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DETECTION OF LUMPY SKIN DISEASE VIRUS GENOME IN FIELD SAMPLES COLLECTED FROM CATTLE IN THE RUSSIAN FEDERATION

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SUMMARY

Results of laboratory tests of pathological and biological material samples for lumpy skin disease virus genome with the FGBI "ARRIAH" lumpy skin disease real-time PCR test system are presented. Samples were collected from cattle in 16 regions of the Russian Federation in 2016–2017. A total of 848 stabilized blood, serum, skin (nodule) scrape, nasal washing samples were tested. In the process of antemortem diagnosis lumpy skin disease virus genome was detected in nasal washings (29.2%), serum (19.5%) and stabilized blood (24.4%). Lumpy skin disease virus genome was detected in skin lesion samples (77.7% of cases) during postmortem diagnosis. No lumpy skin disease virus genome was detected in trachea, spleen and aborted fetus samples. Thus, in case of lumpy skin disease suspicion serum and nasal washing samples shall be tested first for antemortem diagnosis, while nodules (lumps) shall be primarily tested for postmortem diagnosis.

Key words: lumpy skin disease, real-time PCR, genome, sample.

INTRODUCTION

Lumpy skin disease of bovines (LSD) is a highly dangerous transboundary infectious disease of bovines that is specified by fever, nodules on the skin, lymphadenitis, inflammation of conjunctiva and mucous membranes of respiratory, digestive and reproductive organs [10, 21].

Lumpy skin disease is caused by DNA-containing enveloped virus of *Poxviridae* family, genus *Capripoxvirus*. The latter includes closely related sheep pox and goat pox viruses [7]. The virus genome is a 151 kbp double-stranded DNA [9].

The disease affects bovines and buffaloes [5], however, among bovines the most susceptible are dairy cows, and the disease incidence may vary from 3% to 80% [12, 18, 21], thus indicating potential role of other still unstudied factors affecting the severity of the clinical signs.

The prevailing route of the infection with LSD virus is bites of blood-sucking insects [4, 14, 15].

All LSD outbreaks involving clinical signs are subject to notification to the OIE. According to the OIE data, the LSD outbreaks are currently reported in Albania, Macedonia, Greece, Turkey, etc. [3, 6, 8].

In the Russian Federation the disease was first reported in the Republic of Dagestan in 2015 [1]. In 2016, 313 disease outbreaks were reported in 17 Subjects of the Russian Federation [22]. Total of 17,853 bovine animals were diseased, 1,559 animals died and 30 animals were euthanized; the disease incidence amounted to 10%, lethality – 8.7%, mortality – 0.9%. In 2017, 43 outbreaks in six Subjects of the Russian Federation were notified.

The disease causes significant economic damage to livestock breeding as it involves dramatic decrease in milk production (up to complete agalactia) as well as loss of body weight, and it leads to restrictions on trade in live animals and their slaughter products. Abortions resulting in temporal or permanent infertility are reported in pregnant cows [13].

In case of systemic disease, 2–7 cm nodules appear on the body of the animals: mostly on the head, neck, udder and in the perineum. On some body parts the nodules merge with each other and form ulcers. The affected sites of the skin are painful for the animals thus aggravating their health condition and resulting in their performance reduction [10]. Bulls can excrete the virus with the semen for a long period of time [17].

Unprecedented LSDV spread, including spread due to latent virus carrier state, requires development of systemic approaches to the disease early detection and monitoring aimed at prevention of the virus spread and reduction of economic losses due to the disease progression. As far as LSD is an underinvestigated disease it is critical to determine, what samples should be used for early and reliable virus detection in the field.

Therefore, the work was aimed at detection of LSDV DNA using previously developed Real-time PCR LSD test-kit (FGBI "ARRIAH") in biological samples collected from the animals demonstrating the disease clinical signs.

MATERIALS AND METHODS

Test object. Test samples were submitted from a number of livestock farms where LSD was reported in 2016–2017.

