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LUMPY SKIN DISEASE: CHARACTERIZATION AND POSSIBLE RISKS FOR CENTRAL AND EASTERN EUROPE

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Summary. Lumpy skin disease (LSD, nodular dermatitis of cattle) — is the contagious poxviral disease of cattle. It is characterized by severe losses and different ranges of mortality and morbidity. The disease is endemic in many Asian and African countries.

The article is devoted to explanation of LSD history, epizoothology, and distribution, risks associated with the disease, diagnostics, differential diagnostics and prevention. The situation regarding LSDV introduction to Ukraine is likely to be non-optimistic. Russia, Caucasian countries, and Bulgaria high LSD-associated risks put our territory on high range of risk regarding LSDV introduction. Disease introduction probabilities could be estimated as extremely high, and high from the side of Russia.

NSC 'IECVM' in collaboration with SSRILDVSE developed the in house PCR-based protocol for LSDV detection that requires fast implementation. Joint collaboration in area of development regional LSDV distribution control policy and contingency plan are required.

Keywords: lumpy skin disease, risk analysis, epidemiology, diagnostics, animals

Lumpy skin disease (LSD, nodular dermatitis of cattle) — is the contagious poxviral disease of cattle. Severe losses and different ranges of mortality and morbidity characterize LSD. It is endemic in many Asian and African countries, and it is rapidly spreading throughout the Middle East. Turkey, Bulgaria, Russia and Caucasian countries are affected with the disease (AHAW, 2015; OIE, 2016).

LSD clinical picture is supported by following symptoms: fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, oedema in the skin. The epicrisis of the disease is sometimes committed with death of infected animals.

The disease has the economic importance because of temporary reduction in milk production, temporary or permanent sterility in bulls and fertility of cows. LSD could be increased in the damage level in association with secondary bacterial infections (Coetzer, 2004).

Historically, the first cases of LSD were described in 1929 in Zambia. In the beginning, LSD signs were considered to be the consequence either of poisoning or a hypersensitivity to insect bites. Same clinical signs were occurred in Botswana, Zimbabwe and the Republic of South Africa between 1943 and 1945, where the infectious nature of the disease was recognized in these outbreaks (Al-Salihi, 2014).

In South Africa, LSD occurred as a panzootic, which affected eight million cattle. The disease continuous until 1949, and generate massive economic losses. In 1957, LSD was identified in East Africa in Kenya. In 1972, the disease was reported in Sudan and West Africa in 1974. Nowadays, LSD occurs in most countries in Africa (except Libya, Algeria, Morocco and Tunisia) (Tuppurainen and Oura, 2011), Asia and Mideast (Ali and Amina, 2013). One of the recent outbreaks of LSD in African continent was occurred in central Ethiopia in 2007 to 2011.

The disease has been reported in Turkey in October 2013, Iran and Iraq in 2014. The expectation of the travelling and invasion of the LSD to free neighbour countries is possible. LSD may invade north and west from Turkey into Europe and the Caucasus and East to Central and South Asia.

LSD causative agent is the virus from family Poxviridae, genus *Capripoxvirus*, called lumpy skin disease virus (LSDV). The prototype strain is the Neethling strain. These are antigenic and genetic homology in high rates with the sheep pox and goat pox viruses. LSD has a partially different geographical distribution from sheep and goat pox, suggesting that cattle strains of capripoxvirus do not infect and transmit between sheep and goats (Woods, 1988).

LSDV is susceptible to 55°C for 2 hours and 65°C up to 30 minutes. It can be recovered from skin nodules and kept at –80°C for 10 years. The infected tissue culture fluid can be stored at 4°C for 6 months. The virus is susceptible to high rates of alkaline or acid pH. However, there is no significant.

It demonstrates the susceptibility to ether, chloroform, formalin, and some detergents, e.g. sodium dodecyl sulfate. In addition, it is also susceptible to phenol, sodium hypochlorite, Virkon® (2%) and quaternary ammonium compounds. LSDV has remarkably stable

surviving for long periods at ambient temperature, especially in dried scabs. LSDV is very resistant to inactivation.

