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ORIGINAL ARTICLE

Detection of vaccine-like strains of lumpy skin disease virus in outbreaks in Russia in 2017

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Abstract
Lumpy skin disease (LSD) has affected many regions of Russia since its first occurrence in 2015. The most devastating year for Russia was 2016, when the virus resurged following a modified stamping-out campaign, causing 313 outbreaks in 16 regions. To avoid unwanted adverse reactions following the use of live attenuated vaccines against LSD virus (LSDV), sheeppox-based vaccines were administered during vaccination campaigns. As a result, LSD was successfully contained in all Russian regions in 2017. In the same year, however, LSD emerged anew in a few regions of the Privolzhsky Federal District of Russia along the northern border of Kazakhstan, which then necessitated vaccinating cattle with a live attenuated LSDV vaccine. Although live attenuated LSDV vaccines are prohibited in Russia, several vaccine-like LSDV strains were identified in the 2017 outbreaks, including commercial farms and backyard animals exhibiting clinical signs consistent with those of field LSDV strains. Sequence alignments of three vaccine-like LSDV strains showed clear similarity to the corresponding RPO30 and GPCR gene sequences of commercial attenuated viruses. How vaccine-like strains spread into Russian cattle remains to be clarified.

Introduction

Lumpy skin disease (LSD) has been recognized as a transboundary OIE-listed disease inflicting huge economic losses due to trade restrictions and carcass downgrading [1, 2] and is accompanied by significant morbidity but low mortality [3]. LSD is caused by a virus of the genus *Capripoxvirus*, family *Poxviridae*, with a viral genome consisting of a double-stranded DNA of around 150 kbp [4].

Susceptible animals include cattle and buffalos, which, when infected, display pyrexia, generalized pox lesions of the skin and internal organs, and generalized lymphadenopathy [5–7]. Lactating cows show a reduction in milk yield, pregnant cows may abort and remain anoestrus for several months, and bulls may become permanently or temporarily infertile. The disease tends to be more severe in calves and cows during the peak of lactation [8]. As for the transmission mechanism, live attenuated vaccine against LSD virus (LSDV) is believed to be mechanically transmitted through arthropod vectors [9, 10]. The common strategy for LSDV eradication and control has been vaccination, which can be employed through homologous LSD vaccines (the Neethling vaccine and KSGP O240) and heterologous vaccine preparations (based on sheeppox and/or goatpox viruses) [11, 12].

Historically, LSD had been endemic to Africa. However, due to yet unknown factors, its range had dramatically expanded, recently affecting countries in the Middle East, Azerbaijan, and the European Union [13–15]. Moreover, Russian territories had remained free of LSDV until 2015, with the first incursion of LSDV being reported in the Republic of Dagestan (the Caucasus) in 2015. A total of 18 outbreaks have been documented and contained with the aid of a modified stamping-out policy [16]. Unfortunately, LSD re-emerged in 2016 and massively spread throughout the Caucasus and southern Russia, causing 313 outbreaks in 16 regions throughout Russia [17]. The regions affected in 2016 resorted to vaccination with sheeppox-based vaccines, which successfully contained the disease. The use of heterologous vaccines is the national strategy for LSD control in Russia, and the administration of live attenuated LSDV strains can be considered a legal offense, with detected cases suggesting illegal use or vaccine escape. Fortuitously, we

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have identified, for the first time, a vaccine-like LSDV strain (Neethling type) in Russian cattle that caused an LSD outbreak in 2017 [18]. Notably, the virus was detected in both the house fly *Musca domestica* and cows exhibiting nasal discharge and skin lesions.

The present study follows up on the epidemiological situation since 2016 and further examines samples obtained in 2017 containing vaccine-like LSDV strains in Privolzhsky Federal District, an area that is geospatially outside of the zone affected in 2016 and where live vaccines against LSDV had never been authorized or knowingly used.

Materials and methods

Study area

The 2017 LSD outbreaks were documented to have occurred in a geographic area different from those affected by the 2016 LSD outbreak in Russia. A total of 42 outbreaks were identified by the Russian Federation OIE in 2017, primarily in the Privolzhsky Federal District (Table 1). Not all outbreaks were analyzed by the Federal Center for Animal Health (FGBI ARRIAH) (Vladimir, Russia), with only 13 out of the 42 outbreaks being accessioned in 2017. Samples with an unknown status were examined by regional veterinary authorities without FGBI ARRIAH involvement during confirmation and genotyping (Table 1).

All animals infected with vaccine-like LSDV strains displayed clinical signs resembling LSD: generalized and coalescent skin lesions ranging from 0.9 to 1.5 mm in diameter (Fig. 1), fever of up to 40.5 °C, and depression.

Sampling and polymerase chain reaction

Whole blood, nasal swabs, and scabs were used as samples for testing. Blood samples were collected in EDTA-containing tubes, placed on ice, and transferred to FGBI ARRIAH within 24 h of collection. Samples were obtained from one to two backyard cows and approximately 10 commercial cattle. Total DNA was extracted using a QIAamp DNA Mini Kit (QIAGEN, Germany) following the manufacturer's recommendations. Polymerase chain reaction (PCR) was carried out using a real-time PCR assay directed against the 27-bp insertion in the EEV gene that is unique to field LSDV strains [19], a real-time PCR assay for the detection of vaccine strains and capripoxvirus DNA [20], and duplex real-time PCR assay targeting vaccine and field strains that has been described by Agianniotaki et al. [21]. The Agianniotaki et al. assays were run in monoplex format to avoid possible competitive amplification of abundantly present target sequences in favor of the other target sequences present in a disproportional ratio. The threshold value for positivity was <40 for all the assays used. Further information on primer and probe sequences is given in Table 2.

