Board of the Eurasian economic commission dated September 17, 2019 No. 156 is included in the Directory of especially dangerous, quarantine and zoonotic animal diseases. Animal disease code 160 was assigned. The disease is widespread throughout the world [3, 4, 5, 6, 7, 8]. According to official data from the Rosselkhoznadzor, as of december 2022, 16 countries of the world, including Russia, are unfavorable for this infection. Out of the 16 countries recognized as disadvantaged in terms of LSD, 2 country is located in Africa, in Europe - 2 and in Asia - 12.

In case of penetration into the territory of the country, the disease entails enormous economic losses in animal husbandry, leads to death and premature culling of animals, endangers the preservation of breeding herds, stable selection and breeding work, affecting the development of the economy, interfering with the sale and exchange of animals [9, 10, 11, 12, 13].

The economic damage caused by the disease is made up of the need to implement quarantine-restrictive and veterinary-sanitary measures (separate keeping of certain groups of animals, disinfection of premises, diagnostic tests of animals, etc.). The implementation of these measures complicates the normal production activities of farms and requires significant expenditures.

In this regard, it is required to carry out a full-scale systematic epidemiological monitoring in all regions of the country and a comprehensive analysis of all factors influencing the penetration and spread of the infection. This will make it possible to timely recognize threatened areas where there is a high risk of epizootic foci of LSD. The study of this issue will allow the Republic of Kazakhstan to maintain epizootic well-being among cattle and export environmentally friendly livestock products to foreign countries.

The purpose of the study is to identify the main factors contributing to the emergence and spread of the LSD virus in the territory of the Republic of Kazakhstan.

Material and methodology. The materials for the research were the official data of veterinary reporting of the Committee for veterinary control and supervision of the Ministry of Agriculture of the Republic of Kazakhstan, the Republican veterinary laboratory, the National reference center for veterinary medicine, the results of our own epizootological, clinical and laboratory studies collected during the examination of individual epizootological units.

When performing research work, classical and molecular biological methods for diagnosing LSD recommended by the OIE were used (Guidelines for diagnostic tests and vaccines for terrestrial animals 2019, Chapter 3.4.12.). An assessment of the epizootic situation in terms of LSD of the cattle in the world and in the countries adjacent to the territory of Kazakhstan was carried out according to the official data of the OIE, posted on the website of the Rosselkhoznadzor. Sampling was carried out in such a way as to ensure the maximum probability of obtaining a representative sample according to the guidelines developed and proposed by KazSRVI LLP, Almaty, 2021. For this, districts, rural districts and individual farms in the context of the regions of the Republic of Kazakhstan were selected by random sampling. Then, for each epidemiological unit, the number of animals for blood sampling was determined. At the same time, sampling of biological material was carried out in accordance with the Rules for sampling, transported (transported) objects and biological material, approved by order of the Minister of Agriculture of the Republic of Kazakhstan dated April 30, 2015 No. 7-1/393 for testing for LSD in a veterinary laboratory.

ELISA was performed using a commercial reagent kit ID Screen Capripox Double Antigen Multi-species, produced by ID. Vet - France, according to the manufacturer's instructions. Seroprevalence was calculated as a percentage by determining the ratio between the number of reacting and all the studied animals in the context of epizootological units, rural districts, districts and regions. In addition, during trips from each region of the republic, 5 samples of whole blood were taken in a volume of at least 4 cm³ with EDTA vacutainers to determine the DNA of the virus by PCR. DNA isolation was carried out using the ID Gene™ Spin Universal Extraction Kit SPIN50 DNA extraction kit. Amplification was performed using the "Real-time PCR kit for the detection of LSD virus and Neetling vaccine strain in animal whole blood, nasal and oral mucosal swabs, tissues (infected skin)" ID Gene™ LSD DIVA Triplex PCR kit (ID VET, France).

