Emergence of LSD in Montenegro in 2016 – an example of strengths and weaknesses of Veterinary Sector and potential for sustainable public health in the country

Maša Jovanović[1*], Marko Nikolić[2]

[1]University of Montenegro, MARDS Department, Bulevar Džordža Vašingtona b.b. Podgorica, Montenegro
[2]Department for Microbiology and Parasitology, Diagnostic Veterinary Laboratory, Bulevar Džordža Vašingtona b.b. Podgorica, Montenegro

Abstract. A stable epidemiological situation is a prerequisite for a stable and sustainable development of animal husbandry. Surveillance, monitoring and quick response are the basic characteristics of a successful fight against infectious animal diseases. For this reason, the veterinary profession of each country represents the foundation for preservation of public health, animal health, and therefore the sustainable development of animal husbandry as one of the basic branches of agriculture. The outbreak of Lumpy Skin Disease (LSD) in Montenegro in 2016 is a very significant event, but also a warning about the importance of recognizing the dangers that can threaten the sustainability of this branch of agriculture. This situation showed the adequate reaction of the entire veterinary profession of Montenegro, which is not recognized enough, both from the aspect of its importance for public health, through animal health and welfare, and food safety, as well as its impact on the economy and market stability of the country.

Keywords:
Lumpy Skin Disease, LSD, Veterinary Epidemiology, Public Health, Sustainable Agriculture

1. Introduction

The agricultural sector plays an important role in the economy of Montenegro, with a significant share in the gross domestic product. Although agriculture, together with the tourism sector, is the development and economic priority of the national economy, based on official statistics (data from administrative sources), only 1.6% of the total number of employees in Montenegro is permanently employed in agriculture. However, according to the agricultural survey from 2010, out of a total of 620,029 inhabitants of Montenegro, 98,341 inhabitants are employed on family farms. When this figure is expressed in the

* Corresponding author: masha.jov@gmail.com

number of annual work units, 46,473 annual work units are obtained, whereby one annual unit of work is equivalent to one full-time employee. This means that almost 30% of the total number of employees in the country are actually engaged in agriculture.
As the agricultural sector is the most important sector for the development of rural areas of Montenegro, animal husbandry, given the configuration of the terrain, represents its most important branch. However, this sector is not sufficiently developed. The under-development of livestock farms, and thus of the dairy sector, is caused primarily by fragmented farms and the traditional, extensive method of production, as well as inefficient operations and insufficient utilization of available natural resources. Animal husbandry allows Montenegro to use less productive areas (pastures and meadows), which are dominant in the structure of the total agricultural area in Montenegro.
Comparing the total area under pastures and meadows with the total number of cattle and sheep/goats, we get only 0.23 of cattle/hectare and 0.46 of sheep/goats per hectare. If all types of ruminants were aggregated in conditional heads (one conditional head - CH is equivalent to one adult beef or 10 sheep or 10 goats) where horses are also included, the figure would be less than 0.53 CH/ha , which is a small load on those surfaces.
In order to use its full potential, the state of Montenegro needs to stimulate its habitants to invest their financial and all other capacities in animal husbandry, providing them safe environment, health and welfare protection measures for animals and securing minimal risk of infectious diseases, such as Lumpy skin disease.

2. Basic characteristics of Lumpy skin disease (Problem background)

Lumpy skin disease (LSD) or nodular dermatitis (lat. *Exanthema nodularis bovis*) is an infectious disease of cattle and buffalo caused by a virus from the family *Poxviridae*, subfamily *Chordopoxvirinae* and genus *Capripoxvirus*. In terms of its basic biological characteristics, the Lumpy skin disease virus does not differ from other viruses belonging to the *Poxviridae* family.
In most African countries, LSD is an enzootic disease, but since 2012 it has progressively spread towards the Middle East, southern Europe, the Russian Federation and the Caucasus region.
The source of infection in nature are infected animals. The virus is excreted from the infected organism with saliva, discharge from nose and eyes, sperm, and the highest concentration of the virus is found in the skin nodules and scabs of infected animals. The LSD virus is very resistant in the external environment. Although incomplete, the data on the basic epizootiological characteristics of LSD indicate a high potential for stationing and long-term maintenance of the causative agent in a certain area after the first appearance of the disease, which results in significant difficulties in controlling the spread and suppression of the disease. In the population of susceptible animals, hematophagous insects play the most important role in the transmission of infection. Transmission of the LSD virus takes place during the feeding of insects on the blood of infected or diseased animals, by mechanical means, but the role of certain types of hematophagous insects as biological vectors cannot be completely excluded. Depending on the geographical, climatic and ecological characteristics of the areas where LSD occurs, different types of hematophagous insects may play a role in the transmission of the virus. The seasonal activity of hematophagous insects also determines the seasonal occurrence of diseases in the months when they are most active. It is considered that the transmission of the virus through direct contact between animals does not play a significant role in the epizootiology of LSD, but there have been cases of cattle infection caused by ingestion of contaminated food and water, as well as through sexual contact (natural intercourse and artificial insemination).

The clinical picture of LSD in infected cattle can be manifested from a mild to a very severe form with a fatal outcome. Lumpy skin disease is clinically manifested by an increase in body temperature, a sudden drop in milk production, increased salivation, the appearance of discharge from the nose and eyes, swelling of the lymph nodes and the appearance of nodules on the skin and mucous membranes, which are a characteristic sign of the disease.
Measures to control and eradicate LSD are based on early detection of the disease, application of the stamping out method (euthanasia of sick animals), restriction or complete prohibition of animal movement, vector control and vaccination. Immunoprophylaxis is the most effective way to control LSD in countries where the disease occurs endemically.