Virus propagation

Prior to sequencing, scabs were ground completely and added to sterile saline at a 1:10 ratio. Thereafter, suspensions were subjected to a few freeze/thaw cycles to release viral particles from cells and clarified by centrifugation at 1500 rpm. The virus-containing supernatant was incubated with antibiotics (penicillin and streptomycin at final concentrations of 2000 IU/mL and 2 mg/mL, respectively) at room temperature for 90 min and inoculated onto 80% confluent ovine testis cell monolayers. After a cytopathic effect was observed, positive samples were tested by PCR and sequenced. To ensure that no contamination occurred during handling of the samples, each sample was treated individually.

Sequencing

The original (scabs) and corresponding samples isolated in cell culture were sequenced and compared to verify the results. Apart from verifying the absence of coinfection, each positive sample was additionally sequenced at least 10 times. Viral DNA was extracted using a DNA Mini Kit with proteinase K following the manufacturer's recommendation (QIAGEN, Germany). Nucleotide sequences of RPO30 [22] and GPCR [23] were amplified as described. DNA sequencing was performed using an ABI Prism BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an Applied Biosystems Genetic Analyzer (Applied Biosystems, USA). Sequences were aligned using the ClustalW algorithm in BioEdit software with the default parameters. After completing the alignment, the sequences were manually truncated to obtain a common position. Phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replicates in the MEGA 6.06 program. Numbers at the branches show bootstrap support values for neighbor-joining analyses higher than 65.

All GPCR and RPO30 sequences determined in this study were submitted to the GenBank database, and their accession numbers are given in Figs. 2 and 3.

Results

Polymerase chain reaction

FGBI ARRIAH examined samples from 13 out of the 42 outbreaks reported in 2017. Four outbreaks, two in backyard cattle and two in commercial cattle, were caused by vaccine-like LSDV strains (Table 1, Fig. 1), whereas the other nine

Table 1 Characteristics of LSDV outbreaks in Russia in 2017

Oblast	Rayon	Genotype of LSDV[2]	Affected	Total
Saratovskaya	Dergachevskii	**Vaccine Recombinant**[3]	**1**	**12**
		Undetermined	1	3
		Undetermined	1	8
		Field	1	16
		Undetermined	1	3
		Undetermined	1	4
		Undetermined	1	2
		Field	1	5
	Aleksandrovo-Guyskii	Field	1	3
		Field	1	8
		Undetermined	8	407*
		Field	1	441*
	Fedorovskii	Undetermined	1	1
	Ozinskii	Field	1	100*
		Undetermined	3	165*
	Piterskii	Undetermined	1	4
		Undetermined	1	597*
	Pugachevskii	Undetermined	1	6
		Undetermined	1	18*
	Novouzenskii	Undetermined	1	9
		Undetermined	1	16
	Sovetskii	Undetermined	1	3
	Samoilovskii	Undetermined	1	8
	Krasnokutskii	Undetermined	2	11
Orenburgskaya	Sol-Iletskii	Undetermined	1	1256*
		Field	12	1338*
		Undetermined	1	136*
	Tashlinskii	Field	19	542*
		Undetermined	10	86*
	Novosergeevskii	Undetermined	1	558*
	Akbulakskii	Undetermined	2	419*
	Belyaevskii	Undetermined	1	198*
	Orenburgskii	Undetermined	1	160*
	Saraktashskii	**vaccine**	**19**	**1533***
Volgogradskaya	Kirovskii	Field	3	5
	Kalachaevskii	Undetermined	2	35*
	Kletskii	Field	11	70*
Samarskaya	Bolshechernigovskii	**Vaccine**	**1**	**9**
	Bezenchukskii	Undetermined	1	1505*
	Sergievskii	Undetermined	11	132*
Bashkortostan rep.	Ermekeevskii	**Vaccine**	**77**	**579***
Ulyanovskaya	Melekesskii	Undetermined	5	7

*An asterisk denotes a commercial farm

[1]Genotyping was performed using real-time PCR assays (see Materials and methods)

[2]Outbreaks caused by vaccine-like strains are in bold

[3]Reference [25]

outbreaks were attributed to field LSDV strains. Among the backyard cattle, 1 of 12 and 1 of 9 cows were affected in Saratovskaya and Samarskaya oblasts, respectively (Table 1). Among commercial farms in Bashkortostan and Orenburgskaya oblasts, 77 of 579 and 19 of 1533 cows were affected, respectively (Table 1).