Results and its discussion. According to the data of veterinary reporting, to date, no cases of LSD of the cattle have been registered in the territory of the Republic of Kazakhstan. However, the threat of outbreaks of LSD in the country remains. The virus can enter at any time as a result of unauthorized importation of cattle from the territories of countries that are unfavorable for this disease, their slaughter

products, semen, milk and dairy products. The virus is well preserved in the external environment and can be transmitted not only by the alimentary method, but also aerogenically and transmissibly [14, 15, 16, 17, 18, 19]. Therefore, in the system of veterinary measures, the leading place is occupied by general preventive measures. These measures are primarily aimed at protecting the country's territory from the introduction of the LSD virus.

First of all for the Republic of Kazakhstan, the Russian Federation poses an epizootic danger. New epizootic LSD foci have appeared on its territory, where vaccine-like and untyped genotypes of the virus circulate, in particular the Privolzhsky strain, which is capable of being transmitted without the participation of insect vectors. So, in 2022, outbreaks of LSD were registered in the Amur Region and the Republics of Buryatia, Tatarstan, Tyva [20]. There is also a threat of bringing the infection into the country from such states as Mongolia, China and Turkey. Therefore, the country's veterinary service constantly monitors the epizootic situation for LSD cattle in the world and implements the planned set of measures aimed at preventing the penetration of infection from disadvantaged states. Conducted clinical, virological and serological surveillance, as well as vaccination of cattle.

Kazakhstan has the longest land border with Russia. On both sides in the border areas are actively engaged in animal husbandry, including cattle breeding. There are 30 road, 19 rail and 1 river checkpoints between the two countries. Through these checkpoints, goods move from one country to another, including farm animals, products and raw materials of animal origin and feed. These objects represent a potential hazard and may be the reason for the entry of the LSD virus. Purchased animals with unclear epizootological characteristics play an important role, especially when they are placed among a prosperous livestock.

According to the information provided by the Committee for veterinary control and supervision of the Ministry of Agriculture of the Republic of Kazakhstan, in 2018, almost 21,859 heads of cattle were imported as part of the beef cattle breeding program, in 2019 - 38,800 heads, in 2020 - for the first 7 months 7,728 heads of cattle. In 2019, 60% of imported livestock was imported from Russia, from Australia - 12%, from the USA and the Czech Republic - 7% each, from other countries - 10% [more: <https://www.kursiv.kz/news/vlast-i-biznes/kazakhstan-rezko-uvlichil-vvoz-importnogo-skota>].

In 2020-2022, livestock was distributed by regions of import as given in table 1.

Table 1 – Information on the importation of cattle to the Republic of Kazakhstan from foreign countries from 2020-2022

Region name	7 months 2020	2021	2022	
			Sending country	
	number of imported cattle			
Atyrau region	37	31	90	RF, Tatarstan
Mangistau region	0	0	0	-
Almaty region	2383	129	586	No data
Zhetysu region			247	Denmark
			33	Germany
Jambyl region	1146	990	209	Buryatia
			31	Czech Republic
Pavlodar region	718	4905	2085	No data
Northern Kazakhstan region	645	2595	480	Germany
			96	Czech Republic
			24	Lithuania
			66	Ukraine
Western Kazakhstan region	628	1635	165	Belarus, Czech Republic
Eastern Kazakhstan region + Abay region	592	3190	64	Germany, RF Altai Territory
Aktobe region	523	2764	847	Russia
Karaganda region + Ulytau region	380	2219	1399	No data
Akmola region	294	0	25	No data
Turkestan region	200	2318	50	RF, Tatarstan

Kostanay region	169	-	50	Czech Republic
			68	Denmark
Kyzylorda region	50	100	-	-
Total	7 728	20876	6923	

Table 1 shows that cattle are annually imported into Kazakhstan from near and far abroad countries. Among these animals may be virus carriers or latent patients. Such animals, when moving, contribute to the transmission of the virus through blood-sucking insects and are transported over considerable distances by road and rail, ships, etc. Therefore, it is practically impossible to completely exclude the possibility of the LSD virus introduction into the country. Here, one should also take into account the possibility of introduction of the bovine LSD pathogen to the border areas through wild ruminants, which may be potential carriers of the infection.