3. Distribution of Lumpy skin disease

3.1 Lumpy skin disease in the world

Lumpy skin disease of cattle was first described in the territory of Zambia (formerly Northern Rhodesia) in 1929 and was for a long time limited to the southern part of Africa. During the 1940s, LSD spread to the central and northern parts and is currently present everywhere in Africa, including Madagascar. In East Africa, LSD appeared for the first time in Kenya in 1957, then in Sudan in 1970, and on the territory of West Africa the disease spread in 1974 when it was diagnosed in Nigeria. During 1977, LSD was also registered in Mauritania, Mali, Ghana and Liberia. In the 1980s, the first cases of the disease occurred in Tanzania, Somalia and Cameroon. Lumpy skin disease has long been limited to the area of Africa south of the Sahara, while today it is present throughout Africa, with the exception of Libya, Algeria, Morocco and Tunisia, which are still considered to be LSD-free countries. Despite the measures to control and eradicate the disease, in 1988 there was a large-scale epizootic in Egypt [1] from where the disease spread for the first time outside the borders of the African continent, only a year later, when it appeared in the territory of Israel [2]. In Saudi Arabia, LSD was confirmed in 1992 [3] , in Kuwait in 1991, Lebanon in 1993, Yemen in 1995, the United Arab Emirates in 2000, Bahrain in 2003, Israel in 2006- in 2007 and Oman in 2010 [4].

3.2 Lumpy skin disease in Europe and neighboring countries

Special attention was paid to monitoring the epizootic situation of LSD in the world in the period from 2015 to 2016, when the disease showed significant potential for spreading to the Balkan Peninsula and was a threat for further spreading to Europe. During 2013, LSD was registered in the southern part of Turkey [5], and in the following two years, the epidemic would cover the entire territory of Turkey. The emergence of LSD in Turkey increased the possibility of disease transmission to European Union (EU) member states [6].
In November 2014, the disease was confirmed on the island of Cyprus, and in June 2015, in the Turkish district of Edirne, 8 km from the border with Greece. In August 2015, the disease was registered for the first time on the territory of Greece, in the area of Evros at a distance of 15 km from the border with Turkey. It was the first appearance of LSD on the territory of the EU. In Greece, the disease was initially limited to the territory of the Evros area, and then, despite the implemented control measures, it continued to spread to the west of the country (Xanthi, Kavala, Limnos, Rhodope, Halkidiki and Thessaloniki) [7]. In July 2015, LSD was registered for the first time on the territory of the Russian Federation, in the border areas with Georgia and Azerbaijan, and then spread to the Caucasus region (Dagestan, Chechnya and North Ossetia) [8]. In April 2016, the LSD epizootic appeared in the northern regions of Greece, which was the beginning of the LSD epizootic in the area of

Southeast Europe. In less than 4 months, the disease was registered in Bulgaria, North Macedonia, Serbia, Albania and Montenegro [9]. The first appearance of LSD in Bulgaria was recorded in April 2016 in the province of Blagoevgrad, which borders North Macedonia and Greece. In North Macedonia, LSD appeared in April 2016 in the southeastern part of the country, and then spread to the territory of the entire country. In the Republic of Serbia, the first case of LSD was registered at the beginning of June 2016, in Albania at the end of June 2016, and in Montenegro in July 2016. During 2017, there were only sporadic cases of the disease in North Macedonia (4 hotspots) and Greece (2 hotspots), while there were no outbreaks of the disease in other countries of Southeast Europe (except Turkey and Albania). Albania was the only country in Southeast Europe where a large number of LSD outbreaks (379 outbreaks) were registered in 2017.

4. Epidemiological characteristics of Lumpy skin disease

The natural hosts for LSD virus are domestic cattle (*Bos indicus* and *Bos taurus*) and Asian water buffalo (*Bubalus bubalis*). It has been proven that European breeds of cattle (*Bos taurus*) are more receptive than Asian breeds (*Bos indicus*). Certain studies have shown that certain European breeds of cattle with thin skin, such as the Holstein-Friesian breed, fall ill with more severe clinical symptoms of the disease than indigenous African cattle breeds [10]. There is data that indicates that some species of wild ruminants can get infected by LSD, however, the susceptibility of wild ruminants and their role as potential reservoirs of the virus are not fully known.

The presence of LSD virus genome was determined in skin nodules of springbok antelope (*Antidorcas marsupialis*) [11]. In the Oryx antelope (*Oryx leucoryx*), the presence of Capripox virus particles in the skin nodules was determined by electron microscopy, but it was not proven whether it was the LSD or SPPV virus [12]. Antibodies to CaPV have been found in some wild ruminant species such as wildebeest (*Connochaetes taurinus*), white-tailed wildebeest (*Connochaetes gnu*), eland antelope (*Taurotragus oryx*), impala and African buffalo (*Syncerus caffer*) [13]. The presence of antibodies against CaPV in different animal species indicates their susceptibility to the virus, but does not confirm the role of wild animals as reservoirs and their role in virus transmission. However, monitoring the course of the infection, as well as determining the presence of the disease with the appearance of mild clinical symptoms in wild ruminants is problematic, so it remains disputed whether they become ill in nature.

