Bolest kvrgave kože u Europi – stanje i izazovi
Lumpy Skin Disease in Europe – present situation and future challenges

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Experiences of diagnosing and control of lumpy skin disease in Serbia

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Lumpy skin disease (LSD) is a viral disease of cattle caused by capripoxvirus, transmitted mainly by mechanical vectors, and characterised by severe losses, especially in naive animals. LSD is characterised by fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, oedema of the skin, and sometimes death. The disease mainly spread to infection-free areas by transport of infected animals and by vectors. LSD is endemic in many African and Asian countries, and Middle East including Turkey, but it is exotic in Europe. In just one year period, starting from August 2015, the disease was spread from Turkey to the territory of 6 countries on Balkan Peninsula (Greece, Bulgaria, FYR Macedonia, Serbia including Kosovo, Montenegro and Albania).

After the appearance of the LSD in Bulgaria and FYR Macedonia, based on the risk of LSD introduction in Serbia, the Veterinary Directorate increased the active surveillance of the health status of cattle in the territory of southern Serbia. After the first LSD clinical suspicion on 4th of June in settlement Ljiljance, municipality Bujanovac, the first case was laboratory confirmed in VSI Kraljevo on 7th of June 2016. The first decision on designation of the infected zone of Municipality of Bujanovac, and Pčinjski District as the surveillance zone was done on 8th of June.

Based on the Rules of procedure in crisis situations, the hierarchical system within the decision-making process, and implementation of measures of prevention, early detection, monitoring and eradication of disease was established. The national crisis centre, located in the Veterinary Directorate, the regional crisis centre in Veterinary Specialized Institute “Nis”, and the local crisis centres in the five Districts of south part of Serbia, were established before first onset of disease in Serbia on 1st of June 2016. In addition, immediately after the first clinical suspicion on LSD, on 7th of June, 14
the expert's team was formed at the national level from the veterinary epidemiologists, laboratory diagnosticians and members of Veterinary Directorate. The expert's team, as well as the national and regional crisis centres, were in permanent session, starting with the first case of the outbreak of the disease, whether through conventional daily meetings, either on line through the web-based communication system, through which were all team members become familiar with up to date information at all times, and where the communication of expert opinions and documentation were performed in real time each day during 24 hours. Later on, as the disease spread, the new regional and local crisis centres were established. Due to the fast spread of disease, influence on the whole society and necessity of inclusion of more human but especially legal power and resources, as part of the Government of the Republic of Serbia the Operational Headquarters for coordinating the implementation of measures to prevent, control and to eradicate LSD was established. The Operational Headquarters meets on weekly basis, and was responsible for coordination of purchase of vaccine and coordination of activities of veterinary service with activities of local communities, police and army that were also, through this Headquarters, included in control of disease.

At the beginning of the outbreak, only the early detection and stamping out method was used for control of the disease. In that very early phase of disease control, non-vaccination policy was used. Due to the long incubation period of the infection and transmission of the virus by vectors, even the fast laboratory diagnostic and stamping out procedure for the whole herds with detected infected animal, in a time frame of only 48 to 72 hours, was on place during that period, the restrictive measures for animal movement were not enough to stop the virus transmission. After the first 7 days of LSD outbreak, when new cases were rapidly arisen all over the infected district, and due to the experiences of LSD control in Greece and Bulgaria, the expert group was decided to include the vaccination strategy to control LSD. The Governmental Operational Headquarters accepted the decision of the expert group and OBP LSD vaccine was introduced in the country. The whole country were divided in three parts: part A (two first infected districts planned to be vaccinated with 50,000 doses received from EU for emergent vaccination), part B (endangered districts that are near Bulgarian border and around Kosovo, and up to approximately 100 - 200 km from infected districts, as well as biggest cattle farms all over the country - planned to be vaccinated with 400,000 vaccine doses purchased by Veterinary Directorate until the end of June 2016), and part C (regions around Belgrade, parts of central and north-eastern part of Serbia, and
northern part of Serbia - planned to be vaccinated with additional 600,000 vaccine doses purchased by Veterinary Directorate, during July and August 2016).

The measures for control of disease included: stamping out procedure of all cattle in herd with confirmed case of LSD, regardless of category, age and health status of animals; destruction of animals products; safe disposal of carcasses, by-products and waste; disinfection and disinfestation; control of vectors; animal movement restriction and intensive active surveillance in infected and protection zone. Since the decision was made for vaccination of cattle in the entire Republic of Serbia with vaccine based on Neethling strain, measures to combat the disease were redefined. In the case of the occurrence of the disease in unvaccinated herd, the euthanasia and safe removal of all cattle in herd was performed, regardless of category, age and health status of animals. After conducting vaccination, in case if the clinical manifestation and laboratory confirmation of the disease arose in the previously vaccinated herd, and if more than 28 days passed after the vaccination, only the clinically sick animals were euthanized and safely disposed (partial euthanasia). The rest of the animals in such a herd was under the active supervision of the veterinary service.

After the occurrence of LSD in Serbia, three veterinary institutes, VSI Kraljevo in Kraljevo, NIVS in Belgrade and NIV-NS in Novi Sad were designated for the laboratory diagnosis of LSD. Protocol of sampling and samples transportation, prepared by the expert group was issued in the first week after the disease occurrence. The laboratory diagnosis of LSD cases was based on virus detection in biopsy samples of skin nodules and EDTA blood samples (obligatory samples) and in some cases nasal swabs (additional samples). For laboratory detection of LSDV, the real-time PCR methodology (protocol of Bowden et al., 2008), as well as conventional PCR methodology (protocol of Ireland end Binepal, 1998), were introduced in the labs shortly before the first onset of disease. Both of these methods are capripox virus specific, but since Serbia is free of sheep and goat pox, there was no need to include capripox virus differential methods. Mostly, the mentioned real-time PCR methodology, as highly sensitive, fast and robust method was used for detection of all LSD cases. Due to the possibility of mild or systemic post-vaccination reactions in vaccinated animals, after the implementation of the vaccination of cattle against LSD, application of new laboratory diagnostic procedures was necessary for differentiation of field from vaccine (Neetling) virus strain. For that purpose, the nested PCR protocol with RFLP analysis (described by Menasherow et al., 2014) was introduced in labs. Since the nested PCR / RFLP protocol is time consuming and prone to cross contamination, the
real-time PCR DIVA protocol to distinguish field for vaccine LSD strain is developed and introduced in the labs as in house method.

In total, LSD outbreak in Serbia in 2016 resulted with 221 outbreaks with 257 cases, 699 destroyed and safely disposed animals, and 1,080,398 vaccinated animals starting from first occurrence of disease on 4th of June until 23rd of September 2016 (111 days). During LSD outbreak, the whole veterinary service showed that is highly competent and capable to deal with animal disease emergent situations and to successfully control the disease.

**Keywords:** LSD outbreak, diagnostic, control measures, Serbia

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