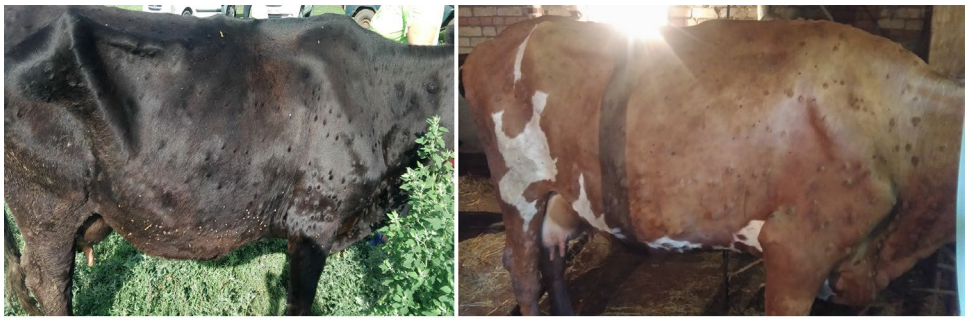

Fig. 1 Clinical signs caused by a vaccine-like LSDV strain and a recombinant vaccine strain in backyard cows (A, Samara/2017, B, Saratov/2017)

Table 2 Primers and probes used in this study

Assay	Sequence	Primer/probe name	Gene	Length, bp	Reference
Sprygin vaccine assay	(5′-TGTTTCCATTCTCCACTGCT-3′)	fnee3	LSDV008	185	[16]
	(5′-TACTTACTAAAAAATGGGCGCA-3′)	rnee3			
	(5′-6-FAM-TCGCTGACATCGTTAGTCCACTC-BHQ1-3′)	Nee_probe			
Sprygin capripoxvirus assay	(5′-ATGAAACCAATGGATGGGATA-3′)	Capr_f	P32	92	[18]
	(5′-CGAAATGAAAAACGGTATATGGA-3′)	Capr_r			
	(5′-6-FAM-ATGAGCCATCCATTTTCCAA- BHQ1-3′)	Capr probe			
Pestova field assay	(5′-AGAAAATGGATGTACCACAAATACAG-3′)	f2	EEV	96	[17]
	(5′-TTGTTACAACTCAAATCGTTAGGTG-3′)	r33			
	(5′-6-FAM-ACCACCTAATGATAGTGTTTATGATTTACC- BHQ1-3′)	lsdv probe			
Agianniotaki field assay	(5′-AGTACAGTTAGTAGCGCAACCATGTATAATAG-3′)	LSwildup	GPCR	134	[19]
	(5′-GTATTTTCATAAGTTGAAGGCGTTGTAACA-3′)	LSdo			
	(5′-6-FAM-TTGAAATTG+T+ACT+AA+T+AATTGT+AG-IBFQ-3′)	LSwildPr			
Agianniotaki vaccine assay	(5′-ACAGTTAGTAGCGCCACCATGTATAATAG-3′)	LSvacup	GPCR	134	[19]
	(5′-GTATTTTCATAAGTTGAAGGCGTTGTAACA-3′)	LSdo			
	(5′-HEX-TG+TACTGAGAATT+GTG+CT-IBFQ-3′).	LSvacPr			

bp, base pairs

The results obtained by testing samples for vaccine and field LSDV DNA using different real-time PCR assays are given in Tables 3 and 4. Three vaccine samples (Samara/2017, Orenburg/2017, and Bashkortastan/2017) and all field LSDV isolates showed 100% agreement among the assays. No mixed infections were detected in either animal or culture samples using real-time PCR assays. Samples from the remaining 30 outbreaks investigated by regional veterinary labs were not available for genotyping.

Sequencing

All virus-containing samples containing vaccine and field LSDV strains were readily cultivable and generated RPO30 and GPCR sequences for phylogenetic analysis that were 100% identical to those obtained from clinical samples, confirming the identity of the recovered virus isolates. The RPO30 and GPCR targets were sequenced twice to ensure accuracy. Phylogenetic analysis based on the RPO30 gene sequence showed that the three vaccine-like isolates clustered together and were 100% identical to commercial vaccine strains, such as Lumpyvax (Fig. 2). GPCR sequencing results showed that all three of these vaccine-like strains (Samara/2017, Orenburg/2017, and Bashkortastan/2017) displayed a strong homology to the commercial vaccine strains (Fig. 3). Meanwhile, sequencing of the RPO30 and GPCR genes of field LSDV strains showed that they were all identical and were similar to the previously sequenced Dagestan/2015 strain of LSDV (Figs. 2 and 3) [24].

An amino acid (aa) sequence comparison revealed that the predicted proteins encoded by the RPO30 and GPCR genes of the field strains LSDV/Saratov_field/2017, LSDV/Orenburg_field/2017, and LSDV/Volgograd_field/2017 were identical to those of LSDV/Dagestan/2015. All vaccine-like