It is thought that due to natural selection, wild ruminants are genetically more resistant to LSD virus infection. Lumpy skin disease is clinically manifested in all age categories and in both sexes of domestic cattle. According to some research, it was found that the disease manifests itself in a more severe form in dairy cows at the peak of lactation as well as in younger animals. Although some studies indicate a higher sensitivity of younger categories [14], in certain studies no difference in prevalence between different age categories was determined [15]. Examining the seroprevalence of LSD in cattle of different age categories proved that seropositivity was higher in adults compared to young cattle (up to 12 months of age), which is explained by the fact that calves are kept in closed areas [16]. In a mathematical modeling study evaluating different routes of transmission of LSD virus, trials showed that suckling calves were less likely to become ill because they were kept in closed areas away from infected animals and protected from insects [17].

According to current knowledge, LSD belongs to the group of vector-borne diseases, where climatic factors, i.e. temperature and rainfall affect the cycle of reproduction of insects in habitats, determining the seasonal occurrence of the disease. In addition to the activity of hematophagous insects, the seasonal occurrence of the disease is also influenced by the way the animals are kept, i.e. reared. In the Balkans, cattle are kept in the traditional way, i.e. in

a semi-open or open system, which allows contact with vectors, but also contact of animals from different herds. Thus, for example, in Greece, it was found that the morbidity of LSD was higher on farms where animals are kept on pasture, compared to farms where animals do not use pasture [18].

According to reports from countries where the disease has emerged in the last few years, it is observed that the speed of spread, the overall prevalence as well as the mortality and lethality rates depend on the geographical area, climatic conditions, the activity and abundance of vectors, the way of keeping livestock, the breed of cattle, the immune status of animals and the speed of implementation of disease control measures [19, 20, 21]. In countries where LSD is endemic, morbidity ranges from 5 to 45%, and mortality from 1 to 3%, exceptionally up to 10%. In areas where the disease appears for the first time, morbidity can reach 100%.

Although LSD is one of the most economically significant diseases of cattle, the dynamics of occurrence, as well as the ways of spreading, are not completely known. In enzootic areas, the disease has been found to occur periodically, with periods between epizootics of several years. It is not known whether there is a specific reservoir of the LSD virus, nor how and where the virus survives between epizootics, but it is known that the presence of non-immune populations, numerous hematophagous vectors and the movement of animals from infected regions are the main drivers of extensive LSD epizootics [22].

In an epidemiological study that covered the period from the appearance of LSD in Turkey (2015) to the appearance of the disease in the Balkan Peninsula (2016), the degree of spread of LSD in the Balkan Peninsula was shown. A seasonal spread of the disease has been recorded, with peaks during the summer periods and cessation of the disease during the winter, which is associated with the cessation of vector activity. Areas with a larger number of outbreaks (so-called hot spots) were observed, which are mainly localized in
border areas. The average rate of spread of LSD was 7.3 km/week (4.4 to 12.5 km/week), and the highest recorded rate of spread of LSD was 543.6 km/week. It was assessed that the low degree of spread of LSD in certain areas is associated with local transmission of the causative agent by vectors and possibly direct contact of infected and uninfected animals, while the high degree of spread of LSD is associated with the movement and trade of infected animals. Also, the possibility that the disease could have spread over a long distance by carrying the infected vector by wind is not excluded [23].

In general, no studies about vector species of LSDV have been performed in Europe and particularly in the LSD-affected countries, so specific evidence on vector competence is still missing. The probability of recurrence of LSD linked to the probability of wildlife being carriers of LSDV or the occurrence of a sylvatic cycle of the virus cannot be assessed because of lack of information. The probability of recurrence of LSD due to the role of subclinically infected animals is low, as the most likely source of virus transmission is linked to the high levels of virus present in skin lesions, so in animals with evident clinical symptoms that are usually removed from the population. The probability of recurrence of LSD linked to the virus remaining viable in the external environment (e.g. in shaded pens or beddings) is not known, as well as the probability of an animal acquiring the infection by contact with contaminated bedding is unknown, although it is known that transmission by direct contact or indirect contract with fomites is less effective than by vector transmission [24].

5. Outbreak of Lumpy skin disease in Montenegro (Methodology and results)

5.1. General notes

The first case of Lumpy skin disease in Montenegro appeared on July 21, 2016, in the territory of the municipality of Gusinje. After the outbreak in North Macedonia, the Administration for Food Safety, Veterinary and Phytosanitary Affairs concluded that there was a very high risk of disease outbreaks in Montenegro. All measures and recommendations by the World Organization for Animal Welfare (OIE) and the European Food Safety Agency (EFSA) were adopted, and intensive work was also done to ensure a sufficient amount of vaccines for disease prevention. At the same time, the Government appointed an expert team on Lumpy skin disease, which consisted of employees of the Administration for Food Safety, Veterinary and Phytosanitary Affairs, experts from Diagnostic Veterinary Laboratory, and veterinary inspectors. Despite the intention to start preventive vaccination in Montenegro, and to finalize arrangements by the established contacts with the relevant institutions of the European Union and OIE, this did not happen. Before the start of preventive vaccination, there was an outbreak of the disease.

Montenegro has an area of 13,812 km², of which 38% is agricultural land, of which 62% is pasture land (Figure 1). Agricultural farms in Montenegro are extremely fragmented, the average farm covers 4.6 ha, with an average of 3.3 breeding head of cattle. According to the data after the vaccination, the number of cattle in the territory of Montenegro was 93,350. As the relief of Montenegro is mostly mountainous, dominated by high mountain ridges with wide plains, agriculture is dominated by the traditional way of keeping cattle, which involves raising cattle to mountain pastures. - katuns from May to October. During the winter, the animals stay in lower areas and are kept indoors.