It also can remain viable for long periods in the environment. Meanwhile, the virus is susceptible to sunlight and detergents containing lipid solvents, while, in dark environmental conditions, such as contaminated animal sheds, it can persist for many months.

The LSDV genome is presented by 151 kbp dsDNA. It includes the central coding region bounded by identical 2.4 kbp-inverted terminal repeats. Viral genome contains 156 putative genes. LSDV genes share a high degree of colinearity and amino acid identity (average of 65%) of its genomic region with genes of other known mammalian poxviruses, particularly suipoxvirus, yatapoxvirus, and leporipoxviruses. LSDV is closely related to other members of the Chordopoxvirinae, it contains a unique complement of genes responsible for viral host range and virulence. The complete genome sequences of several capripoxviruses, including LSDV, sheep poxvirus and goat poxvirus, have been published (Tulman et al., 2001, 2002).

Epidemiology of LSD. The significant variation in the morbidity and mortality rates of LSD outbreaks has been observed. It depends on different factors, such as geographic location and climate, the conditions of livestock management, nutritional and keeping factors, general condition of the animal, breed of affected animals, and their immune status. Also the population levels and dissemination of putative insect vectors in the various habitats create a great level of influence. Virus virulence rate also play the significant role. The morbidity rate for LSD is ranging from 5 to 45%. However, the morbidity rates 1–5% are considered more usual.

The significant morbidity and mortality rates were described in 2009 in Holstein cattle in Asian countries (Wainwright, 2013).

Disease has been observed in various Middle East countries, where it could be recognized as the endemic. Turkey, Bulgaria, Russia and Caucasian countries are affected with the disease (Fig. 1).

The risk of introduction of LSD into the EU via the illegal movement of animals was modeled. The number of animals that need to be moved to have a probability of introduction of LSD into Europe greater than 0.95 or lower than 0.05 would be above 1'300 and below 25, respectively (seroprevalence equal to 30%), or above 7'800 and below 140, respectively (seroprevalence equal to 5%).

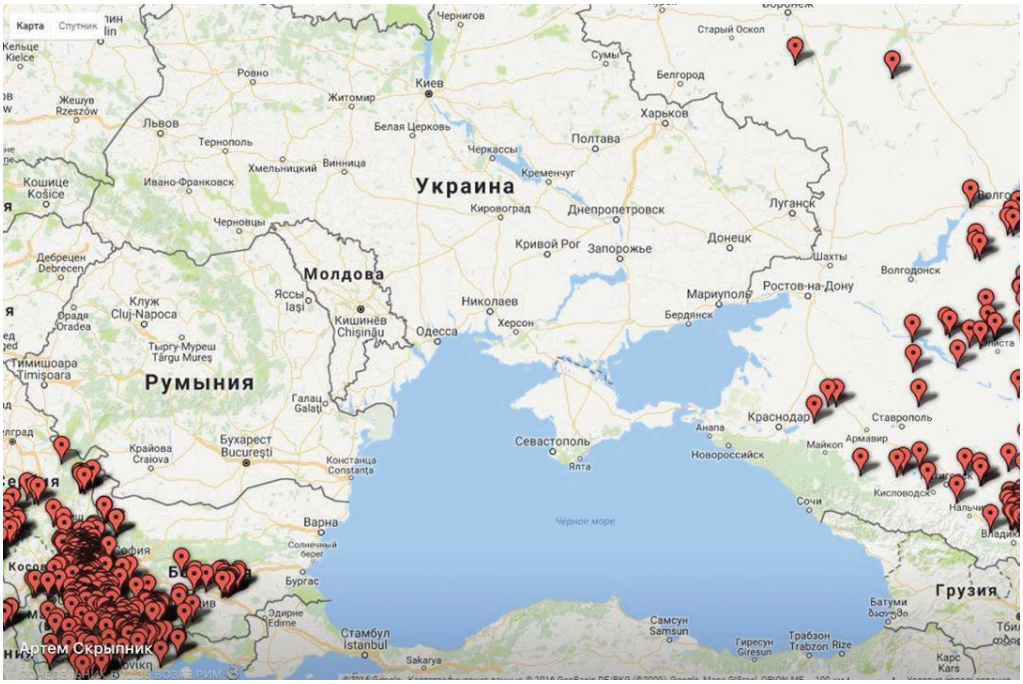


Figure 1. LSD outbreaks map (2nd September, 2016, OIE)