This danger is confirmed by the cases of detection of seropositive animals in the regions of the republic where cattle are not vaccinated against LSD. For example, in 2018, according to the data of the Republican veterinary laboratory, when examining blood sera in ELISA selected from the territory of the Pavlodar region, 10 animals responding to LSD were identified, which gave a negative result in PCR.

Also, employees of KazSRVI LLP in 2019 found 9 positive samples in the ELISA. Samples were obtained from 3 animals located in the economic entities of the Zhambyl rural district, Rayymbek district of Almaty region and 2 samples from the Turksib district, microdistrict Kairat, as well as 4 samples, were delivered from the rural district of Tegishhil, Saryagash district of the Turkestan region. PCR diagnostic was carried out, as a result, all positive samples obtained by ELISA turned out to be negative.

The next case occurred in 2020, when 129 heads of cattle were imported from the Russian Federation: to the East Kazakhstan region, Kokpektinsky district, Meirbek farm - 82 heads; in the Kurchum district, the village of Kurchum, the farm "Ansar" - 2 heads; to the North-Kazakhstan region, Akkuly district, Sherbakty village, farm "Dastarkhan" - 8 heads; to Atyrau region, Indersky district, village of Zharsuat, farm "Agatay" - 37 heads. Cattle were imported from the Altay territory, Kemerovo and Penza regions, where epizootic foci of LSD were registered. In general, 1884 heads of cattle were brought to the East Kazakhstan region that year. Cattle were imported mainly from the Altay territory, as well as the Republic of Buryatia, where there were epizootic foci of LSD. The National reference center for veterinary medicine by ELISA and PCR identified 16 positive animals among the cattle imported to the Atyrau region from the Russian Federation, which were then destroyed by slaughter in a sanitary slaughterhouse.

Considering that only cattle have epizootological significance in the distribution of LSD, we studied the number and density of susceptible animals in the context of the regions of the republic. So, by January 1, 2022, on the territory of the country, in farms of all forms of ownership, the number of cattle amounted to 8,185,100 heads. Its largest number is in Almaty, Zhetysu, East Kazakhstan, Abay and Turkestan regions, 731400, 565900, 484800, 806200, 1141800 heads, respectively. Almaty and Turkestan regions have the highest cattle densities of 4.3 and 8.57 head/km², respectively. Also, a relatively high density of livestock is observed in the East Kazakhstan, West Kazakhstan, Pavlodar and North Kazakhstan regions (3.15-3.58 heads/km²). A lower density of cattle stock is noted in Aktobe, Atyrau, Karaganda and Kyzylorda regions (1.18-1.44 heads/km²). The Mangistau region has the lowest cattle density - (0.11 heads/km²).

The presence of such a number of susceptible cattle increases the likelihood of infection in these areas. Therefore, in order to protect animals from LSD, all cattle are annually immunized with a live homologous vaccine from an attenuated capripoxvirus strain. At the same time, most of the animals are vaccinated in the spring before the appearance of blood-sucking insects.

As for 07.01.2022, only 5588218 heads of cattle or 59.23% of the total livestock kept in the republic - 9433200 were immunized in the country. A high percentage of coverage of animals with vaccination against LSD is observed in Atyrau - 89.01, Akmola - 86.07, East Kazakhstan and Abay - 82.64, Turkestan - 71.79, Zhambyl - 71.09 regions. We note a low percentage of vaccination coverage of animals in the North Kazakhstan region - 30.80, Karaganda and Ulytau regions - 30.82. In the Karaganda region, according to the reporting data of the State Enterprise "Vetstansion of the Aktogay district", vaccination against LSD of the cattle in the district is not planned.

