HRVATSKA AKADEMIJA ZNANOSTI I UMJETNOSTI
CROATIAN ACADEMY OF SCIENCES AND ARTS

RAZRED ZA MEDICINSKE ZNANOSTI
DEPARTMENT OF MEDICAL SCIENCES

ODBOR ZA ANIMALNU I KOMPARATIVNU PATOLOGIJU
COMMITTEE OF ANIMAL AND COMPARATIVE PATHOLOGY

Bolest kvrgave kože u Europi – stanje i izazovi

Lumpy Skin Disease in Europe – present situation and future challenges

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Josip Madić, Chair of the Organizing Committee

Predsjedaju / Moderators: Teodor Wikerhauser i Josip Madić

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Emergentne zarazne bolesti – izazovi sadašnjosti i budućnosti

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Emergentne zarazne bolesti su one koje se pojavljuju po prvi put, odnosno pojavljuju u novom domaćinu, značajno šire na nova područja ili čija se incidencija naglo povećava. Posljednjih desetljeća emergentne zarazne bolesti su u značajnom porastu, a čak 75% njih su zoonoze. Uz značajan porast zoonoza jasno se uočava i kontinuirani trend porasta emergentnih bolesti koje se prenose vektorima. Taj jasno izražen trend zasigurno je uvjetovan promjenama u okolišu koje su posljedica prvenstveno ljudske djelatnosti. Kao čimbenike emergencije treba istaknuti socio-demografske promjene i njihov utjecaj na okoliš, gospodarske aktivnosti kojima se mijenjaju ekosustavi i promjene klimatskih prilika odraz kojih je globalno zatopljenje s nesagledivim posljedicama. Uz navedeno, značajan doprinos pojavi emergentnih bolesti daje i globalizacija prometa ljudi, životinja i proizvoda te razvoj tehnologije i industrije bez potpune projekcije utjecaja na okoliš. Nadalje, neprimjerenom uporabom ljekovitih pripravaka, prvenstveno antibiotika, doprinosi se poticanju izmjena mikroorganizama i nastanku rezistentnih vrsta. Uz navedeno, sve izraženije razlike u kvaliteti sustava javnog zdravstva, ovisno o gospodarskim mogućnostima pojedinih država, ili njegovo potpuno urušavanje u primjerice područjima oružanih sukoba zasigurno pogoduju pojavi i širenju emergentnih zaraznih bolesti. Spomenuti trendovi su sastavni dio razvoja moderne civilizacije što iziskuje obvezu uspostave sustava kontrole emergentnih zaraznih bolesti čije pojavljivanje nas neminovno očekuje i u budućnosti.

Sve te objektivne okolnosti, kao i kompleksnost pojave i širenja emergentnih zaraznih bolesti, jasno su prepoznate i od svjetskih krovnih organizacija za zaštitu zdravlja ljudi i životinja te se multidisciplinarnost nametnula kao jedini moguć odgovor na nove izazove. Intenzivna i bliska multidisciplinarna suradnja rezultirala je uvođenjem pristupa „Jednog zdravlja" koji podrazumijeva suradnju stručnjaka iz različitih područja na lokalnoj, nacionalnoj i globalnoj razini sa zajedničkim ciljem postizanja najboljih rezultata u očuvanju zdravlja ljudi i životinja te očuvanju okoliša.

Za razliku od zaraznih bolesti koje se dulje vrijeme pojavljuju na određenim područjima, značajke emergentnih zaraznih bolesti, poput primjerice potpuno novih uzročnika ili pojave u novim epizootiološkim/epidemiološkim uvjetima, zahtjeva brzu i usklađenu reakciju kako bi nadzor i suzbijanje uopće bili mogući.

