Acta Veterinaria-Beograd 2022, 72 (4), 485-492
UDK: 636.09:616.5-022.3(470)"2018/2019"
578.821.083.3
DOI:10.2478/acve-2022-0039

Research article

EVIDENCE OF LUMPY SKIN VIRUS DNA IN BLOOD-FEEDING FLIES DURING OUTBREAKS IN RUSSIA IN 2018-2019

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(Received 15 August, Accepted 30 November 2022)

In this study we report the testing of blood-feeding and synanthropic flies captured near animals affected by lumpy skin disease virus (LSDV) in Russia during the outbreaks in the Kurgan region in 2018 and Saratov region in 2019. The insects of interest were the stable fly *Stomoxys calcitrans*, *Tabanidae* horse flies, *Culicoides* midges and the house fly *Musca domestica* examined as individuals or pools. The obtained findings demonstrate that viral DNA was found in pools of *S.calcitrans* and *M. domestica* and in the head and abdomen of stable flies. This is the first report of LSDV DNA detection in *Tabanidae* flies from the field. The presented data are envisaged to help further guide the search for putative vectors of LSDV in different climatic regions and interpret laboratory-controlled experiments on vector-borne transmission of LSDV.

Key words: Lumpy skin disease virus, entomology, vector, horse fly, stably fly, house fly, midge

INTRODUCTION

Lumpy skin disease is a capripoxvirus infection of cattle and buffaloes [1]. The capripoxvirus genus is currently comprised of sheeppox virus, goatpox virus and lumpy skin disease virus (LSDV). The LSDV genome is about 150 kilo base pairs long and contains 156 putative genes [2]. Due to the economic damage the disease inflicts on affected countries, it must be reported to the World Organization for Animal Health upon occurrence. The clinical symptoms usually include a rapid eruption of cutaneous nodules, generalized lymphadenitis and edema of lumps [3]. The epidemiological profile of LSD is characterized by high morbidity and low mortality. A unique feature of LSD is that only 50% of infected animals are likely to develop clinical signs, with all remaining viremic [4,5].

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Historically, LSD was first described in the 30s of the 19th century in South Africa, followed by a wide spread through the African continent and beyond [6,7]. In 2015 first LSD outbreaks were reported in the Balkans, Turkey and Europe [8,9].

From 2015-2016 LSD occurred in the Caucasus and South of Russia causing 313 outbreaks in 16 regions [10]. Since then South Asian countries such as China, India, Bangladesh, Vietnam and Sri Lanka have reported LSD outbreaks [11-13].

The climate and environmental factors, as well as humidity and vegetation favor the emergence of LSD [14], which lead to the concept that LSD is a vector borne disease [15]. Laboratory controlled experiments have demonstrated a role for *Stomoxys* flies [16,17], Haematopota spp. horseflies [18], and ticks [5]. Viral DNA has also been discovered in non-blood-feeding *M. domestica* flies collected in the field [19,20]. With the emergence of novel naturally occurring recombinant vaccine-like LSD virus strains, the concept of vector-borne transmission needs revision because recent experiments demonstrate that recombinant vaccine-like LSD virus strains can use alternative modes of transmission without insects' bites [21,22]. Unfortunately, entomological data from active outbreaks are lacking in literature, impeding the accurate interpretation of epidemiological findings on LSD in novel climatic and environmental conditions.

In this study we report field evidence of LSD virus DNA identification in collected flies during outbreaks in the Kurgan region in 2018 and Saratov region in 2019.

MATERIAL AND METHODS

Insects

The stable fly *S. calcitrans*, *Tabanidae* horse flies*, Culicoides* midges and the house fly *M.domestica* were used for the study. Entomological trapping *Tabanidae* horse flies, *Culicoides* midges and the house fly *M. domestica* was carried out as previously described [19]. Stable flies were trapped using Starbar Bite Free (Central Life Sciences, USA). The traps were set 1 meter off the ground and at a distance of no more than 5 m from the viremic animals. The weather conditions at both sites were windy and sunny, light wind and temperature was 15-21 C. Flies were identified using the key by Zimin L.S [23].