Since 2020, the Republican veterinary laboratory has stopped serological testing of cattle for lumpy skin disease in almost many regions of the country, because the applied vaccine Lumpivaxtm of Kenyan production (KEVEVAPI) turned out to be in fact a strain of the virus obtained from goats.

Therefore, serological tests were not suitable to detect infected animals after the vaccination campaign started because the recombinant virus present was indistinguishable from other strains of capripoxvirus [21]. Currently, there are no vaccines that provide a strategy for differentiating postvaccination antibodies from postinfection antibodies [22]. In addition, in some areas where cattle were first immunized against LSD, approximately 10% experienced complications after administration of the vaccine.

In this regard, employees of KazSRVI LLP annually conduct screening studies for lumpy dermatitis. Selected biomaterial samples are examined by serological and molecular genetic methods.

In 2022, in accordance with the sampling plan of KazSRVI LLP, studies were conducted in the ELISA of 3045 samples of cattle blood serum taken from the territory of 13 regions of the Republic of Kazakhstan. The results of serological studies are presented in table 2.

Table 2 – Results of serological tests of blood serum of cattle for 2022

Region name	Researched	Revealed	Animal characteristics	% response alive
Kostanay region	285	81	after vaccination	28.42
Northern Kazakhstan region	285	77	after vaccination	27.01
Aktobe region	285	90	after vaccination	31.58
Akmola region	15	15	before vaccination	100.00
	270	31	after vaccination	11.48
Kyzylorda region	30	3	after vaccination	10.00
	255	103	before vaccination	40.39
Karaganda region + Ulytau region	150	19	before vaccination	12.60
	135	34	after vaccination	25.18
Pavlodar region	285	39	after vaccination	13.68
Zhetysu region	195	44	after vaccination	22.56
Jambyl region	285	57	after vaccination	20.00
Turkestan region	15	8	before vaccination	53.33
	270	73	after vaccination	27.03
East Kazakhstan region + Abay region	285	40	after vaccination	14.03
Total for Kazakhstan after vaccination	2610	588	after vaccination	22.53
Total for Kazakhstan before vaccination	435	145	before vaccination	33.33
Total for Kazakhstan	3045	733		

As can be seen from table 2, seroprevalence was detected in 733 animals, including 2610 after and 435 before vaccination. 588 vaccinated animals or 22.53% of cattle developed immunity to LSD. The remaining 145 positive samples (33.33%) were found in unvaccinated cattle.

After vaccination, about 80-90% of cattle should have antibodies. But in fact, not all immunized

animals, as was the case in 2021, develop an immune response to the same degree to the vaccine [23]. There are some rural districts where specific antibodies were not detected in any of the samples by examining vaccinated cattle in ELISA. So in the Zhambyl region, out of 15 samples taken by us in the village of Moiyunkum, post-vaccination antibodies were not found in any of them. Post-vaccination antibodies were also not detected in animals after vaccination in some rural districts of the Ayagoz district of the East Kazakhstan region and in other regions of Kazakhstan. It is likely that these animals may have been absent at the time of mass vaccination. Also, some emaciated animals may not respond at all to vaccine administration. In addition, the LSD virus encodes a number of proteins that help it to avoid the host's immune response. Such animals, which are not immune, remain at risk of infection even in areas with relatively high vaccination coverage.

Meanwhile, in Kyzylorda region 40.39% of unvaccinated animals were found to have antibodies to cattle LSD virus. This may be due to previous immunizations of animals and the immunobiological properties of the live attenuated vaccine itself, as well as the ability of ELISA to cross-react with some antibodies of other poxviruses. In connection with the above arguments, amendments and additions were made to the Veterinary Rules in 2021, where it is proposed to use vaccines against LSD certified by the OIE reference centers and produced according to the GMP standard.