Based on the transmission patterns of LSD as investigated in Israel, a mathematical model was developed to simulate LSD spread between farms over space after an incursion in Greece. When the control measures entail the removal of animals showing generalized clinical signs, approximately 90% of epidemics remain confined to the region around the initial site of incursion. However, the remaining 10% of simulated epidemics are more extensive, with the virus spreading up to approximately 300–400 km from the

site of introduction by six months after the incursion. This identifies the potential for disease outbreaks to spread in Bulgaria and Greece.

Regarding the risk of LSD becoming endemic in animal populations in the EU, owing to a lack of data regarding the ability of potential European vectors of disease transmission, the international data cannot be extrapolated directly to the European situation. Nevertheless, under the current EU policy and according to the scenarios produced using the spread model, if the situation and ability of vectors was the same as in Israel, LSD would most likely not become endemic in the EU.

Situation regarding LSDV introduction to Ukraine is likely to be non-optimistic. Russia, Caucasian countries, and Bulgaria high LSD-associated risks put our territory on high range of risk regarding LSDV introduction. Disease introduction probabilities could be estimated as extremely high, and high from the side of Russia. The first way for possible introduction could be potentially associated with warm and wet summer-spring period, sufficient for growing of the population of different insects, potentially could be LSDV transmission factors in the wildlife and farming animals, especially backyards kept on free pastures.

Also the Eastern way of introduction is actual because of absence of proper antiepizootic control measures in occupied territories of antiterrorist operation in Lugansk and Donetsk regions of Ukraine.

Table 1 – Transboundary risks estimation for LSD introduction from Central Europe in Ukraine

Risk factor	High level	Midlevel	Low level
Existing of the disease in the wildlife	+		
Existing of the disease in domestic animals	+		
Existing of the transmission vectors	+		
Existing of the transboundary transmission ways (migratory wildlife)	+		
Transport communications (migration of people and trade)	+		3+3+3+3+3=15 Extreme risk (confirmed)

The transmission of LSD virus (LSDV) could be potentially executed via insects, or by the natural contact transmission in the absence of insect vectors.

Lesions and signs of the disease. LSD gross lesions include the skin nodules that usually may fuse into large irregular and circumscribed plaques; they have different sizes and ranges. The cut surface of the nodules is reddish-gray. They contain serous fluid and edema in the subcutis layer, after induration they may form deep ulcers. The typical circular necrotic alimentary lesions may also be seen on the muzzle, nasal cavity, larynx, trachea, bronchi, inside of lips, gingiva, dental pad, forestomach, abomasum, uterus, vagina, teats, udder and testes (Fig. 2) (Ali et al., 1990).

Table 2 – Transboundary risks estimation for LSD introduction from Russia in Ukraine

Risk factor	High level	Midlevel	Low level
Existing of the disease in the wildlife		+	
Existing of the disease in domestic animals	+		
Existing of the transmission vectors	+		
Existing of the transboundary transmission ways (migratory wildlife)	+		
Transport communications (migration of people and trade)		+	3+2+3+3+2=13 High risk (confirmed)

The regional lymph nodes are grossly enlarged in 3–5 times from their usual size. The oedematous and pyaemia changes are occurred. Muscle tissue and the fascia over limb muscle may show nodular lesion that are grey-white surrounded by red inflammatory tissue. The same nodules are distributed throughout the carcass. It is about 10–30 mm diameters in the kidney. Interstitial or bronchopneumonia associated with 10–20 mm diameter lesions are also scattered in the lungs.