Fly processing and DNA extraction

Stable and houseflies were tested in pools of five. Horse flies were tested individually, with separate analyses of heads and abdomens. DNA was extracted using TRIzol reagent following the manufacturer's instructions (Thermo Fisher Scientific, USA). PCR was performed as described [24].

Sequencing

Nucleotide sequencing was performed based on the targets described previously [25] from samples positive by real-time PCR. PCR products were purified on Quick Gel columns (Qiagen, Germany). Sequencing was carried out using the amplification primers in an automatic sequencer (ABI Prism 3130, Applied Biosystems, USA). The identity of the insect-derived LSDV sequences were verified by Blastn analysis to the corresponding sequences deposited in GenBank: ORF19, GPCR and RPO30 [26].

RESULTS

Entomological investigation in the Kurgan region in 2018

During the 24- h period a few *Culicoides* individuals and 39 *Tabanidae* horse flies were trapped in July 2018. A picture of the abundant *Tabanidae* horse fly is given in Figure 1. *S. calcitrans* was not captured.

Figure 1. The abundant *Tabanidae* horse fly

The trapped *Culicoides* midges and *S.calcitrans* from the Kurgan region tested negative for LSDV DNA, whereas some of 37 *Tabanidae* flies gave positive results: one horse fly contained viral DNA both in the head and abdomen, two – only in the head (Cts ranged from 27.6 to 29.8 across the positive samples). The remaining 34 horse flies were negative. Sequencing showed the identity of ORF19 of the insect-derived sequence to the corresponding target of Kurgan/2018 reported previously [26].

Entomological investigation in the Saratov region in 2019

A total of 33 *S. calcitrans* L. flies (stable fly), 5 *M.domestica* L. flies (house fly) were collected during a 24-h period in July 2019. No *Tabanidae* and Culicoides midges species were captured. *S. calcitrans* were sorted into pools of five each, with one pool of three. *M. domestica* flies were treated as one pool.

PCR analyses showed viral DNA in one pool of *S. calcitrans* and the pool of *M. domestica* (Table 1).

Table 1. Molecular identification of the presence of LSDV DNA in *S. calcitrans* and *M.domestica* groups

Group number	Species	Ct value
1	*S. calcitrans*	no
2	*S. calcitrans*	no
3	*S. calcitrans*	**32.56**
4	*S. calcitrans*	no
5	*S. calcitrans*	no
6	*S. calcitrans*	no
7	*S. calcitrans*	no
8	*M. domestica*	**29.19**

DISCUSSION

This is the first work reporting the testing of field-collected blood-feeding dipteran insects putatively implicated in the transmission of LSDV from northern latitudes. Although, *M.domestica* flies were also trapped and tested positive for LSDV DNA, with regard to non-biting flies, our study lends support to previously published work by Sprygin et al [19] and Wang et al [20] that this group of insects can become contaminated while feeding on erupted lesions, however, their conclusive implication in LSDV transmission still requires clarification. Considering non-vector-borne transmission of recombinant vaccine-like strains [4], this aspect gains additional relevance. In this case, more data from the field are required to broaden the understanding of LSDV biology and fill in the current gaps in the knowledge of LSDV transmission. *Culicoides* biting midges were not abundant in our study and their role was negligible while these dipterans are enjoying considerable interest as putative vectors [27,28]. It is likely that when the trapping was conducted, the temperature, climate or time or season was not optimal for midges [29], although *Culicoides* biting midges are abundant across regions of Russia and their seasonal activity pattern well fits with the frequency of LSDV outbreaks in Russia (data not shown) [30].