Opći trend pojave i širenja emergentnih zaraznih bolesti nije zaobišao ni Republiku Hrvatsku i njeno okruženje. Samo u posljednja dva desetljeća, na području RH zabilježena je pojava bolesti plavog jezika, autohtoni slučajevi Dengue, širenje bolesti Zapadnog Nila te dokaz Usutu virusne infekcije konja i oboljenja ljudi. Samo tijekom 2016. godine zabilježeni su i prvi importirani slučajevi oboljenja ljudi uzrokovani Chikungunya virusom i Zika virusom. Stoga, pojava i širenje bolesti kvrgave kože u državama jugoistočne Europe 2016. godine ne predstavlja izdvojeni slučaj nego je samo nastavak svjetskog trenda širenja emergentnih zaraznih bolesti koji je očit i u našoj zemlji. Štoviše, svim navedenim bolestima zajednički su prvi opisi bolesti i uzročnika u Africi, prijenos artropodima i virusna etiologija te doista možemo govoriti o pravilnosti u njihovom pojavljivanju.

Stečena iskustva tijekom pojave i širenja navedenih bolesti, a posebice iskustva uspješnog zaustavljanja širenja bolesti kvrgave kože 2016. godine, moraju biti model brze i usmjerene reakcije stručnjaka na svim razinama s obvezom brze prilagodbe novonastalim okolnostima, kako bi u budućnosti spremno dočekali pojavu neke sljedeće emergentne zarazne bolesti i bili u mogućnosti očuvati zdravlje ljudi, životinja i okoliša te izbjeći nesagledive gospodarske posljedice.

Ključne riječi: emergentne zarazne bolesti, čimbenici emergencije, mjere nadzora i suzbijanja, pristup jedno zdravlje

Emerging infectious diseases – present and future challenges

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Emerging infectious diseases are an infectious diseases that have newly appeared in a population or in a new host or that have been known for some time but are rapidly increasing in geographic range or incidence. Over the last few decades appearance of the numerous emerging infectious diseases is reported and more than 75% of these are zoonoses with majority of recent emerging infectious diseases being vector-borne. In the most cases specific factors precipitating disease emergence could be related to human activities. For instance, influence of socio-demographic and economic development factors have detrimental influence on environment and resulting global warming with tremendous consequences. Furthermore, globalisation on transport levels, with the increasing movement of people, animals and goods and development

of technology and industry definitely contribute to emergence of infectious diseases. The widespread use of drugs especially antibiotics results in microorganism adaptation and emergence of the resistant pathogens. Differences in public health systems due to economic development between countries and breakdown of public health measures due to war or civil conflict are also important emergence factors. Majority of above listed factors in infectious diseases emergence are unavoidable as the consequence of humankind development and introduction of control system are prerequisite for the future.

All before mentioned facts and complexity of emergence process are recognised between international organisations for human and animal health protection. The multidisciplinary approach is now considered as only possible response to the new challenge. Close interdisciplinary collaboration resulted with "One health" approach, which considers the cooperation of different specialists on the local, national and global level with the aim to protect human, animal health and their environment.

In contrast to well-known infectious diseases the nature of emergent infectious diseases, new infectious agents or new epidemiological/epizootiological circumstances, warrant fast and synchronized reaction to be able to control and confine spreading.

Global trend in emergence and spreading of emergent infectious diseases has been seen in Croatia and surrounding countries as well. Over last two decades blue tongue disease, autochthonous Dengue, West Nile and Usutu virus infections in horses and humans are recorded. Recently in 2016 imported cases of Chikungunya and Zika virus human infections have been confirmed for the first time. Occurrence and spreading of lumpy skin disease in Southeast European countries in 2016 is just another case confirming global trend of infectious diseases emergence also present in Croatia. Arthropod borne nature, viral etiology and African origin for all previously mentioned diseases can't be guarded as coincidence.

Knowledge gained during outbreaks of previously mentioned emergent infectious diseases especially during last emergence of lumpy skin disease in 2016 should be used as a model for fast and focused experts action at all levels. Fast adjustment of mode of action to new circumstances is needed to be able to protect human, animal and environmental health and to avoid huge economic losses in future infectious disease emergence cases.

