Genomic characterization of a lumpy skin disease virus isolated in southeast China

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SUMMARY

Lumpy skin disease virus (LSDV) is of high economic importance and has spread rapidly to many European and Asian countries in recent years. LSDVs spread to China in 2019 and have caused severe outbreaks in multiple provinces. The LSDVs in China have not been well investigated. Here we isolated an LSDV (GD01/2020) in southeast China and investigated its features in replication, phylogenetics, and genomics. GD01/2020 caused a typical LSD outbreak and replicated well in MDBK cells as detected by a novel quantitative real-time PCR assay targeting the viral GPCR gene. GD01/2020 was similar in phylogenetics to the one circulating in Xinjiang, China in 2019, and distinct from the LSDVs identified in other countries. In genomics, GD01/2020 was a vaccine-recombinant similar to those identified in Russia. A total of 13 major putative recombination events between a vaccine strain and a field strain were identified in the genome of GD01/2020, which could affect the virulence and transmissibility of the virus. The results suggested that the LSD outbreaks in China caused by a virulent vaccine-recombinant LSDV from the same unknown exotic source, and virulent vaccine-recombinant LSDVs obtained transboundary transmissibility. This report shed novel insights into the diagnosis, transmission, and control of the disease.

Introduction

Lumpy skin disease (LSD) has been listed as a notifiable viral disease of cattle by the World Organization for Animal Health (OIE). It is a serious transboundary disease and causes significant economic losses from decreased milk production, abortions, infertility, and damaged hides. Farmers in developing countries whose livelihood rely on cattle bear the heaviest burden (Babiuk et al., 2008). The etiological agent of LSD is lumpy skin disease virus (LSDV), a member of the genus *Capripoxvirus* within the family *Poxviridae*. It is genetically similar to the other two *Capripoxvirus* species Goatpox virus (GTPV) and Sheeppox virus (SPPV). LSDV harbors a double-stranded DNA genome, which is about 151,000 bp in size, encoding approximately 156 proteins (Tulman et al., 2001). LSDV is mainly transmitted via arthropod vectors, such as flies, mosquitos, and ticks (Chihota, Rennie, Kitching, & Mellor, 2001; Lubinga et al., 2015; Sprygin, Pestova, Wallace, Tuppurainen, & Kononov, 2019). Other transmission pathways, such as direct or indirect contact between infected and susceptible animals, were also possible (Aleksandr et al., 2020; Carn & Kitching; Sprygin et al., 2019).

LSD was first described in Zambia in 1929, and spread slowly in Africa thereafter (Davies, 1982). In 1989, LSD spread to Israel and subsequently circulated in the Middle East (Rweyemamu et al., 2000). In 2013, LSD spread to Europe and subsequently circulated in 11 European countries, including Turkey, Greece, and Russia (Sevik & Dogan, 2017; Sprygin, Pestova, Prutnikov, & Kononov, 2018; Tasioudi et al., 2016). From 2019 on, outbreaks of LSD have been reported by several Asian countries, such as China, India, Bangladesh, and Nepal (Acharya & Subedi, 2020; Lu et al., 2020; Sudhakar et al., 2020).

Live attenuated vaccines (LAVs) were widely used for LSD control in various countries, such as Greece, Serbia, Croatia, Kazakhstan, and Armenia (Agianniotaki et al., 2017; Sprygin, Babin, et al., 2018; Tasioudi et al., 2016). These LAVs were developed by serially passing a Neethling-type field isolate in tissue culture and embryonated chicken eggs (Hunter & Wallace, 2001). Some LAV strains caused clinical symptoms in South Africa (van Schalkwyk et al., 2020). Moreover, two LAV recombinant stains, Saratov/Russia/2017 and Udmurtiya/Russia/2017, caused typical outbreaks of LSD in Russia (Sprygin, Babin, et al., 2018; Sprygin et al., 2020).

In July 2019, LSD outbreak was first identified in Xinjiang, northwest China, near to Kazakhstan and Russia. Despite great efforts to contain the disease, this disease spread to southeast China in 2020 and caused outbreaks in multiple provinces (Lu et al., 2020) (Appendix Figure 1). It remains unknown the genomic and other features of the LSDVs circulating in China. To address this issue, we isolated an LSDV in southeast China and investigated its features in replication, phylogenetics, and genomics.

Materials and Methods

Sample collection

A suspected LSD outbreak on a cattle herd with 70 animals was reported in June 2020 in southeast China. Skin nodules (*n*=23), skin wound swabs (*n*=19), Ocular swabs (*n*=17), nasal swabs (*n*=16), oral swabs (*n*=19) and rectal swabs (*n*=14) were collected from the affected cattle (*n*=6). All the samples were immediately immersed in 1 mL of RPMI medium 1640 (Gibco, USA). These samples were crushed with a sterile grinder, and then suspended as 10% homogenates with RPMI medium 1640 and 1% antibiotic solution (penicillin G-sodium 100 IU/mL and streptomycin sulphate 100 mg/mL), for DNA extraction and virus isolation.