Interestingly, LSDV DNA was discovered in both the heads and abdomens of *Tabanidae* horse flies. In the context of mechanical transmission, this finding clearly points to contaminated mouthparts, whereas the abdomen is likely positive because

of the viremic blood meal. Issimov et al. showed that LSDV can be recovered from another putative vector - *S.calcitrans* mouthparts post-feeding on a viremic animal [17]. Since trapping was performed near the affected animals, these positive horse flies might have picked up the virus from viremic animals. Of note, mouthparts contamination in dipteran insects can also be due to regurgitation during blood meals [29]. Moreover, the large size of *Tabanidae* flies points that their flight patterns can cover a bigger distance, further increasing the chances that they can travel and transmit LSDV from one herd of animals to another within 1-2 km [31]. Since the identity of insect-derived LSDV sequences aligned with those previously reported for the regions of study [3,32], it can be concluded that the positive insects were carrying, possibly spreading, the virus actively circulating in the region, taking into account that since 2017 no two same recombinant strains have been detected within one region [33]. On the other hand, segregation of animals effectively prevents the mechanical transmission of pathogens by tabanids [34]. Having said that, caution is recommended when interpreting entomological findings from the field and laboratory.

Considering the availability of classical LSD strains for sharing within research communities, more and more works are being devoted to the entomological aspects of LSD virus transmission. The available literature shows that the majority of experiments are conducted under laboratory conditions that tap into the understanding of what actually occurs under field conditions with a variety of environmental factors that may escape the researcher's eye. However, different lineages of LSDV are now on the rise and it is them that should be evaluated as a priority. This is not only about entomological aspects but also alternative modes of transmission [21,35].

Overall, in this work we provide findings on the detection of LSDV DNA in field-collected *Tabanidae* flies, *S.calcitrans* and *M.domestica* flies. When tested in pools, *Stomoxys flies* and *M.domestica* showed PCR positive results, whereas *Tabanidae* flies tested positive in the heads and abdomen. These field findings should further guide the search for putative vectors of LSDV in different climatic regions.

Acknowledgments

This work was supported by the grant no. 075-15-2021-1054 from the Ministry of Education and Science of Russia to implement objectives of the Federal Scientific and Technical Program for the Development of genetic technologies during 2019–2027.

The authors express thanks and appreciation to A. Van Schalkwyk for comments on the manuscript.

Authors' contributions

AN carried out sample preparation for PCR analysis, participated in drafting the manuscript. IS and PP did the trapping of insects at outbreaks sites. NV and OB participated in discussion and interpretation of published evidence, drafted the

manuscript. AS designed the study, coordinated its execution, drafted and reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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DOKAZ PRISUSTVA DNK VIRUSA NODULARNOG DERMATITISA KOD MUVA KOJE SE HRANE KRVLJU TOKOM EPIDEMIJE U RUSIJI 2018-2019

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U ovoj studiji izveštavamo o rezultatima testiranja muva koje se hrane krvlju i sinantropskih muva uhvaćenih u blizini životinja zaraženih virusom nodularnog dermatitisa (LSDV) u Rusiji tokom izbijanja epidemije u Kurganskoj oblasti 2018. i Saratovskoj oblasti 2019. Ispitivali smo štalske muve (*Stomoxys calcitrans)*, *Tabanidae* (konjske mušice), *Culicoides* mušice i kućne muve (*Musca domestica*) kao jedinke ili zbirne uzorke. Dobijeni nalazi pokazuju da je virusna DNK pronađena u zbirnim uzorcima *S.calcitrans* i *M. domestica*, kao i u glavi i abdomenu štalskih muva. Ovo je prvi izveštaj o detekciji LSDV DNK kod *Tabanidae* sa terena. Predviđeno je da predstavljeni podaci pomognu u daljem vođenju traganja za navodnim vektorima LSDV u različitim klimatskim regionima i tumačenju laboratorijski kontrolisanih eksperimenata o vektorskom prenosu LSDV-a.