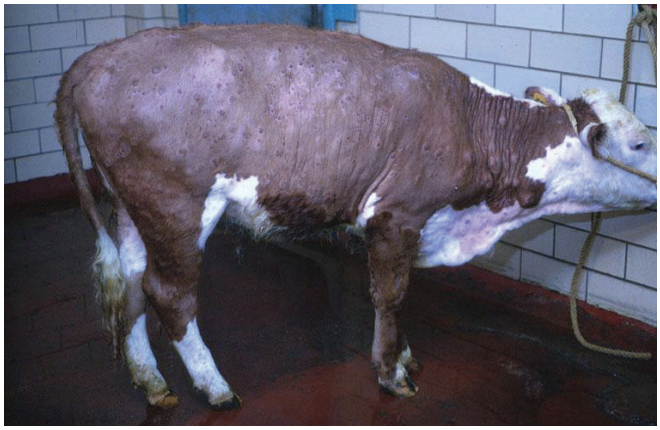
The lesions are separated from the necrotic epithelium far from the healthy tissue. The necrotic tissue sloughs away to leave an ulcer that slowly heals by granulation. Severely infected animals show secondary bacterial pneumonia, tracheal stenosis, acute and chronic orchitis, mastitis with secondary bacterial infection, and similar lesions in the female reproductive tract (Al-Salihi, 2014).

Pathological lesions of the LSD disease in microlevel vary considerably depending on the stage of development. In the acute stage of the disease,

it is mostly characterized by lesions of vasculitis, thrombosis, infarction, and perivascular fibroplasia. Inflammatory cell are infiltrated the infected areas, which includes macrophages, lymphocytes and eosinophils.

A

B



C

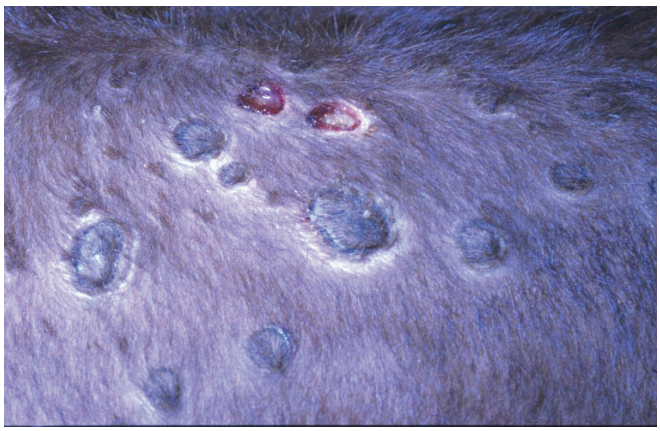


Figure 2. A LSD lesions (A — photo by Orap Zenzele from http://orapzimbabwe.blogspot.com/2014/06/wh-t-is-lump-y-skin-disease-lsd-it-is.html; B and C — photos by Noah's Arkive, PIADC from http://www.cfsph.iastate.edu/DiseaseInfo/disease-images.php?name=lumpy-skin-disease).

The oedema and infiltration of the epidermis and dermis with large epithelioid macrophage type cells, presented in LSV-affected animals, are well described for sheep pox. They are found with plasma cells and lymphocytes in early lesions, and in older lesions, fibroblasts and polymorphonuclear leucocytes with some red cells predominate (Al-Salihi, 2014; Ali and Amina, 2013).

Diagnostics. The diagnostics of LSD should be managed in the complex way, including data of epidemiological investigation, pathological examination (on macroscopic and microscopic levels), and confirmatory laboratory diagnostics, using different tests and tools.

The laboratory testing and LSDV identification are based on OIE Terrestrial Manual (OIE, 2016). Confirmation of lumpy skin disease in a new area requires virus isolation and identification. Samples for virus isolation should be collected within the first week of the occurrence of clinical signs, before the development of virus-neutralising antibodies (Davies, 1991). Skin biopsies of early lesions could be used for the virus isolation. In addition, LSD virus can be isolated from buffy coat from the blood sample collected into EDTA or heparin during the viraemic stage of LSD. Samples should be taken at least from three animals belonging to affected herd. Samples aspirated from enlarged lymph nodes can be also used for virus isolation. LSD virus grows in tissue culture of bovine, ovine or caprine origin. Bovine dermis cells or lamb testis (LT) cells (in primary or continuous culture), are considered to be the most susceptible cells. LSD capripoxvirus have been also adapted to grow on the chorioallantoic membrane of embryonated chicken eggs and Vero cells. But these are not recommended for primary isolation (OIE, 2016; Al-Salihi, 2014).

Transmission electron microscopy (TEM) can be used for confirmatory diagnosis of LSD. Skin specimens or mucosa swabs are used. Mature capripox virions have an average size 320×260 nm and are a more oval profile and larger lateral bodies than orthopox virions (OIE, 2016).