Keywords: emerging infectious diseases, emergence factors, control measures, one health approach

Vektori emergentnih zaraznih bolesti životinja i njihova rasprostranjenost u Hrvatskoj

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Bolesti prenosive vektorima privlače sve veću pozornost u posljednjih nekoliko godina zbog globalnih promjena koje neposredno utječu na ekologiju uzročnika i njihovo širenje. Klimatske promjene pospješuju širenje vektora u nova područja, a sama globalizacija omogućava brže prenošenje vektora i životinjskih rezervoara. Razvojem molekularnih metoda te njihova redovita primjena u istraživanjima i redovnoj dijagnostici značajno je doprinijela otkrivanju novih uzročnika i/ili novih izolata što je potaknulo detaljnija istraživanja vektorskog potencijala ponajprije komaraca, krpelja, komarčića (*Culicoides*) ali i ostalih člankonožaca. Procjenjuje se da gotovo 39 000 različitih vrsta artropoda može parazitirati na ljudima te domaćim i divljim životinjama što jasno ukazuje na njihov globalni značaj. Republiku Hrvatsku odlikuju velike razlike u reljefu, tlu i klimi pojedinih regija čemu su se prilagodile i brojne vrste člankonožaca. Poznato je da su u Europi najzačajniji vektori krpelji, uspješni vektori uzročnika bolesti u ljudi i životinja. Danas je poznato 907 vrsta s različitim vektorskim potencijalom. Najznačajnija vrsta *Ixodes ricinus* uspješno prenosi zoonotske uzročnike poput bakterije *Borrelia burgdorferi* sensu lato, virus krpeljnog encefalitisa, rikecije *Anaplasma phagocytophilum, Rickettsia monacensis*, kandidata vrste *Neoehrlichia mikurensis* te protozoe poput vrste *Babesia microti* i *B. venatorum* (EU 1). Morfološka determinacija vektora je zahtjevna i često subjektivna. Stoga se danas provode brojna istraživanja sa ciljem razvoja novih objektivnijih metoda za njihovo razlikovanje kao i istraživanja njihova vektorskog kapaciteta. Dokaz uzročnika u vektoru ili njegove DNA ne znači da ga može uspješno prenijeti stoga su neophodna pokusna istraživanja i objektivan dokaz mogućnosti prijenosa. Vrste komarčića unutar Pulicaris kompleksa nije moguće morfološki razlikovati, a samo pojedine vrste mogu prenijeti virus bolesti plavog jezika stoga je nužna primjena genskih metoda za razlikovanje vrsta. Na ovaj način je u RH dokazano najmanje 6 novih vrsta komarčića kao i nove genske varijante poznatih vrsta čija uloga u širenju i održavanju virusa je u potpunosti nepoznata. Komarac *Aedes albopictus* (azijski tigrasti komarac) predstavlja invazivnu vrstu, ali i uspješnog prijenosnika parazitarnih i virusnih bolesti. Ovaj komarac posjeduje podjednak afinitet za ubadanje životinja i ljudi čime omogućava uspješniji prijenos zoonotskih uzročnika poput oblića *Dirofilaria repens.*

Vektori za emergentne bolesti često i nisu poznati ili nisu istraživani stoga je teško procijeniti širenje, ali i preživljavanje uzročnika u potencijalnim vektorima. Na primjeru bolesti kvrgave kože jasno je vidljivo da nepozavanje vektora otežava kontrolu i suzbijanje bolesti. Osnovni način prijenosa je mehanički, a postoji sve više dokaza da se virus može prenijeti i putem različitih stadija krpelja, odnosno da može perzistirati u populaciji krpelja. U krpelja *Rhipicephalus appendiculatus* i *R. decoloratus* dokazan je transstadijski i transovarijski prijenos, što znači da virus mogu prenijeti na novu generaciju ličinke krpelja (transovarijski), a zatim i na druge razvojne stadije krpelja (nimfe, adulte). Pokusno je dokazano da ličinke hranjene na viremičnim govedima, nakon presvlačenja u nimfe, mogu prenijeti virus na neificirana goveda koja su kasnije razvila klasičan oblik bolesti. Navedene vrste nisu prisutne u Europi no može se pretpostaviti da druge vrste krpelja iz roda *Rhipicephalus* mogu imati vektorski kapacitet. Sadašnjim istraživanjima roda *Rhipicephalus* otkrili smo postojanje potencijalno „nove" vrste za koju se do sada smatralo da je *R. sanguineus* temeljem morfoloških analiza. Nalaz je značajan jer dovodi u pitanje prijenos novih uzročnika kao što su *Anaplasma centrale*, *A. ovis* i *A. bovis.*