Biomaterial samples collected from clinically diseased live and dead animals were used including stabilized blood, blood sera, skin (nodule) scrapings, nasal and ocular swabs, milk, lymph nodes, lungs, trachea, spleen and aborted fetuses. As soon as the disease suspects were identified, the samples were collected and delivered to the laboratory on ice within 24 hours.

DNA extraction. Samples were prepared in the laboratory. Sera, stabilized blood and 5–10% biomaterial suspension were used for testing.

The suspension was made by the sample homogenization until mashy using sterile mortar and pestle. Then nuclease free water was added to the mortar and mixed with homogenate in order to obtain 10% suspension. DNA was extracted using AllPrep DNA/RNA Mini Kit (Qiagen, Germany) according to manufacturer instruction.

Polymerase chain reaction (PCR). LSD virus genome was detected using test-kit for detection of field isolates of the lumpy skin disease virus genome using real-time polymerase chain reaction "lumpy skin disease Real-time PCR" (FGBI "ARRIAH").

Real-time PCR was run using Rotor Gene (Qiagen, Germany) according to manufacturer's instructions. The reaction was performed according to the following protocol: activation at 95 °C – 10 min; 40 cycles at 95 °C – 15 sec, at 60 °C – 1 min. The results were interpreted basing on the fluorescence intensity. LSDV DNA deemed confirmed and the sample positive in case *Ct* value was less than 35; and negative if *Ct* value was absent or did not exceed 37.

Presence of LSDV genome in the samples was additionally confirmed by virus isolation and sequencing of RPO30 gene fragment using genetic analyzer (Applied Biosystems, USA) [20].

RESULTS AND DISCUSSION

In 2016–2017, 848 biological samples from 16 regions of the Russian Federation (Astrakhan, Volgograd, Voronezh, Ryazan, Rostov, Samara, Saratov, Orenburg and Tambov Oblasts, Krasnodar and Stavropol Krais, Republics of Dagestan, Kalmykia and Bashkortastan, Karachay-Cherkess and Chechen Republics) were tested. The samples were collected from cattle demonstrating the disease clinical signs.

LSD virus genome was detected in 268 samples that amounted to 31% of the total number of samples tested (see the Table). Tests of samples collected from live animals typically demonstrated LSDV in nasal swabs (29.2%), blood sera (19.5%) and stabilized blood (24.4%).

While testing pathological samples 77.7% of skin samples (nodules) demonstrated LSD virus genome. No LSD virus genome was detected in samples of trachea, spleen and aborted fetuses.

Eleven milk samples collected from clinically diseased cattle were additionally tested, of which six samples demonstrated LSD virus genome. Out of five lymphoid tissue samples, the virus genome was detected in two ones; out of six lung samples LSD virus genome was detected in three samples.

Table
Detections of LSD virus genome in filed samples collected from cattle

Type of sample	Total number of samples	Positives	Positive samples, %
Nodules	108	84	77.7
Stabilized blood	417	102	24.4
Blood sera	128	25	19.5
Nasal swabs	195	57	29.2

It is worth mentioning that LSD virus demonstrates epithelial cell tropism. Subcutaneous and intradermal infection of cattle on 4–7 dpi results in inflammation involving epidermis, dermis and subjacent muscles. Exudate is accumulated in the formed nodules and hereafter necrosis is developed. The process is generalized on day 7–19 post infection and it is specified by fever. The virus appears in the blood on day 3–4 post the rise of the body temperature and mass formation of nodules. The virus is blood born all over the organism entering mucosa in the mouth, nose, eyes, vagina and preputium; into salivary and mammary glands, testes and other organs and tissues causing thrombosis and coagulative necrosis of the adjacent tissues. The virus reproduction in the above mentioned organs results in formation of new necrotizing skin nodules, development of generalized lymphadenopathy, limb swelling, lesions of eyes and mucosa of the respiratory, reproductive and digestive organs.

The diagnosis is based on the analysis of epidemic data, clinical signs and post mortem lesions. The final diagnosis is made following the laboratory tests. Affected skin, lymphoid tissue, nasal and ocular swabs, stabilized blood (sera) and semen or milk samples are recommended for use for the virus detection and/ or isolation.