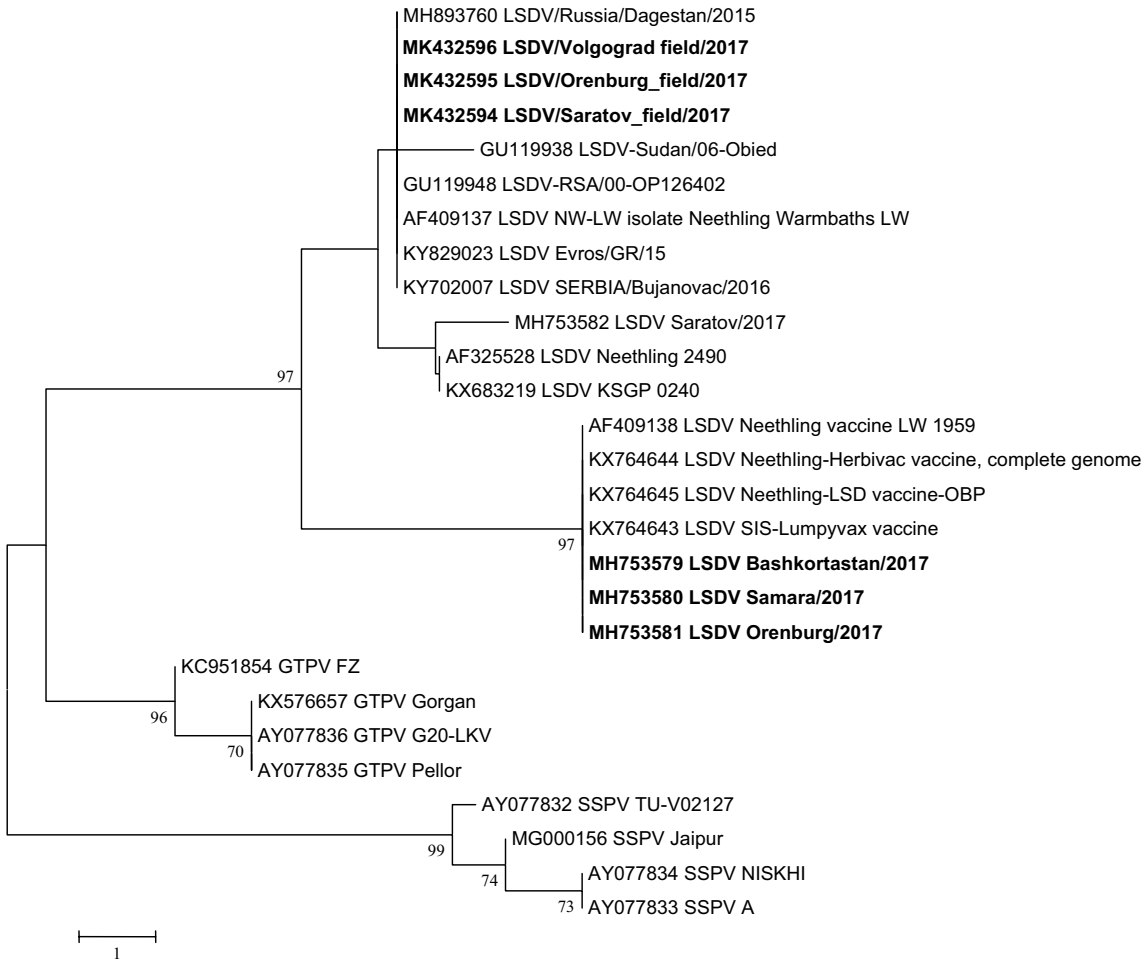

Fig. 2 Neighbour-joining tree showing phylogenetic relationships based on the RPO30 region for LSDV strains detected in 2017. The sequences obtained in this study are highlighted in bold

strains were identical to Neethling 1959 based on both gene sequences (Figs. 4 and 5).

Clinical signs

Clinical signs in cows from Samarskaya and Saratovskaya oblasts infected with vaccine-like strains were moderate and severe, respectively (Fig. 1). Figure 4 clearly shows that no reliable differentiation was possible based on comparison of clinical signs in these animals.

Discussion

The present work is the first to report on the widespread presence of vaccine-like LSDV strains in Russian cattle despite prohibitions against the use of live LSDV vaccines. The first case of vaccine strain detection in Russia was reported in 2017 by Sprygin et al. [18] in the Bashkortostan Republic, a region sharing a border with Kazakhstan. Moreover, it was detected not only in affected cattle but also in abundantly present, promiscuous-landing house flies (*Musca domestica*) collected at the outbreak site. Non-blood-sucking insects are generally not considered important for LSDV transmission because skin barrier penetration is believed to be necessary for infection. Notably, LSD outbreaks in both 2015 and 2016 were exclusively attributed to field LSDV strains (data not published).

In 2016, a total of 313 outbreaks had occurred in 16 regions mainly within the southern and central federal districts. Following a vaccination campaign in which sheeppox vaccine "SheepPox-LSD vac" (FGBI ARRIAH, Russia) was administered at a dose of 10^4 $TCID_{50}$, none of the regions affected in 2016 [17] reported reemergence of LSD in 2017, except for Volgogradskaya oblast, where the vaccination campaign was conducted using a sheeppox-based vaccine by a different manufacturer. Notably, our findings showed that the documented LSDV field strain outbreaks