Culicoides punctatus pripada skupini *Culicoides pulicaris* kompleks te predstavlja dominantnu skupinu komarčića u RH, a *C. punctatus* je jedna od najčešćih vrsta. U 2015. godini bile su prisutne već u travnju u kontinentalnoj Hrvatskoj. DNA virusa bolesti kvrgave kože dokazana je u Turskoj tijekom epidemije iz 2015. godine u vrsti *C. punctatus*. Na primjeru emergentne bolesti kvrgave kože, jasno je vidljivo da nedostaju temeljne spoznaje o vektorima tog do sada nepoznatog uzročnika u Europi.

Vectors of emerging infectious diseases and their distribution in Croatia

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Diseases transmitted by vectors are attracting increasing attention in recent years due to global changes that directly affect the ecology of pathogens and their spread. Climate change promotes the spread of the vector in new areas, and at the same time globalization enables faster transmission of vectors and their animal hosts. The development of molecular methods and their use in research and routine diagnostics significantly contributed to discovery of new pathogens and/or new isolates which

encouraged more detailed studies of the vector potential especially mosquitoes, ticks, biting midges (Culicoides) or other arthropods. It is estimated that almost 39,000 different species of arthropods parasite humans, domestic and wild animals, which clearly points their global importance. Croatia has large variations in relief, soil and climate of certain regions which allowed adaptation of numerous species of arthropods. In Europe the most important vectors are ticks, able to transmit successfully pathogens to humans and animals. Today it is known 907 species of ticks with different vector potential. The most significant species *Ixodes ricinus* transmit zoonotic pathogens such as bacteria *Borrelia burgdorferi*, the tick-borne encephalitis virus, rickettsial patthogens *Anaplasma phagocytophilum*, *Rickettsia monacensis*, candidate species Candidatus Neoehrlichia mikurensis and protozoa such as *Babesia microti* and *B. venatorum* (EU 1), thus has a significant potential for transmission of zoonotic pathogens. Morphological determination of vector species is demanding and often subjective, therefore, today researchers are focused to develop new more objective methods for their determination and to explore their vector capacity. Detection of pathogen's DNA in the vectors does not mean that it can be transmit to new host therefore experimental studies are needed to evaluate vector capacity of each proposed vector. Culicoides within Pulicaris complex cannot be distinguished morphologically, but only certain species can transmit bluetongue virus, therefore is necessary to apply genetic methods to distinguish species. Using that approach in Croatia at least 6 new species and a new genetic variants of known species have been confirmed, whose role in the dissemination and maintenance of the virus is completely unknown. Mosquito *Aedes albopictus* (Asian tiger mosquito) is an invasive species but also an excellent vector of parasitic and viral diseases. This mosquito has equal affinity for bitting animals and humans, which enables successful transmission of zoonotic pathogens such as nematode *Dirofilaria repens.* Vectors for emerging diseases are often not known or have not been investigated thus is difficult to assess their spread and surviving. For example, in case of viral lumpy skin disease it is clear that without knowledge on vectors is difficult to control disease spreading. Main mode of transmission is mechanical, but there are evidences that the virus can be transmitted through different stages of ticks and can persist in the population of ticks. In *Rhipicephalus appendiculatus* and *R. decoloratus* trans-stadial and trans-ovarial transmission have been proven, indicating the virus can be transmitted to the new generation of larval ticks (trans-ovarian), and then to other developmental stages of ticks (nymphs, adults). Experimental studies has shown that the larvae fed on viremic cattle, after molting into nymphs can transmit the virus to non-

infected cattle which later developed the classic form of the disease. These species are not present in Europe, but it is to assume that other ticks of the genus *Rhipicephalus* possess vector capacity. In the current research of the genus *Rhipicephalus* we discovered the existence of a potential "new" type which is so far considered to be *R. sanguineus* based on morphological analysis. The finding is significant prejudice to the transfer of new pathogens such as *Anaplasma centrale, A. ovis* and *A. bovis*. *Culicoides punctatus* belongs to a *Culicoides pulicaris* complex and the dominant group of biting midges in Croatia, and *C. punctatus* is one of the most common species. In 2015, it was detected in April in continental Croatia and at the same time was the most common type in Slavonia. DNA of lumpy skin disease virus has been confirmed in *C. punctatus* during the 2015 outbreak in Turkey. On the example of emerging lumpy skin disease, it is clear that there is lack of basic knowledge of vectors for, so far unknown pathogen in Europe.