The *Capripoxvirus* antigen can also be identified on the infected cover slips or tissue culture slides using fluorescent antibody tests. An agar gel immunodiffusion (AGID) test has been used for detecting the precipitating antigen of capripoxvirus, but has the disadvantage that this antigen is common with parapoxvirus.

The recombinant antigen for the production of P32 monospecific polyclonal antiserum and monoclonal antibodies (MAbs) was developed for virus antigen detection using ELISA (Carn et al., 1994).

Polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), as very fast tools for agent's identification are widely used and recommended by OIE. The assay have been used for detection of capripoxviruses with higher sensitivity (Bowden et al.,

2009). In house PCR test has been development in NSC 'IECVM' and SSRILDVSE based on FAO protocol (Stegniy B. T., Nevolko O. M. et al., pers. comm., APHL, 2014).

Multiple serological examination tools are also developed for surveillance and control of LSD: ELISA, VN, Western blot analysis (Tuppurainen and Oura, 2011).

There are many diseases causing similar signs of LSD. It is important to obtain a definite ***differential diagnosis*** to ensure the best preventative and control measures for susceptible herds.

LSD can be confused with the following diseases:

- Pseudo-lumpy-skin disease,
- Bovine virus diarrhoea/mucosal disease,
- Demodicosis (Demodex),
- Bovine malignant catarrhal fever (Snotsiekte),
- Rinderpest,
- Besnoitiosis,
- Oncocercariasis,
- Insect bite allergies.

Prevention of LSD. As far as LSD vaccines are concerned, only live attenuated vaccines against LSD are currently commercially available. RM-65 attenuated sheep pox vaccine at the recommended dose for sheep has limited effectiveness in protecting animals from LSD. There is field evidence that 10 times the dose of RM-65 is more effective in terms of protection, although is less effective than vaccination with a homologous strain. The Neethling attenuated lumpy skin disease virus vaccine is highly effective in the prevention of morbidity, thus confirming the need to use homologous vaccines for the control of *Capripoxvirus* infections. Nevertheless, some safety issues have been reported that are linked to generalize clinical reactions due to vaccination with LSD strains that can be observed.

Concerning the effectiveness of control measures, according to Israeli experience, while using the attenuated RM-65 vaccine at the recommended dose for sheep; culling only those animals with generalized skin lesions has controlled epidemics of limited extent. Large epidemics can be controlled by the use of effective vaccination. Epidemics are not self-limiting when effective vaccination or culling are not applied. Although insecticides are frequently used to control LSD outbreaks, there is no evidence to date to prove their effectiveness in controlling LSD spread.

The AHAW Panel recommends further investigation into the potential relevant vector species for LSD transmission in controlled environments and the mode of transmission, besides the ecology of different blood feeding and biting arthropod species in the cattle farming setting. In relation to this, the effectiveness of insecticides for LSD control should also be investigated.

Owing to the risk of LSD spreading from the Middle East to the rest of Asia or to Europe, the development of safe, efficient and non-replicating 'differentiating infected from vaccinated animals' (DIVA) vaccines against LSDV is required, as well as an associated diagnostic test. Furthermore, the efficacy of currently available live vaccines in cattle against LSDV should be evaluated using challenge experiments in controlled environments (AHAW, 2015).

Conclusion. LSD is the high risks associated emergent disease of cattle. It demonstrated high trends of transboundary distribution in Central and Eastern Europe and requires development of new strategies of surveillance and control. NSC 'IECVM' in collaboration with SSRILDVSE developed the in house PCR-based protocol for LSDV detection that requires fast implementation. Joint collaboration in area of development regional LSDV distribution control policy and contingency plan are required. It needs development of the multi-authorities collaborative effort.

References

AHAW (EFSA Panel on Animal Health and Welfare) (2015) 'Scientific opinion on lumpy skin disease', *EFSA Journal*, 13(1), p. 3986. doi: 10.2903/j.efsa.2015.3986.