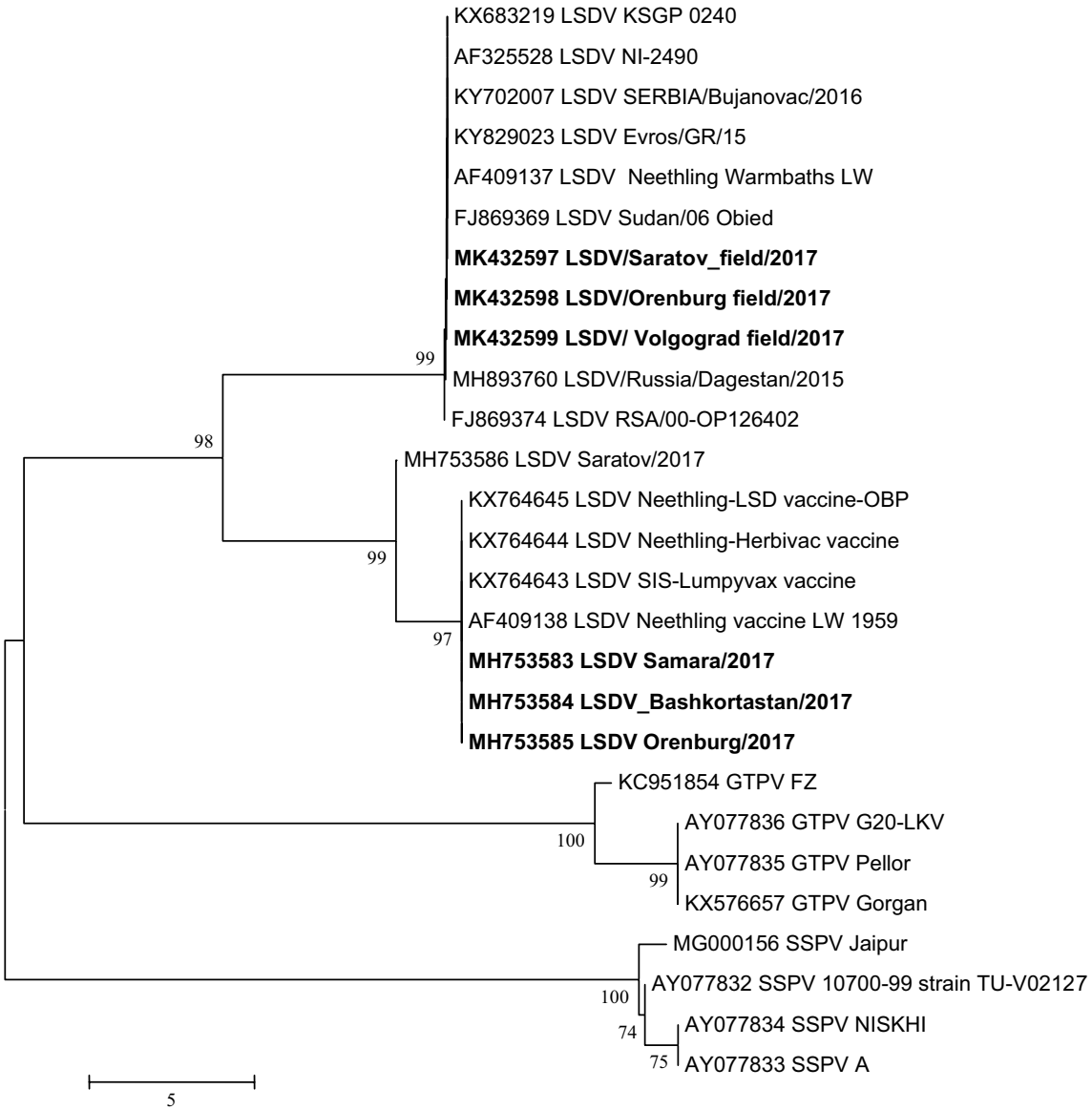

Fig. 3 Neighbour-joining tree showing phylogenetic relationships based on the GPCR region for LSDV strains detected in 2017. The sequences obtained in this study are highlighted in bold

Table 3 Results of real-time PCR assays targeting field and vaccine strain DNA of LSDV

LSDV isolate	Capripoxvirus assay[4]	Vaccine LSDV[2]	Anniotaki vaccine assay[3]	Anniotaki field LSDV[3]	Field LSDV1
Saratov/2017	+	-	+	-	-
Samara/2017	+	+	+	-	-
Orenburg/2017	+	+	+	-	-
Bashkortastan/2017	+	+	+	-	-
All other field LSDV strains detected in 2017	+	-	-	+	+

[1]Pestova et al., 2018 [17]

[2]Sprygin et al., 2018 [16]

[3]Agianniotaki et al., 2017 [19]

[4]Sprygin et al., 2017 [18]

Table 4 Ct values of samples from cows infected with vaccine-like strains

LSDV strain	Nasal discharge			Blood			Scab		
	Sprygin capripoxvirus assay	Sprygin vaccine assay	Agianniotaki vaccine assay	Sprygin capripoxvirus assay	Sprygin vaccine assay	Agianniotaki vaccine assay	Sprygin capripoxvirus assay	Sprygin vaccine assay	Agianniotaki vaccine assay
Orenburg	34.1, 33.5,	35.67	35.18	24.03	24.78	25.17	20.4	19.90	21.19
		34.05	34.81	-	-	-	17.7	17.08	17.66
Samara	31.14	30.25	31.11	25.89	25.05	24.71	21.56	21.64	21.73
Bashkotostan	NI	NI	NI	28.88	27.53	27.01	23.3	23.21	23.44
Saratov	NI	NI	NI	27.10	-	28.09	19.40	-	19.09