Lumpy skin disease – epidemiology and clinical findings

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Lumpy skin disease (LSD) is an important transboundary disease of cattle, caused by LSD virus from the *Capripox* genus. Its presence results in substantial economical losses caused by reduced milk production, infertility and abortion, emaciation, permanent damage to hides, as well as restrictions or total ban of trade with live animals and animal products [1,2].

The clinical appearance of the disease is rather specific, thus enabling easy recognition and initial suspicion. LSD is characterized by persistent fever, enlarged lymph nodes, necrotic lesions (mainly localized in the mucous membranes of the oral cavity and upper respiratory tract), and the most prominent sign - development of multiple, firm skin nodules. Severity of the disease could differ from subclinical (up to 50% of experimentally infected cattle) to fatal (less than 10%). LSD is not highly contagious and it has relatively low morbidity which varies between 5 and 45%. The main mode of LSDV transmission is mechanical via blood-feeding insects, but little is known about the role and importance of other transmission routes (direct contact, seminal transmission, infected fomites)[2, 3, 4]. Until recently, LSD remained primarily confined to the African continent, with occasional but short lasting incursions in the Middle East countries. In 2012, new and severe outbreaks were reported in Israel and Lebanon, marking the beginning of a new Middle East LSD epidemic. Political unrest and collapsed veterinary services in Syria and Iraq, further complemented with the absence of effective control measures in the most of the neighbouring countries, aided for a rapid disease spread and increased risk for LSD introduction in Europe [5,6]. In August 2015, only one year after becoming endemic in Turkey, LSD was reported in Greece and the first introduction of LSD in Europe was announced. The disease continued to spread despite the control efforts, but remained limited to the southern Greek provinces. In April 2016, new disease outbreaks appeared unexpectedly in the northern regions, indicating the beginning of a wide Balkan LSD epidemic. Within less than 4 months, many Balkan countries reported their first LSD outbreaks: Bulgaria (13 April), Macedonia (18 April), Serbia (4 June), Kosovo (June), Albania (6 July) and Montenegro (21 July). Mass vaccination campaigns using homologous, attenuated LSD vaccines were launched in the affected Balkan countries, in response to the

disease introduction. Vaccination, along with the removal of infected animals/herds and strengthened movement controls, are the principle tools used in the control of the Balkan LSD epidemic, and their effectiveness is yet to be assessed. For now, it remains clear that current LSD epidemic is a serious threat for the rest of Europe. Elucidation of the key knowledge gaps related to the LSD transmission, efficacy and safety of the available vaccines, development of safe inactivated DIVA vaccines and serological tests for rapid screening, presents a great challenge, but it is essential for success in LSD control in Europe.

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Outbreaks of lumpy skin disease in Europe and the support of the Joint FAO/IAEA Division in dissemination of early detection and response tools

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The Lumpy Skin Disease of cattle was traditionally considered endemic in Africa and as an exotic disease in Europe. The disease was introduced in Turkey in August 2013, and until July 2016 it caused 237 outbreaks in the country. In August 2015, the disease spread to Greece, causing 218 outbreaks until August 2016. The disease has rapidly spread to Bulgaria and Macedonia (April 2016), in Serbia (June 2016), Montenegro (July 2016) causing 202, 181, 219 and 63 outbreaks respectively*.

The European countries did not have experience with the control of the disease (strategies to be used, basically test-and-slaughter or vaccine strategy), however, it seems that the techniques for rapid and sensitive detection of the virus, essential for scientifically based decision making and response to the disease were mostly lacking.