Currently, molecular and genetic tools are used for LSD diagnosis. LSD diagnosis is deemed confirmed in case either LSDV or its antigen or genome is detected in the samples collected from the diseased or suspected animals. PCR is used for this purpose [2].

Therefore, the paper demonstrates LSDV genome test results for field samples collected from live and emergently euthanized animals. The tests were performed using real-time PCR LSD test-kit. Rapid LSD laboratory diagnosis, especially in live disease suspects, is essential for presumptive diagnosis confirmation as well as for urgent actions for the virus spread prevention. However, insufficient knowledge on LSDV biological properties and its pathogenicity requires joint efforts of the laboratories and veterinary practitioners for better understanding of typical properties of the virus. LSD virus genome was most frequently reported in skin lesions (77.7% of the samples) that is consistent with the results of other studies and supports the expressed virus tropism for epithelium of the skin [16]. In addition, E. S. Tuppurainen et al. demonstrated that PCR allowed detection of the genome in the blood of animals starting from the moment of the nodule appearance [19]. As for other biological samples, the virus genome was detected in 19–29% of the samples. Genetic material of the virus was also detected in the samples of the lungs, lymph nodes and milk. However, conclusive statistical assessment of the results cannot be made due to the limited number of the samples. It should be emphasized that the samples were delivered in the period of the clinical sign onset that could influence the performance of the LSDV detection. E. g. absence of virus genome in the majority of samples (swabs, sera and blood) can be explained by the fact that the virus failed to accumulate in such an amount so as its excretions in the body fluids were sufficient to exceed the limit of detection of the test-kit. Moreover, S. Babiuk et al. determined that under experimental infection the virus excretion from mucous membranes starts after nodule formation. Herewith, low concentration of viral DNA was detected in swabs for several days and viremia lasted for about 9 days and was specified by inconsistent presence of

the virus during the experiment [16, 19]. Such properties could explain low virus transmission among the animals in case of absence of the insect-vector emergence.

The OIE recommended conventional gel-based PCR was applied for the analysis of the results obtained following the use of the test-kit. The analysis demonstrated agreement of the results with the method proposed by D. C. Ireland and Y. S. Binpal [11] in 96% of cases; in 4% of cases the conventional method demonstrated no DNA. That makes sense as it is well known that conventional PCR is less sensitive and at the early stage of the infection the virus concentration range is below the sensitivity level of the test. This can be confirmed by the fact that E. S. Tuppurainen et al. [19] previously determined the 4–6 day viremia period using conventional PCR while S. Babiuk et al. [16] obtained data using real-time PCR. Subsequent analysis of uncertain samples (23 serum samples and swabs) using virus isolation and sequencing (unpublished data) confirmed the virus presence and indicated higher sensitivity of the real-time polymerase chain reaction run using LSD test-kit (FGBI "ARRIAH") as compared to the conventional PCR. It should be pointed out that virus isolation, which is usually successful in case of LSD virus [1], results in detection of viable virions, while PCR results in detection of virus genome DNA without differentiation of its viability thus being preferable for diagnosis as even presence of unviable virions in animal samples can be indicative of the infection.

Of note is the fact that not all infected animals can demonstrate the disease signs or such signs can be mild thus complicating differential diagnosis of bovine herpesvirus 2 (pseudo-lumpy skin disease / herpes mammilitis). Therefore, clinically healthy animals should be also considered while planning monitoring in the high risk zones as such animals can be within the incubation period or recovered without any visible signs [19] thus aiding to the virus transmission with the blood sucking animals. It is of the specific practical importance because ante-mortem diagnosis is performed during the LSD epidemic control and it allows for significant reduction of economic losses due to the disease.

CONCLUSION

Thus out of 848 tested samples 268 samples demonstrated positive result amounting to 31.6% of the total amount of the samples. LSDV was most frequently isolated from the following samples: skin lesions (77.7%), nasal swabs (29.2%), blood sera (19.5%) and stabilized blood (24.4%). The virus genome was detected in milk, lymphoid tissue and lungs. No LSD virus genome was detected in the samples of trachea, spleen and aborted fetuses.

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