Figure 1: Map of Montenegro
(Source *https://commons.wikimedia.org/wiki/File:Montenegro_Map.png)*

5.2. First cases

The outbreak of Lumpy skin disease coincides with the period of grazing of animals on high pastures near the border with Albania. That part of the border between Albania and Montenegro is characterized by the mountain ranges of Prokletije with an altitude of over 2000 m. Since the illegal movement of animals between the two countries through that terrain is not possible, the occurrence of the first case in Montenegro can be linked to the movement of hematophagous insects. The expert team, in accordance with the situation and recommendations of OIE, decided to send all the animals on the infected farms to the slaughter, and the infected animals were stamped out in accordance with the legislation. This principle was applied to the first ten cases on ten different farms.

After that, the disease unexpectedly appeared in another part of the country in the municipalities that gravitate towards the mountain massif of Bjelasica. In this part the largest number of cases appeared during the entire duration of the epidemic. About 80% of the total registered cases of the disease occurred in the territory of four municipalities: Kolašin, Mojkovac, Berane and Bijelo Polje (Tables 1 and 2).

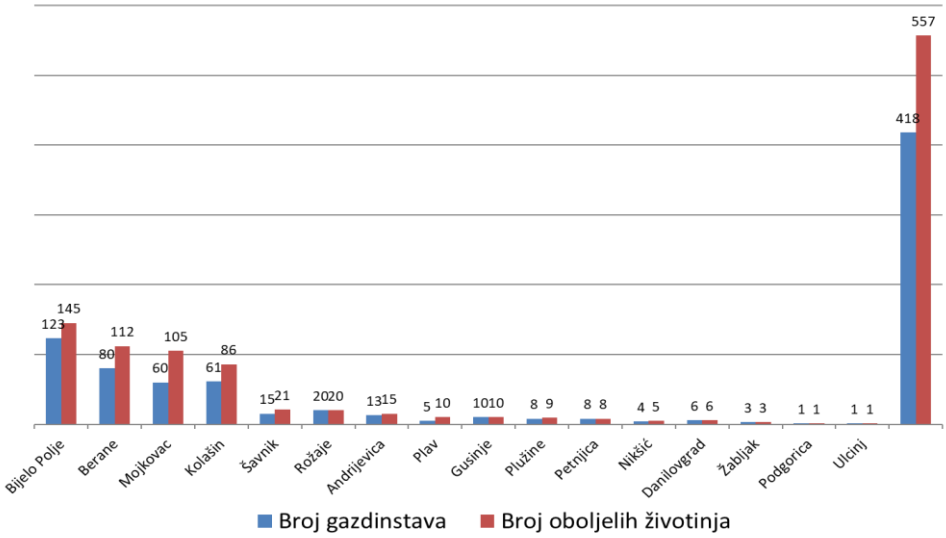
Table 1: Distribution of LSD outbreaks on Bjelasica mountain

Municipality	**Number of affected households**	**Number of infected/stamped out animals**
Bijelo Polje	123	145
Berane	80	112
Mojkovac	60	105
Kolašin	61	86
Total	**324**	**448**

Table 2: Distribution of registered cases in Montenegro

Municipality	Number of affected households	Number of infected/stamped out animals
Bijelo Polje	123	145
Berane	80	112
Mojkovac	60	105
Kolašin	61	86
Šavnik	15	21
Rožaje	20	20
Andrijevica	13	15
Plav	5	10
Gusinje	10	10
Plužine	8	9
Petnjica	8	8
Nikšić	4	5
Danilovgrad	6	6
Žabljak	3	3
Podgorica	1	1
Ulcinj	1	1
Total	**418**	**557**

Another unexpected outbreak occurred in the north-eastern part of Montenegro on the territory of the municipality of Žabljak in the remote village of Mala Crna Gora, at an altitude of 2000m, which caused additional concern. However, there were no other cases in that locality except for one locus where three animals became ill. In the first thirty days of the epidemic, 522 outbreaks were recorded (Figure 2). At the end of August, two hotspots appeared near the border with Republika Srpska (Bosnia and Herzegovina), which is why one could suspect that the disease already existed in that territory, however, there were no reported cases. The last case of the disease in Montenegro appeared on October 1 in an unvaccinated cow, so the total number of cases of Lumpy skin disease was 557 (Graph 1).