The Animal Production and Health Sub-programme (APHS) of the Joint FAO/IAEA Division in Vienna, Austria has a historical experience in the development and dissemination of such tools among Member States (MS) of FAO and IAEA. Therefore, in accordance with its mandate, several emergency actions were undertaken to support the MS in their efforts to control LSD. These actions included:

- Two emergency expert visits (Bulgaria and Serbia) to evaluate on-the-site the preparedness of the veterinary services in the affected MSs to manage the outbreaks, especially related to the capacities of the officially designated laboratories to early detect LSD infections;
- Upon request of both MSs, an emergency support through the Technical Cooperation Programme of IAEA was delivered (priority needs in equipment and consumables in a value of 50.000 euros per country);
- Two training courses on the techniques for detection / differentiation of the LSD virus were organized at the IAEA laboratories in Seibersdorf, from 15-19 August 2016 and 22-26 August 2016. Thirty-seven (37) participants from 23 MS of the IAEA European region attended the training courses;

- Emergency diagnostic toolkit to each participating laboratory at the training courses was delivered comprised of complete kit of reagents sufficient for ~ 500 runs, as well as the harmonized set of SOPs for the delivered techniques;
- Access to a free-of-charge genetic sequencing services to enable counterparts in depth understanding of the molecular epidemiology of circulating LSD viruses;
- A workshop discussing the experiences, success and the actions for the future will be held from 21-25 November 2016. The workshop will be supported by 10 experts with long term experience in capripox virus infections and LSD.

The techniques delivered included a diagnostic algorithm for detection and differentiation of LSD infections, as follows:

- A pan-capripox quantitative real-time PCR (qPCR) TaqMan assay.-The assay amplifies an 89 bp fragment of the SPPV, GTPV and LSDV ORF074 which encodes the intracellular mature virion envelope protein P32 [*Bowden T et al. (2008): Virology 371, 380–393; Stubbs S et al. (2012): Journal of Virological Methods 179, 419– 422*];
- The short fragment conventional PCR for amplification of a portion of the 30kD RNA polymerase of the RPO30 gene [*Lamien CE et al. (2011): Veterinary Microbiology 149, 30–39*]. The technique is used for simultaneous detection of all capripox viruses and allows for differentiation of SPPV from GTPV/LSDV. The same can be used for sequence analysis.
- The long fragment conventional PCR for amplification of a portion of the 30kD RNA polymerase of the RPO30 gene [*Esayas G. et al. (2015): Antiviral Research 119, 28–35*]. The technique is used for sequence analysis.
- Real time PCR method for simultaneous detection, quantitation and differentiation of capripoxviruses using fluorescence melting curve analysis [Lamien CE. et al. (2011): Journal of Virological Methods, Volume 171(1), 134–140].
- Training in the use of the free-of-charge sequencing services package of the APHS, which includes sample preparation, submission, sequence assembly and alignment, and interpretation of the genetic trees,

The Joint FAO/IAEA Division will continue supporting MS in their preparedness for response to upcoming animal disease challenges, including diseases with zoonotic impact.

* Records based on the reports from FAO-EMPRES-i

Current knowledge on the safety and effectiveness of lumpy skin disease vaccines

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The experience gathered to date suggests that appropriate vaccines are needed for the control of large lumpy skin disease (LSD) epidemics. Currently, only live attenuated vaccines based on attenuated sheep pox virus (SPPV), goat pox virus (GTPV) or lumpy skin disease virus (LSDV) are used for the control of lumpy skin disease. The main prerequisites from such vaccines are to be safe, effective and not too expensive. Unfortunately, reliable data on the effectiveness of most of the currently used vaccines is lacking. A large portion of the available knowledge is based on non-controlled field observations, causing erroneous conclusions on vaccine effectiveness. In this talk I summarize the evidence based knowledge on the currently available LSD vaccines and control methods. Literature review revealed only one randomized field study, one analysis of vaccine effectiveness during and epidemic and several challenge studies of lumpy skin disease vaccines. The randomized field study, conducted on 5000 cows in 15 dairy herds in Israel, compared an LSDV attenuated Neethling strain vaccine and an SPPV attenuated RM-65 vaccine administered in X10 of the dose administered to sheep (both administered at $10^{3.5}$ TCID). The Neethling vaccine was 4.3 times more effective in preventing laboratory diagnosed LSD than the 10xM-65 vaccine, and 11.2 times more effective in preventing severe LSD cases. This vaccine, however caused adverse effects in 0.5% of the vaccinated cows, which was characterized by a very mild and transient LSD-like disease. Analysis performed by EFSA during an outbreak of LSD in Greece showed that the Neethling vaccine was 80% effective for preventing occurrence of LSD in a herd. The vaccine reached its highest effectiveness only 30 days after vaccination. A challenge study performed recently in Ethiopia showed that an attenuated Gorgon strain GTPV vaccine is effective in preventing LSDV. The results of this study are in accordance with previous results of challenge studies with the Kedong and Isiolo goat pox vaccines which showed to be protective against LSD challenge. However, the results of these studies need to be re-confirmed by natural challenge in the field. Another challenge study performed during the 90's showed that the KGSP-240 (LSDV) attenuated vaccine is protective against LSD. However, small sample size of this study as well as severe adverse effects observed in KGSP-240