NI, not investigated

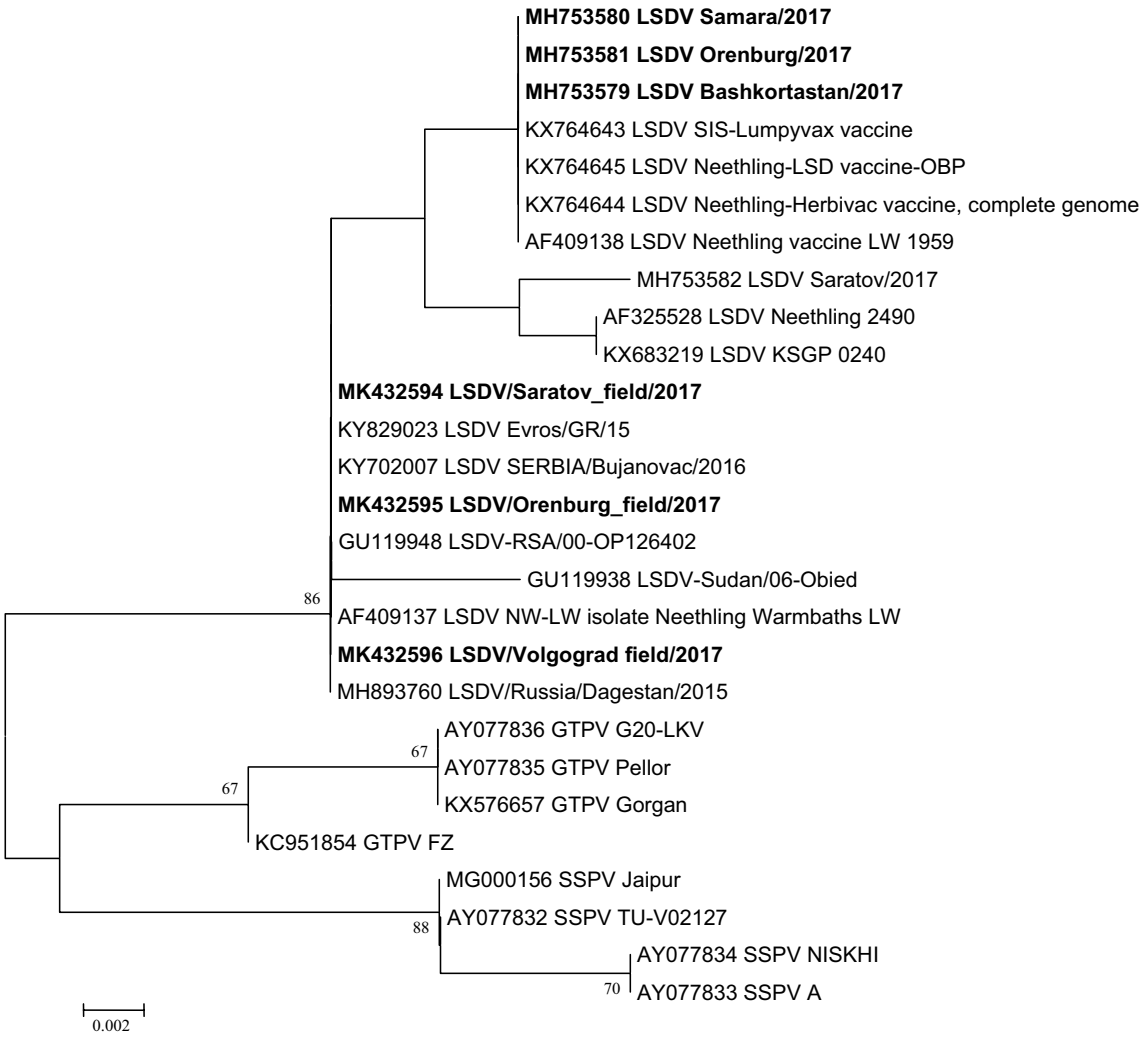

Fig. 4 Neighbour-joining tree showing phylogenetic relationships based on amino acid sequences of the RPO30 region for LSDV strains detected in 2017. The sequences obtained in this study are highlighted in bold

in Volgogradskaya oblast occurred within the same areas as in 2016. This failure might have been due to animals being vaccinated for the first time in summer, when the average daily temperatures reached up to 40 °C. Therefore,