Graph 1: Number of infected households / animals in Montenegro

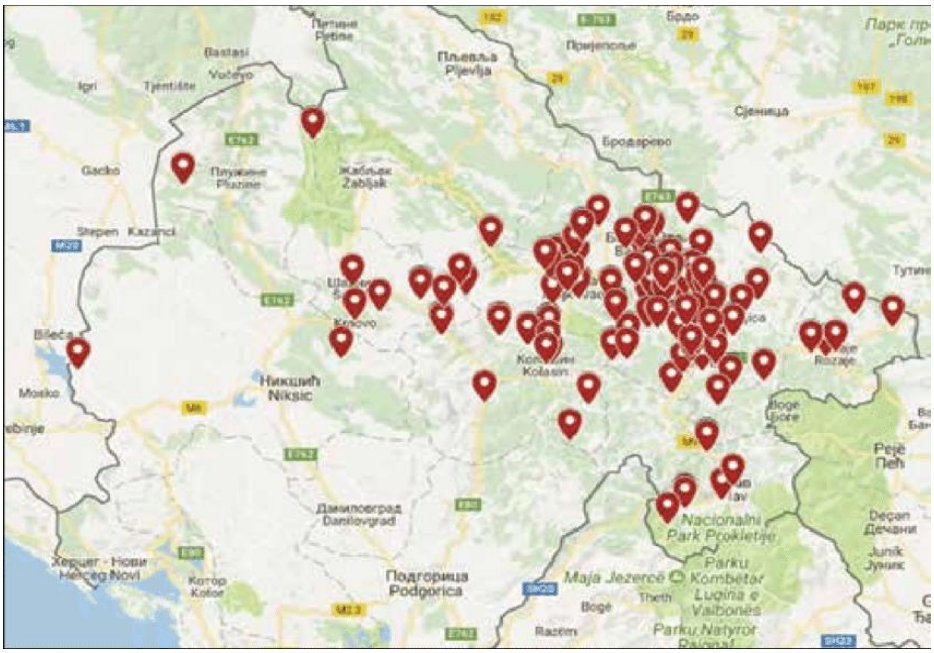


Figure 2: Distribution of the outbreaks 30 days post-epidemic

During the duration of the disease, all samples were tested in the Diagnostic Veterinary Laboratory in Podgorica. A total of 1402 samples was analyzed using PCR, half of which were sampled during the month of August. In the first 4 weeks after the disease outbreak, only the LSD Real-Time PCR protocol was used in laboratory diagnostics [25]. Three weeks after the start of mass vaccination (August 22), a new KV protocol was introduced (Veterinary Institute Kraljevo, Serbia) for the purpose of detecting the "wild strain" of the

virus. At the beginning of September, an additional Hoffmann protocol (FLI) was introduced to distinguish the "wild" strain of the virus from the vaccine strain. After August 26, 2016 (2-3 weeks after vaccination), only skin/bump samples were examined.
The vaccination campaign started on August 1, 2016 (10 days after the appearance of the first case of the disease). For the vaccine, a homologue of the live attenuated vaccine against the Lumpy skin disease virus (Neethling strain) "Lumpy Skin Disease Vaccine For Cattle" was used (Onderstepoort Biological Products, Republic of South Africa). Emergency vaccination was carried out in the infected and endangered area where the disease was registered in the period from July 21 to August 1, 2016. Continuation of mass vaccination on August 5 in the entire territory of Montenegro was undergone (Figure 3).

Figure 3: Vaccination campaign

All cattle (without visible clinical symptoms) were vaccinated, regardless of breed, age, pregnancy or production status, and in accordance with the instructions of the vaccine manufacturer. Vaccination on the entire territory of Montenegro was completed on August 15. 91,410 cattle on 21,367 farms were vaccinated. The total percentage of vaccinated cattle in relation to the total population was over 99%. After the end of the vaccination campaign, the veterinary service continued to vaccinate newborn calves, as well as those cattle imported from countries where vaccination against bovine Lumpy skin disease has not been carried out or is not carried out. Numerous cases of the disease were registered after the start of the vaccination campaign. These cases, to the greatest extent, can be related to:
– infection with the "wild strain" of the virus before the vaccination,
– infection with the "wild strain" of the virus before reaching full immunity after vaccination,
– infection with the vaccine strain of the virus originating from the vaccine.
Post-vaccination reactions were most common in infected areas. The greatest number of reactions appeared in the period 10-14 days after vaccination: swelling at the vaccination site, which persists for more than 15 days, large swellings on the lower side of the neck and abdomen, which persist up to 30 days after vaccination, reduction of milk production up to 30%, which lasts more than 2 weeks, the appearance of skin bumps, from a few to dozens, which disappear after more than 7 days, elevated temperature >39.5°C, lasting 2-3 days. Vaccination continued during 2017, a total of 78,451 cattle were vaccinated, and 69,261

during 2018. In accordance with the recommendations of the GFTADs meeting held in Ohrid on 18-19 October, vaccination continued in 2019, and the total number of vaccinated animals was 64,886.

5.3. Program for the surveillance of Lumpy skin disease in Montenegro

Since 2020, a program of passive and active monitoring of Lumpy skin disease has been implemented in Montenegro. The goals of this program are: proving the absence of the LSD virus from the territory of the country and confirming the country's LSD-free status, early detection of possible virus circulation. The program consists of passive and active surveillance of cattle in the territory of Montenegro. Passive surveillance includes the exclusion and reporting of suspicion to LSD, which is mandatory in the entire territory of Montenegro. Active surveillance of LSD is carried out by sampling and clinical examination of cattle in areas of Montenegro that are considered endangered and less endangered for the occurrence of LSD with the aim of early detection of the LSD virus.

5.3.1 Passive monitoring

It is mandatory to rule out any suspicion due to the determination of clinical signs that may point to LSD: changes on the skin - nodules and/or ulcerative changes on the skin and mucous membranes, general infectious syndrome, drooling, increased discharge from the nose and eyes, swelling, enlarged lymph nodes, decrease in milk yield, lameness, etc.. In case of suspicion, i.e. observed signs of disease, the authorized veterinarian must perform a clinical examination and sampling of material from the suspected animal. Suspicion of LSD is reported in accordance with the Rulebook on the Classification of Animal Diseases, the Method of Reporting and Notification of Infectious Animal Diseases. Passive surveillance is carried out in the entire territory of Montenegro.