vaccinated herds in Israel and its ineffectiveness for preventing LSD as observed in Ethiopia, suggest that this vaccine is not a good candidate for the control of LSD in the field. Using the Neethling and 10xRM65 vaccines, with minimal stamping out (i.e. no stamping out in grazing cattle and modified stamping out in dairy cattle), LSD was eliminated from Israel with the last case occurring in August 2013. A mathematical model performed recently by EFSA showed that indeed when pre-emptive vaccination is used, culling has only minor influence on the development of the epidemic. In conclusion, LSD can be effectively controlled using the attenuated Neethling vaccine and minimal culling. However, contamination found in some of the commercial vaccine batches used in Israel, emphasizes the need for a GMP production of such a vaccine. Minor adverse effects may rarely follow vaccination, but these should not prevent the use of this effective vaccine for LSD outbreak control.

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,

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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Preventivne mjere s ciljem sprječavanja pojave bolesti kvrgave kože goveda u Hrvatskoj

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Bolest kvrgave kože (BKK) na teritoriju EU po prvi je put zabilježena ljeti 2015. godine u Grčkoj. U razdoblju od travnja do kolovoza 2016. godine proširila se na područje Bugarske, Makedonije, Albanije, Srbije, Kosova i Crne Gore, stigla na granice Rumunjske i Bosne i Hercegovine te je prepoznata kao ozbiljna prijetnja govedarstvu u Hrvatskoj, Mađarskoj, Austriji i Sloveniji. Pravila o mjerama suzbijanja ove bolesti u EU zakonodavstvu su izuzetno stroga, budući da mehanizmi i mogućnosti širenja uzročnika nisu u potpunosti istraženi, a potencijalne ekonomske posljedice u slučaju širenja BKK vrlo velike. Na temelju znanstvenih podataka i mišljenja relevantnih institucija (EFSA) tijekom 2016. godine pokrenute su izmjene kako EU legislative tako i postavki Svjetske organizacije za zdravlje životinja (OIE) u odnosu na BKK. Ključne izmjene u pristupu jesu prepoznavanje preventivnog cijepljenja kao najboljeg načina za suzbijanje širenja bolesti te redefiniranje opasnosti od mogućeg širenja BKK prometom cijepljenih živih goveda, odnosno prometom svježeg mesa i mlijeka cijepljenih životinja. Hrvatska je, kao zemlja visokog rizika u odnosu na pojavu BKK, tijekom ljeta 2016. godine u dogovoru s EK pravovremeno započela s preventivnim cijepljenjem populacije goveda u rizičnim županijama, a uspostavom zakonskog okvira za mogućnost slobodnog prometa proizvoda cijepljenih životinja mjera je proširena na cijelu zemlju te će cijepljenje čitave populacije goveda biti dovršeno tijekom studenoga. Na taj su način moguće posljedice za mljekarski sektor svedene na minimum. U provedbi cijepljenja trenutno se koristi živo cjepivo, koje sadrži oslabljenog uzročnika. Kod cijepljenih životinja potvrđene su nuspojave (prijavljene reakcije u 0.068 % cijepljenih goveda), pa i uginuća (prijavljeno u 0.019 % cijepljenih goveda). Reakcije u krava u laktaciji češće su u odnosu na reakcije u tovnih goveda i teladi. Novo cijepljenje bit će provedeno početkom 2017. godine, prije početka sezone vektora – prijenosnika bolesti, s ciljem nadocjepljivanja populacije i zaštite podmlatka. Iako je za tekuću godinu rizik od novih slučajeva BKK u regiji sve manji, bolest je sezonskog karaktera i nužno ju je promatrati kao dugoročni problem, kroz koordinirani zajednički pristup i suradnju s ostalim zemljama u regiji.