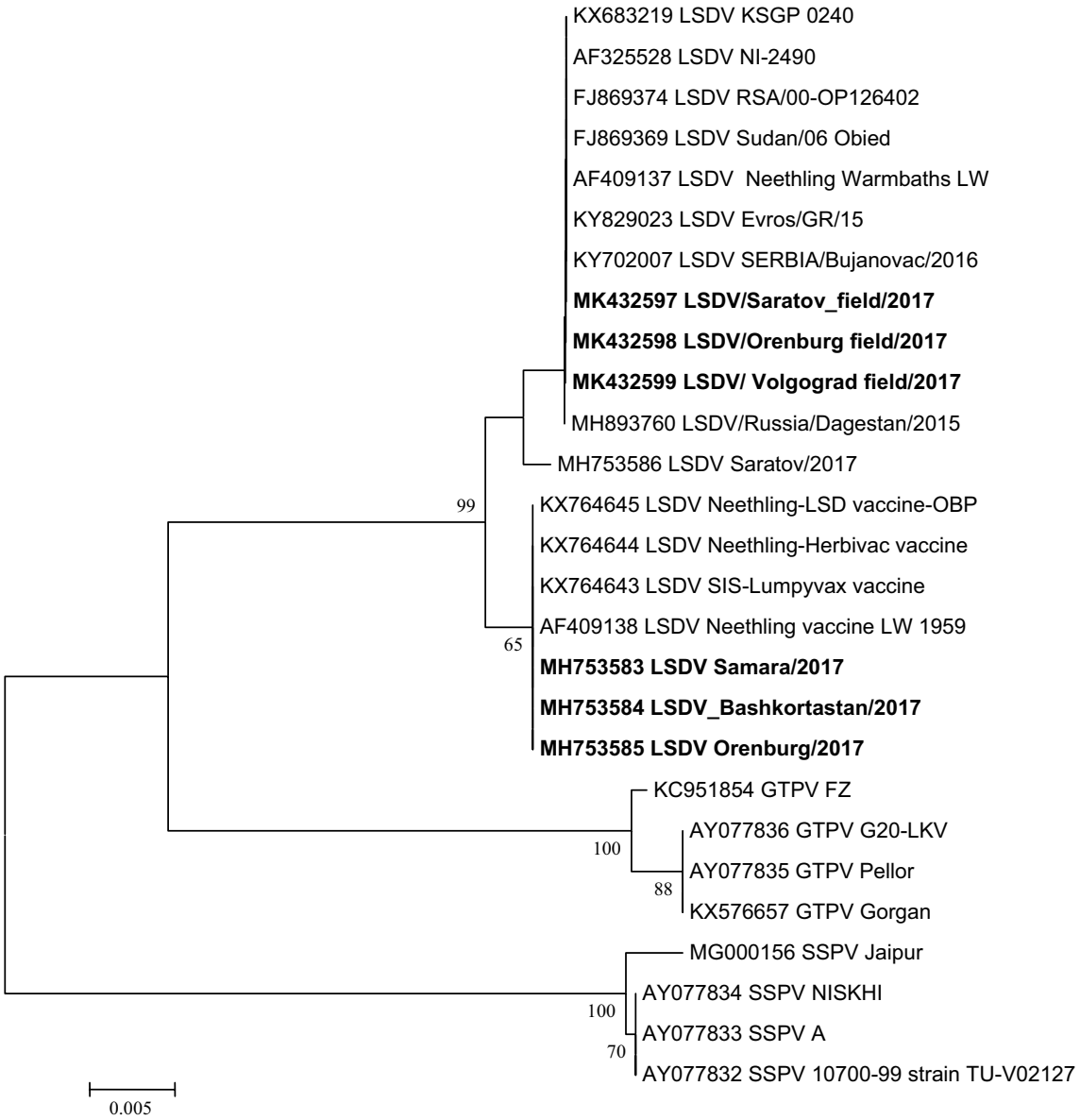

Fig. 5 Neighbour-joining tree showing phylogenetic relationships based on amino acid sequences of the GPCR region for LSDV strains detected in 2017. The sequences obtained in this study are highlighted in bold

the virus might have already been circulating, possibly in vectors, when vaccination was first initiated. Orenburgskaya and Saratovskaya oblasts, which had opted out of vaccination, were among those most affected by LSDV (Table 1). Although the Bashkortostan Republic did not resort to vaccination, the single outbreak documented there was surprisingly found to be caused by a vaccine-like LSDV strain [18]. Data on Chelyabinskaya oblast, where one outbreak was reported, were not available. However, considering the current epidemiological situation, a vaccine-like strain was likely to be involved in that outbreak.

The 2017 outbreak of LSDV in Russia differed dramatically from that of 2016 in terms of geographical distribution and frequency of outbreak reports [17, 18]. Unfortunately, a large proportion of samples could not be genotyped because testing was performed by regional veterinary laboratories that rely on domestic commercial assays with unknown performance characteristics. Such gaps in the data strongly impede our understanding on how both strains were introduced and spread throughout the Russian Federation. Although an association between outbreaks and the main roads along which LSDV might have traveled have been suggested [18], this may not necessarily be the case, considering that non-blood-sucking flies, the role of which warrants further study, may accompany affected animals and tag along with them on trucks. Interestingly, two of the four outbreaks

caused by vaccine-like strains occurred in backyard cows, with the other two occurring on commercial farms (Table 1), affecting only one backyard cow and low teens of commercial cows, thus limiting the extent of sampling. Given that samples from only one affected cow each were available from Saratovskaya and Samarskaya oblasts, we were unable to confirm the presence of the same isolate in apparently healthy cows that were in contact with the diseased animals. Commercial farms in Bashkortostan and Orenburgskaya oblast submitted a few samples from cows with severe clinical signs, the test results of which are listed in Tables 2 and 3. Interestingly, a mix of circulating recombinant and field strains was detected in Saratovskaya oblast, whereas vaccine and field strains were identified in Orenburgskaya. In other regions that had samples for genotyping, a single genotype was found (Table 1).

Live attenuated vaccines against LSDV have been known to induce symptoms indistinguishable from those of field strains [25]. Our study showed that the identity of the virus could not be distinguished based on clinical signs in either backyard (Fig. 1) or commercial cattle. The farmers reported coalescent cutaneous lesions and crusts measuring 1.5–2.5 cm in diameter, accompanied by fever and decreased milk production.

Importantly, no mixed infections or evidence of illegal use of commercial live attenuated vaccines against LSDV were found using real-time PCR assays as described previously. Moreover, regional veterinary officials that supervise vaccinations in backyard and commercial settings reported nothing suspicious regarding the use of live attenuated vaccines.

The "vaccine" outbreaks in the present study seemed to be epidemiologically unrelated and occurred more than 300 km apart from each other and 100 to 300 km from the Kazakhstan border, where live LSDV vaccine strains are officially approved and used on a large scale. Another interesting observation is that the majority of the field LSDV outbreaks largely congregated along the border of neighboring Kazakhstan, which remains officially free of the disease.