5.3.2 Active monitoring

Endangered areas are defined based on the epidemiological situation, size of the area, geographical location, number of cattle population, method of spread and number of positive cases of the disease in 2016. In accordance with the estimated risk, the number of samples that need to be tested for LSD is determined.
Supervision of LSD in the risk area - the border zone with Albania and Kosovo* - municipalities Ulcinj, Tuzi, Podgorica, Andrijevica, Gusinje, Plav, Berane, Rožaje, as well as the municipalities of Bijelo Polje, Petnjica, Mojkovac, Šavnik, Kolašin and Bar- blood is sampled from a total of 150 animals (95% probability of detecting the LSD virus if it is present in 2% of the cattle population). The cattle were selected in such a way that they were not vaccinated against LSD, were older than 6 months and were born after August 1, 2019.
LSD surveillance in a less threatened area - the areas of the municipalities of Plužine, Nikšić, Danilovgrad, Cetinje, Budva, Tivat, Kotor and Herceg Novi- blood is sampled from a total of 60 animals (95% probability of detecting the LSD virus if it is present in 5% of the cattle population). Cattle were selected in such a way that they are unvaccinated, older than 6 months and born after August 1, 2019.
A total of 210 cattle were included in the risk-based surveillance of LSD for the purpose of early detection of the LSD virus. Cattle sampling is repeated in such a way that samples are taken from the same cattle, during the vector season, from April to October. During each

sampling, two tubes of blood are taken, one for serological and the other for virological examination.

5.3.3 Clinical examination of cattle

In all farms where cattle blood sampling is carried out according to the previously mentioned protocol, a clinical examination must also be carried out on the certain number of animals in the herd, which enables the detection of clinical signs if LSD is present in 10% of the population with a 95% probability. A clinical examination of animals is carried out in all cases of suspected LSD. In case of suspicion of LSD, a clinical examination is also performed in all animals in the herd of origin of the suspected cattle. Clinical examination of healthy cattle includes:
– examination of the visible mucous membranes of the eyes and nose,
– inspection of the udder, perineum and genitals,
– skin palpation.

If clinical signs are detected, in addition to the above, a complete clinical examination is carried out, which includes:
– examination of the visible mucous membranes of the eyes and nose,
– inspection of the udder, perineum and genitals,
– skin palpation,
– palpation of lymph nodes,
– triassic values in ruminants.

5.3.4 Results of the program for the year 2020 and 2021

Through passive surveillance, during 2020, at the request of the field service, the Diagnostic Veterinary Laboratory examined only one suspicious case (two samples), the nasal swab of a cow and full blood of a cow from the area of the municipality of Cetinje, while in 2021, 5 samples were processed. The Real-Time PCR method did not detect the DNA sequence of the Lumpy skin disease virus in these samples.
Through active monitoring in 2020, out of a total of 330 submitted and tested bovine blood samples (208 from the endangered area and 122 from the less endangered area), the presence of specific antibodies against the *Capripox* virus was determined in a total of 27 samples (25 from the endangered area and 2 from the less endangered area). In 2021, a total of 404 submitted and tested cattle blood samples (277 from the endangered area and 127 from the less endangered area), the presence of specific antibodies against the *Capripox* virus was determined in a total of 6 samples (3 from the endangered area and 3 from the less endangered area) (Table 3).
All cattle in which the presence of specific antibodies to the *Capripox* virus was determined by serological tests were additionally examined by molecular diagnostic techniques for the presence of DNA of the field strain of the virus that causes Lumpy skin disease from EDTA blood samples of the same cattle that were delivered to the Laboratory at the same time as the blood samples for serological tests. A total of 33 seropositive animals were examined. Tests were performed using the Real-time PCR method. DNA extraction was performed using the High Pure Viral Nucleid Acid Kit, and amplification using the Qiagen kit and primers and probes from Operon.

Table 3: Results of the program for 2020. and 2021.

Surveillance of the LSD		2020.	2021.
Active surveillance	Serological	330	404
	Molecular	27	6
Passive surveillance	Serological	-	-
	Molecular	2	5

6. Further work

If a country identifies an outbreak of LSD, prompt reaction in setting measures is of crucial importance. Availability of vaccines and a vaccination plan for quick implementation should be in place (unlike what happened in Montenegro in 2016), since vaccination is by far the most effective measure. Because of these reasons, to properly choose the best option for surveillance, it would be important to estimate the expected delay of disease recognition and notification under different introduction and surveillance scenarios, given the direct relationship between such delay and the extent of population to be submitted to emergency vaccination for limiting the spread of the infection.

The veterinary services should implement programs in order to raise awareness among farmers and stakeholders who have everyday contact with livestock, as well as veterinary technicians, veterinarians and diagnosticians, who should report promptly any suspicion of LSD.

Knowledge gaps recognized by now include within-herd transmission parameters, duration of protective immunity from vaccination and natural infection, duration of passive immunity in calves, role of vectors, diagnostic test performance under field conditions, exact farm location and farm type in all affected and at-risk countries and the epidemiological status of neighboring countries. Further research studies and data collection should be encouraged on these aspects.