Ključne riječi: bolest kvrgave kože, mljekarski sektor, preventivno cijepljenje

Measures for the prevention of the outbreak of lumpy skin disease in Croatia

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Lumpy skin disease (LSD) in the EU has been confirmed for the first time in summer 2015 in Greece. In period April – August 2016 LSD has been confirmed in Bulgaria, Macedonia, Albania, Serbia, Kosovo and Montenegro, it reached the borders of Romania and Bosnia and Hercegovina and has been recognised as the serious risk for cattle farming in Croatia, Hungary, Austria and Slovenia. Legislative frame for control and eradication of this disease in EU legislation is very strict, as all the possibilities and ways of spreading the agent are still not fully investigated, and potential economic losses in the case of spreading LSD very high. On the basis of scientific data and opinions of relevant institutions (EFSA) during 2016 were initiated changes to EU legislation and setting the World Organisation for Animal Health (OIE) in relation to LSD. Key changes in approach are identifying preventive vaccination as the best way of combating the spread of disease and redefining risk of possible spread of LSD by trading of vaccinated live cattle or trade of fresh meat and milk of vaccinated animals. Croatia, recognised as a country with high risk in relation to the occurrence of LSD, during the summer of 2016, in consultation with the European Commission in due time began with the preventive vaccination of the cattle population in high-risk counties. After changes in a legal framework (possibility of free trade of fresh meat and milk from vaccinated animals) measure was extended to the whole country and vaccination of the entire cattle population will be completed during November. In this way, the possible consequences of the dairy sector is kept to a minimum. In carrying out the vaccination, live atenuated vaccine is currently used. These vaccines can show side effects in vaccinated animals (side effects in 0.068 % vaccinated cattle), even accidental deaths (registered in 0.019 % vaccinated cattle). Reactions in lactating cows are more often, compared to reactions in beef cattle and calves. New vaccination campaigne will be carried out in early 2017, before the start of the vector season, with the aim of revaccination of entire population and protection of offspring. Although the risk of new confirmation of LSD till the end of current year is lower, the disease is seasonal and it should be considered as a long-term problem, through a coordinated joint approach and cooperation with other countries in the region.

Keywords: lumpy skin disease, dairy sector, preventive vaccination

Lumpy skin disease – present situation and future activities of European Commission

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Lumpy Skin Disease (LSD), a disease of cattle previously considered to be exotic, entered continental Europe for the first time in August 2015, in Greece, through its border with Turkey, where the disease is endemic. By December of the same year, thanks to a combination of mass vaccination and favourable winter weather, the disease came to a halt.

On April 2016 however the disease reappeared, initially in Greece, followed by Bulgaria and the former Yugoslav Republic of Macedonia. By the end of July, LSD had spread further, affecting in turn Serbia, Kosovo[1], Albania and Montenegro. All affected countries resorted to mass vaccination against LSD and in view of the situation the competent authorities of Croatia, a country not affected by the disease, also decided to resort to mass vaccination as a preventive measure. By November 2016 the progress of the LSD vaccination campaigns in the affected countries, seems to have stopped further spread of the disease west or north, at least in those areas where high vaccine coverage has been achieved.

During the epidemic the European Commission swiftly adapted the policy and relevant rules to the disease evolution, scientific advice and experience gained so as to ensure a more suitable and sustainable disease control strategy. Support was offered to the affected countries and countries at risk in multiple ways (vaccines donated from the European Union LSD vaccine bank, technical support by experts, trainings, international coordination meetings, co-financing of measures, e.t.c.) and such activities are expected to continue in the future given the specific nature of the disease (transmission by insects) and its likely seasonal character.

[1] This designation is without prejudice to positions on status, and is in line with UNSCR 1244 and the ICJ Opinion on the Kosovo Declaration of Independence.