Of particular concern is the recombinant LSDV Saratov/2017 strain, whose RPO30 and GPCR gene fragments had been previously shown to belong to different phylogenetic clusters (Figs. 2 and 3) [26]. The GPCR gene of this strain grouped with the cluster of vaccine strains (Fig. 3), which explains why the Agianniotaki vaccine assay showed positive results (Table 2). However, the RPO30 gene fragment segregated it into the sub-branch that includes Neethling 2490 and KSGP 0240 (Figs. 2 and 3). Of note, the strains Neethling 2490 and KSGP 0240 have never been known to be used in Russia or adjacent countries.

The amino acid phylogenetic tree was in congruence with the results of genetic clustering (Figs. 4 and 5). Moreover, the Sprygin et al. assay, which targeted unique genetic signatures exclusive to the commercial live attenuated LSDV strains used in current vaccine products, corroborated our finding that these strains were vaccine-like [18].

Despite independently running two assays each for the vaccine and field strain genomes, we were unable to detect coinfection in any sample (Table 2) or obtain evidence pertaining to the illegal use and transfer of live attenuated LSDV vaccines in the investigated outbreaks.

Another important issue that urgently needs to be addressed is whether live attenuated LSDV strains are amenable to vector transmission similar to LSDV field strains [27, 28]. An extensive search of the available literature did not identify any studies regarding the vector-borne potential of LSDV vaccine strains. By contrast, due to the massive use of live attenuated LSDV vaccines, it is tempting to suggest an escape of the LSDV vaccine strain from the vaccination zone via a yet unstudied route and spillover into "sentinel" Russian cattle, in which, after natural coinfection of a host, the recombinant vaccine-like LSDV strain emerged [26]. Therefore, caution should be taken in the interpretation of the current findings considering the lack of published literature ruling out transmission of Neethling-based vaccines.

Accordingly, a different group of researchers, Kolcov and coauthors, also reported that vaccine strains dominated the 2017 outbreaks of LSDV in Saratovskaya oblast [29]. These findings are shedding more light on the extent to which vaccine strains may have been distributed throughout Russia while raising questions about the ability of live vaccine strains to escape from vaccinated herds and spread uncontrollably. Unfortunately, no published evidence has currently proven that attenuated vaccine strains against LSD cannot be transmitted in the same way virulent field strains are, which strongly impedes understanding of the epidemiological consequences of vaccine transmission. Importantly, the issue regarding live vaccine transmissibility has already been raised [30], and it has been questioned whether, despite the claimed benefits of live vaccines, live vaccine evolution can undermine vaccine utility and bring about recombinant progeny.

The vaccine-like strains identified in this study were identical to live vaccine strains at the genetic and amino acid level, whereas the field strains were 100% homologous to Dagestan/2015 (Figs. 2, 3, 4, 5). Considering the data reported by Kolcov et al., it is very unlikely that presumably illegal usage of a live strain took place in half of Saratov oblast. On the contrary, this epidemiological situation strongly suggests that the transmission capacity of live vaccines against LSD had been underestimated. The incursions and spread of vaccine-like strains into Russia could perhaps be attributed the heavy use of live vaccines in neighboring countries without proper DIVA tools. Although the possible mechanisms of transmission may involve insects, it is still not known which insects

contribute to transmission. Another possible mechanism could be contact mode, which is currently receiving considerable attention due to yet unexplained patterns of LSDV spread in the northern hemisphere (WAHID, 2018).

Another important point to discuss is what precipitated the emergence of vaccine escape recombinant Saratov/2017, the virus carrying the backbone of a Neethling-like vaccine strain detected recently [26]. It is likely that a recombination event occurred due to coinfection of a host with an attenuated vaccine strain and a circulating field strain. However, the similarity between the RPO30 gene of the recombinant strain and that of KSGP O-240 or Neethling 2490 is even more intriguing considering that only Dagestan/2015-like field strains had been identified previously in Russia. However, since the dominant parental strain of Saratov/2017 is a Neethling-like vaccine virus, the findings reported in this work add weight to the view that attenuated strains can revert to virulence should coinfection occur. Nevertheless, more epidemiological studies are needed to determine the origin and source of KSGP O-240 or Neethling 2490-like fragments in the genome of the novel LSDV strain [26].

Overall, although the benefits of vaccination with Neethling vaccines, which have been reported to have high efficiency in controlling LSD [31], are likely to outweigh the very low risk of vaccine virus transmission, randomly produced LSDV strains generated by attenuation in cell culture and embryonated chicken eggs [32] are capable of evolving and generating new variants [26].

In conclusion, in the present study, we analyzed LSDV samples obtained from 13 outbreaks documented in different parts of Russia in 2017. We found that not only field LSDV strains but also vaccine-like LSDV strains have been present and circulating in Russian cattle, causing outbreaks. The incursion route, however, remains to be investigated. Given the prohibition of live attenuated vaccines against LSDV, the continuous detection of vaccine-like LSDV strains based on the GPCR gene is of great concern, considering the likelihood that the circulation of field and vaccine strains have driven the emergence of vaccine escape recombinants. Further experiments are needed to determine whether live vaccines against LSDV are truly devoid of the ability to be transmitted to susceptible contacts. This information will help us better understand the epidemiological features of LSDV.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study does not contain any subjects with human participants or animals performed by the authors.

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