7. Conclusion

The outbreak of Lumpy skin disease reduced the cattle stock by 0.5% of the total population. The costs of acquiring vaccines, carrying out vaccination, compensation for killed animals, it all amounted to 1.5 million euros, additionally through monitoring programs more than 0.5 million euros were spent, which in total exceeds the figure of 2 million euros, which is the total annual budget of the veterinary service of Montenegro, determined by the state through the Mandatory Animal Health Protection Program. In addition to the economic one, it is important as well to point out the very large role of the veterinary profession in public health and environment protection. The entire work on LSD epizooty and measures of control and surveillance afterwards were carried out by about 100 people engaged in veterinary field in Montenegro. Veterinary professionals are a very important link in the chain of public health, because of their role in preserving the safety of food of animal origin, but also the health and welfare of animals in general, as well as nature preservation and environment protection in indirect way. The One Health principle has been ongoing in the last decade in developed countries with a vision of progress, and Montenegro should not lag behind either. The poor condition of veterinary field and lack of credits for veterinary professionals' role in society have led to deficiency in personnel and human resources for this branch of medicine. This is also linked to a fact that there is no academic institution providing knowledge and training for future veterinarians in

Montenegro, so the interest and enthusiasm that one may possess for the progress in this direction fades even more in this path full of obstacles. All of this is reflected as well in general progress of the country, whose EU integrations' process is stagnating due to inability to fully cover and upgrade this field, among other. The main aspects to be worked on are certainly the improvement of existing and the development of new animal disease surveillance programs, especially in the part of passive disease surveillance, resolving the ever-going issue of inadequate disposal of by-products of animal origin, including animal carcasses, all in the interest of preserving and protecting the environment, dealing with animal welfare and stray dogs and cats population. Also, long-term goals should include human resources and professional strengthening of administrative and laboratory capacities, life-long learning for all involved, upgrading inspection supervision, establishment of auditory body, expansion of veterinary field service, formation of scientific institutions, raising public awareness about veterinary profession. The main goal from the aspect of sustainable development of public health would be strengthening the link between human and veterinary medicine by including the One Health principle in legislation and implementing it through institutional cooperation and activities.

References

[1] Ali AA, Esmat M, Attia H, Selim A, Abdel-Hamid YM. Clinical and pathological studies on lumpy skin disease in Egypt. Vet Rec. 1990 Dec 1;127(22):549-50. PMID: 2281596.

[2] Yeruham, I & Nir, O & Braverman, Yehuda & Davidson, M & Grinstein, H & Haymovitch, M & Zamir, O. (1995). Spread of lumpy skin disease in Israel dairy herds. The Veterinary record. 137. 91-3. 10.1136/vr.137.4.91. W. Zeng, H. Yu, C. Lin. (2013, Dec 19). Multimedia Security Technologies for Digital Rights Management [Online]. Available: http://goo.gl/xQ6doi

[3] Greth A, Calvez D, Vassart M, Lefèvre PC. Serological survey for bovine bacterial and viral pathogens in captive Arabian oryx (Oryx leucoryx Pallas, 1776). Revue Scientifique et Technique (International Office of Epizootics). 1992 Dec;11(4):1163-1168. DOI: 10.20506/rst.11.4.652. PMID: 1305861.

[4] Tuppurainen ES, Oura CA. Review: lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. Transbound Emerg Dis. 2012 Feb;59(1):40-8. doi: 10.1111/j.1865-1682.2011.01242.x. Epub 2011 Jul 12. PMID: 21749675.

[5] Wainwright, Sherrylin & El-Idrissi, Ahmed & Mattioli, Raffaele & Tibbo, Markos & Njeumi, Felix & Raizman, Eran. (2013). Emergence of lumpy skin disease in the Eastern Mediterranean Basin countries. FAO EMPRES Watch. 29.

[6] Tuppurainen ES, Oura CA. Review: lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. Transbound Emerg Dis. 2012 Feb;59(1):40-8. doi: 10.1111/j.1865-1682.2011.01242.x. Epub 2011 Jul 12. PMID: 21749675.

[7] Tasioudi, K.E., Antoniou, S.E., Iliadou, P., Sachpatzidis, A., Plevraki, E., Agianniotaki, E.I., Fouki, C., Mangana-Vougiouka, O., Chondrokouki, E. and Dile, C. (2016), Emergence of Lumpy Skin Disease in Greece, 2015. Transbound Emerg Dis, 63: 260-265. https://doi.org/10.1111/tbed.12497

[8] Sprygin, A, Pestova, Y, Prutnikov, P, Kononov, A. Detection of vaccine-like lumpy skin disease virus in cattle and *Musca domestica* L. flies in an outbreak of

lumpy skin disease in Russia in 2017. *Transbound Emerg Dis*. 2018; 65: 1137–1144. https://doi.org/10.1111/tbed.12897

[9] EFSA 2016a, EFSA 2016b

[10] Coetzer JAW Tustin RC. *Infectious Diseases of Livestock*. 2nd ed. Cape Town: Oxford University Press; 2004.

[11] Sameea Yousefi, P., Mardani, K., Dalir-Naghadeh, B. and Jalilzadeh-Amin, G. (2017), Epidemiological Study of Lumpy Skin Disease Outbreaks in North-western Iran. Transbound Emerg Dis, 64: 1782-1789. https://doi.org/10.1111/tbed.12565

[12] Greth A, Calvez D, Vassart M, Lefèvre PC. Serological survey for bovine bacterial and viral pathogens in captive Arabian oryx (Oryx leucoryx Pallas, 1776). Revue Scientifique et Technique (International Office of Epizootics). 1992 Dec;11(4):1163-1168. DOI: 10.20506/rst.11.4.652. PMID: 1305861.

[13] F.G. Davies, Lumpy skin disease, an African capripox virus disease of cattle, British Veterinary Journal, Volume 147, Issue 6, 1991.

[14] Gari, G. et al. "Epidemiological Aspects and Financial Impact of Lumpy Skin Disease in Ethiopia." Preventive Veterinary Medicine 102.4 (2011): 274–283. Web.