At the same time, PCR studies of 45 whole blood samples collected from vaccinated cattle from different regions of Kazakhstan did not reveal LSD virus DNA, which proves the effectiveness of the developed system of anti-epizootic measures using homologous live vaccines.

Conclusion. LSD of cattle is still a danger, as evidenced by the positive results diagnostic studies. Therefore, it is necessary to further strengthen veterinary control over export-import operations, places of unloading and quarantine of animals, unloading and storage of products and raw materials of animal origin. Import livestock, products of their slaughter, semen, milk and dairy products only from countries that are free from LSD in the presence of accompanying veterinary documents (certificate). All cattle should be vaccinated with preparations registered in the Republic of Kazakhstan and (or) member states of the Eurasian economic union, which have passed the mandatory certification procedure at the OIE reference centers and are produced at biocombines that meet GMP requirements.

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ТҮЙІН

ҚР АШМ Ветеринария департаментінің, "Республикалық ветеринариялық зертхана" ШЖҚ РМК, "Ветеринария бойынша ұлттық референттік орталық" ШЖҚ РМК ветеринариялық есептілік деректері зерделенді және талданды. Ауруға бейім малы бар облыстар, аудандар, ауылдық округтер бөлінісінде эпизоотиялық жағдайды объективті бағалау үшін елімізде нодулярлық дерматит бойынша мониторингтік зерттеулер жүргізілді. Нодулярлық дерматит вирусының пайда болуына және таралуына ықпал ететін негізгі факторлар анықталды.

Сонымен қатар, вирустың шетелден әкелінген жануарлармен, атап айтқанда Ресей Федерациясынан, инфекцияны тасымалдаушыларымен немесе жасырын науқастармен елге терең ену ықтималдығы өте жоғары. Оның айғағы оның аумағында ірі қара мал ауруларының жаңа эпизоотиялық ошақтарын тіркеу дәлел болып табылады.

ИФТ-дағы қан сарысуларын серологиялық зерттеу арқылы вакцинацияның жануарларда иммунитеттің қалыптасуына әсері зерттелді. Нәтижесінде жануарлардың вакцинаны енгізуге реактивтілігіне баға берілді және жануарларда иммунитеттің қалыптасуын тежейтін кейбір себептер анықталды.

Жануарларды Қазақстан Республикасында және (немесе) Еуразиялық экономикалық одаққа мүше мемлекеттерде тіркелген, ХЭБ референттік орталықтарында міндетті сертификаттау рәсімінен өткен және GMP талаптарына жауап беретін биокомбинаттарда өндірілген вакциналармен иммундау ұсынылды.

РЕЗЮМЕ

Изучены и проанализированы данные ветеринарной отчетности Департамента ветеринарии МСХ РК, РГП на ПХВ «Республиканская ветеринарная лаборатория», РГП на ПХВ «Национальный референтный центр по ветеринарии». Проведены мониторинговые исследования по нодулярному дерматиту для объективной оценки эпизоотической обстановки в разрезе областей, районов, сельских округов, где имеются восприимчивый к болезни крупный рогатый скот. Выявлены основные факторы, способствующие появлению и распространению вируса нодулярного дерматита в стране. При этом очень высокая вероятность проникновения вируса с завозимыми из-за рубежа животными вглубь страны, в частности из РФ, которые могут быть носителями инфекции или латентными больными. Свидетельством тому являются регистрация новых эпизоотических очагов заболеваний крупного рогатого скота на её территории.

Изучено влияние вакцинации на формирование иммунитета у животных путем серологического исследования их сывороток крови в ИФА. В результате, которой была дана оценка реактивности животных на введение вакцины и выяснены некоторые причины, сдерживающие формирование иммунитета у животных.

Предложено иммунизировать животных вакцинами, зарегистрированными в Республике Казахстан и (или) государствах-членах Евразийского экономического союза, прошедшими процедуру обязательной сертификации в референс центрах МЭБ и производимых на биокомбинатах, отвечающих требованиям GMP.