Ali, A. A., Esmat, M., Attia, H., Selim, A. and Abdel-Humid, Y. M. (1990) 'Clinical and pathological studies on lumpy skin disease in Egypt', *Veterinary Record*, 127(22), pp. 549–550. doi: 10.1136/vr.127.22.549.

Ali, M. A. and Amina, A. D. (2013) 'Abattoir-based survey and histopathological findings of Lumpy skin disease in cattle at Ismailia Abattoir', *International Journal of Bioscience, Biochemistry and Bioinformatics*, 3(4), pp. 372–375. doi: 10.7763/IJBBB.2013.V3.235.

Al-Salihi, L. (2014) 'Lumpy Skin disease: Review of literature', *Mirror of Research in Veterinary Sciences and Animals*, 3(3), pp. 6–23. Available at: http://mrvsa.com/upload/3-3-2-2014%20Lumpy%20Skin%20disease%20%20Review%20of%20literature.pdf.

APHL (Joint IAEA/FAO Animal Production and Health Laboratory) (2014) *SOP/IAEA/FAO/Coprpoxvirus/PCR/Standard operation procedure for the detection of Coprpoxvirus*. Seibersdorf, Austria: FAO/IAEA Agriculture and Biotechnology Laboratories.

Bowden, T. R., Coupar, B. E., Babiuk, S. L., White, J. R., Boyd, V., Duch, C. J., Shiell, B. J., Ueda, N., Parkyn, G. R., Copps, J. S. and Boyle, D. B. (2009) 'Detection of antibodies specific for sheeppox and goatpox viruses using recombinant capripoxvirus antigens in an indirect enzyme-linked immunosorbent assay', *Journal*

of Virological Methods, 161(1), pp. 19–29. doi: 10.1016/j.jviromet.2009.04.031.

Carn, V. M., Kitching, R. P., Hammond, J. M. and Chand, P. (1994) 'Use of a recombinant antigen in an indirect ELISA for detecting bovine antibody to capripoxvirus', *Journal of Virological Methods*, 49(3), pp. 285–294. doi: 10.1016/0166-0934(94)90143-0.

Coetzer, J. A. W. (2004) 'Lumpy skin disease', in Coetzer, J. A. W. and Tustin, R. C. (eds.) *Infectious diseases of livestock*. 2nd ed. Cape Town, South Africa: Oxford University Press, pp. 1268–1276. ISBN 9780195761719.

Davies, F. G. (1991) 'Lumpy skin disease, an African capripox virus disease of cattle', *British Veterinary Journal*, 147(6), pp. 489–503. doi: 10.1016/0007-1935(91)90019-j.

OIE (World Organisation for Animal Health) (2016) 'Chapter 2.4.13. Lumpy skin disease', in: *Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees)*. Paris: OIE. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.13_LSD.pdf.

Tulman, E. R., Afonso, C. L., Lu, Z., Zsak, L., Kutish, G. F. and Rock, D. L. (2001) 'Genome of lumpy skin disease virus', *Journal of Virology*, 75(15), pp. 7122–7130. doi: 10.1128/jvi.75.15.7122-7130.2001.

Tulman, E. R., Afonso, C. L., Lu, Z., Zsak, L., Sur, J.-H., Sandybaev, N. T., Kerembekova, U. Z., Zaitsev, V. L., Kutish, G. F. and Rock, D. L. (2002) 'The Genomes of Sheeppox and Goatpox viruses', *Journal of Virology*, 76(12), pp. 6054–6061. doi: 10.1128/jvi.76.12.6054-6061.2002.

Tuppurainen, E. S. M. and Oura, C. A. L. (2011) 'Review: Lumpy skin disease: An emerging threat to Europe, the Middle East and Asia', *Transboundary and Emerging Diseases*, 59(1), pp. 40–48. doi: 10.1111/j.1865-1682.2011.01242.x.

Wainwright, S., Idrissi, A. E., Mattioli, R., Tibbo, M., Njeumi, F. and Raizman, E. (2013) 'Emergence of lumpy skin disease in the eastern Mediterranean basin countries', *EMPRES watch*, 29, pp. 1–6.

Woods, J. A. (1988) 'Lumpy skin disease—A review', *Tropical Animal Health and Production*, 20(1), pp. 11–17. doi: 10.1007/bf02239636.