[15] Elhaig, Mahmoud & Selim, Abdelfattah & Mahmoud, Mohamed. (2017). Lumpy skin disease in cattle: Frequency of occurrence in a dairy farm and a preliminary assessment of its possible impact on Egyptian buffaloes. The Onderstepoort journal of veterinary research. 84. a1393.

[16] Ochwo S, VanderWaal K, Munsey A, Nkamwesiga J, Ndekezi C, Auma E, Mwiine FN. Seroprevalence and risk factors for lumpy skin disease virus seropositivity in cattle in Uganda. BMC Vet Res. 2019 Jul 8;15(1):236. doi: 10.1186/s12917-019-1983-9. PMID: 31286926; PMCID: PMC6615106.

[17] Magori-Cohen, Reuma & Louzoun, Yoram & Herziger, Yael & Oron, Eldad & Arazi, Alon & Tuppurainen, Eeva & Shpigel, Nahum & Klement, Eyal. (2012). Mathematical modelling and evaluation of the different routes of transmission of lumpy skin disease virus. Veterinary research. 43. 1. 10.1186/1297-9716-43-1.

[18] EFSA (European Food Safety Authority), Calistri, P, DeClercq, K, Gubbins, S, Klement, E, Stegeman, A, Cortiñas Abrahantes, J, Antoniou, S-E, Broglia, A and Gogin, A, 2019. Scientific report on lumpy skin disease: III. Data collection and analysis. *EFSA Journal* 2019;17(3):5638, 26 pp. https://doi.org/10.2903/j.efsa.2019.5638

[19] Sameea Yousefi, P., Mardani, K., Dalir-Naghadeh, B. and Jalilzadeh-Amin, G. (2017), Epidemiological Study of Lumpy Skin Disease Outbreaks in North-western Iran. Transbound Emerg Dis, 64: 1782-1789. https://doi.org/10.1111/tbed.12565

[20] 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. Outbreak investigation and molecular diagnosis of Lumpy skin disease among livestock in Saudi Arabia 2016. Transbound Emerg Dis. 2018 Apr;65(2):e494-e500. doi: 10.1111/tbed.12769. Epub 2017 Nov 17. PMID: 29150916.

[21] Sprygin, A, Pestova, Y, Prutnikov, P, Kononov, A. Detection of vaccine-like lumpy skin disease virus in cattle and *Musca domestica* L. flies in an outbreak of lumpy skin disease in Russia in 2017. *Transbound Emerg Dis*. 2018; 65: 1137–1144. https://doi.org/10.1111/tbed.12897

[22] Tuppurainen, E.S.M., Venter, E.H., Shisler, J.L., Gari, G., Mekonnen, G.A., Juleff, N., Lyons, N.A., De Clercq, K., Upton, C., Bowden, T.R., Babiuk, S. and Babiuk, L.A. (2017), Review: Capripoxvirus Diseases: Current Status and Opportunities for Control. Transbound Emerg Dis, 64: 729-745. https://doi.org/10.1111/tbed.12444

[23] Mercier, A, Arsevska, E, Bournez, L, et al. Spread rate of lumpy skin disease in the Balkans, 2015–2016. *Transbound Emerg Dis*. 2018; 65: 240–243. https://doi.org/10.1111/tbed.12624

[24] EFSA (European Food Safety Authority), Calistri, P, DeClercq, K, De Vleeschauwer, A, Gubbins, S, Klement, E, Stegeman, A, Cortiñas Abrahantes, J, Antoniou, S-E, Broglia, A and Gogin, A, 2018. Scientific report on lumpy skin disease: scientific and technical assistance on control and surveillance activities. *EFSA Journal* 2018;16(10):5452, 46 pp. https://doi.org/10.2903/j.efsa.2018.5452

[25] Bowden TR, Babiuk SL, Parkyn GR, Copps JS, Boyle DB. Capripoxvirus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats. Virology. 2008 Feb 20;371(2):380-93. doi: 10.1016/j.virol.2007.10.002. Epub 2007 Nov 7. PMID: 17988703.

Authors:

Maša Jovanović, DVM earned the title of doctor of veterinary medicine at the Faculty of Veterinary Medicine, University of Belgrade on September 27, 2019 (average grade 9.23). She obtained the title of Dr vet. med. spec. in November 2020. Obtained the title of Specialist in internal medicine for dogs and cats in October 2021. Awarded with Erasmus+ scholarships for exchange program studies in Bologna, Italy and Madrid, Spain. Fluent in English, Italian and Spanish, and modest knowledge of Portuguese. Since January 2020, has been attending the "New Acropolis" international School of philosophy. Internship done in Administration for Food Safety, Veterinary and Phitosanitary Affairs. Full-time employee as a practitioner at the veterinary clinic 'RoyalVet' in Podgorica at the moment. Enrolled into multidisciplinary international PhD program-Sustainable Development at the University of Montenegro as a part of the first generation of students.

Marko Nikolić, DVM currently employed as Advisor for Microbiology at the Department for Microbiology and Parasitology in Diagnostic Veterinary Laboratory (since 2019). PhD candidate at the Faculty of Veterinary Medicine, University of Belgrade. Previously engaged as a trainee in Administration for Food Safety, Veterinary and Phytosanitary Affairs, in the Department for Animal Health and Welfare Protection (2017-2019). Member of Montenegrin Microbiology Society and Veterinary Chamber of Montenegro.