Viral DNA detection

DNA were extracted by TIANamp Genomic DNA Kit (TIANGen, China) according to the manufacturer's instruction. The extracted DNA was detected by polymerase chain reaction (PCR) targeting the RPO30 and GPCR genes using the primers as described previously ((Lamien et al., 2011; Le Goff et al., 2009). The positive amplicons were sequenced for phylogenetic analysis. To quantitate LSDV genomic copies, a quantitative real-time PCR (qPCR) assay was developed using SYBR Green I targeting the viral GPCR gene. The qPCR was performed in a volume of 20 µL containing 500 nM each primer, 1×TB green Premix DimerEraser (Takara, China), and 2 µL of DNA template. The amplification was conducted at 95℃ for 30 s, followed by 40 cycles of 95℃ for 5 s, 60℃ for 30 s, and 72℃ for 30 s. The sample was considered positive if the cycle threshold was less than 31.3. The PCR primers used in this study are showed in Appendix Table 1.

Virus isolation

Madin-Darby bovine kidney (MDBK) cells were used for virus isolation as described previously (Fay et al., 2020). Briefly, MDBK cells were cultured in 1640 medium supplemented with 10% fetal bovine serum (Gibco, USA). The sample homogenates described above were inoculated onto the confluent cell monolayer for 2 hours, then the cells were washed with phosphate-buffered saline (PBS) for three times, followed by addition of fresh growth medium and incubated at 37°C in 5% CO_2. Viral-induced cytopathic effects (CPE) were monitored for 10 days following the inoculation. The supernatants were also collected for transmission electron microscopy as described previously (Wang et al., 2019).

Virus replication kinetics

To evaluate the replication kinetics of the virus, MDBK cells were cultured in 24-well plates using a multiplicity of infection (MOI) of 1.0 and 0.2, respectively. The virus was harvested at every 24 hours till the 7 days post infection (dpi), and its titer was quantified using

the qPCR.

Genomic sequencing

Whole-genome sequencing was performed on the Illumina HiSeq PE150 platform. To assemble the genome, reads were mapped to the reference genome (Saratov/Russia/2017, MH646674) and the assembled whole genomic sequence was stored in GenBank (accession number: MW355944).

Phylogenetic analysis

Sequence alignment was conducted by the MAFFT software tool (Katoh & Standley, 2013). Phylogenetic relationships were calculated using the software package Mega X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018), with the maximum likelihood method and the substitution model which was of the lowest Bayesian-Information-Criterion scores (the Tamura 3-parameter model for the GPCR gene sequences, the Hasegawa-Kishino-Yano model for the RPO30 gene sequences, and the General Time Reversible model for the genomic sequences). Rates among sites were set in gamma distribution. Bootstrap values were calculated with 1000 replicates.

Recombination analysis

For the recombination analysis, LSDV genomic sequences were aligned and analyzed using the bootscan/rescan recombination test (Martin, Posada, Crandall, & Williamson, 2005), MAXCHI (Smith, 1992), GENECONV (Padidam, Sawyer, & Fauquet, 1999), CHIMAERA (Posada & Crandall, 2001), and the SISCAN (Gibbs, Armstrong, & Gibbs, 2000) methods within the RDP software package (v4.39) with default settings. The SimPlot software was used to further characterize the potential recombinant events (Lole et al., 1999).

Results

Field observation

We observed that 24 of 70 (34.3%) cattle presented typical clinical signs of LSD, including pyrexia, salivation, nasal and ocular discharges, and skin nodules (1–2 cm to 7–8 cm in diameter). The skin nodules involved the epidermis, dermis, subcutis, and musculature with the appearance of ulcerations and deep scabs (Figure 1). No death was reported in this outbreak.

Virus detection and isolation

All clinical samples were tested by the PCR and qPCR assays, then further inoculated into MDBK cells for virus isolation. Of these samples, 7.1–91.3% were positive for the qPCR detection and 0.0–100.0% were positive for the virus isolation (Appendix Table 2, Figure 2). The LSDV was detected from all the types of collected samples, with high concentration in skin nodules, skin wound swabs, and ocular swabs (Appendix Figure 2). This suggests that LSDV could be transmitted through cattle-to-cattle direct contact or close contact with the virus shed

from skin wounds or body excretion or feces, which has been observed recently in Russia (Aleksandr et al., 2020).

Figure 1. Clinical signs of an LSD outbreak in southeast China. (a, b) Skin nodules covering the entire body of infected cattle; (c, d) The necrotic wounds of skin subject to fly pestering.

LSDV was tough to be isolated until MDBK cells were found to be sensitive for the virus culture (Fay et al., 2020). In this study, we found that GD01/2020 replicated well in MDBK cells (Appendix Figure 3). This confirmed that MDBK cells could be used for the viral culture, disease diagnosis, and neutralizing antibodies quantification. Typical foci-type plaques were observed in infected MDBK cells after three times of blind passage. The foci-type plaques became visible at 2–4 dpi (Figure 2a, b), and were highlighted by crystal violet staining (Figure 2c). LSDV virions in the supernatant were confirmed by the qPCR and transmission electron microscopy (Figure 2d). The LSDV isolate was designated as LSDV/GD01/China/2020 (GD01/2020).

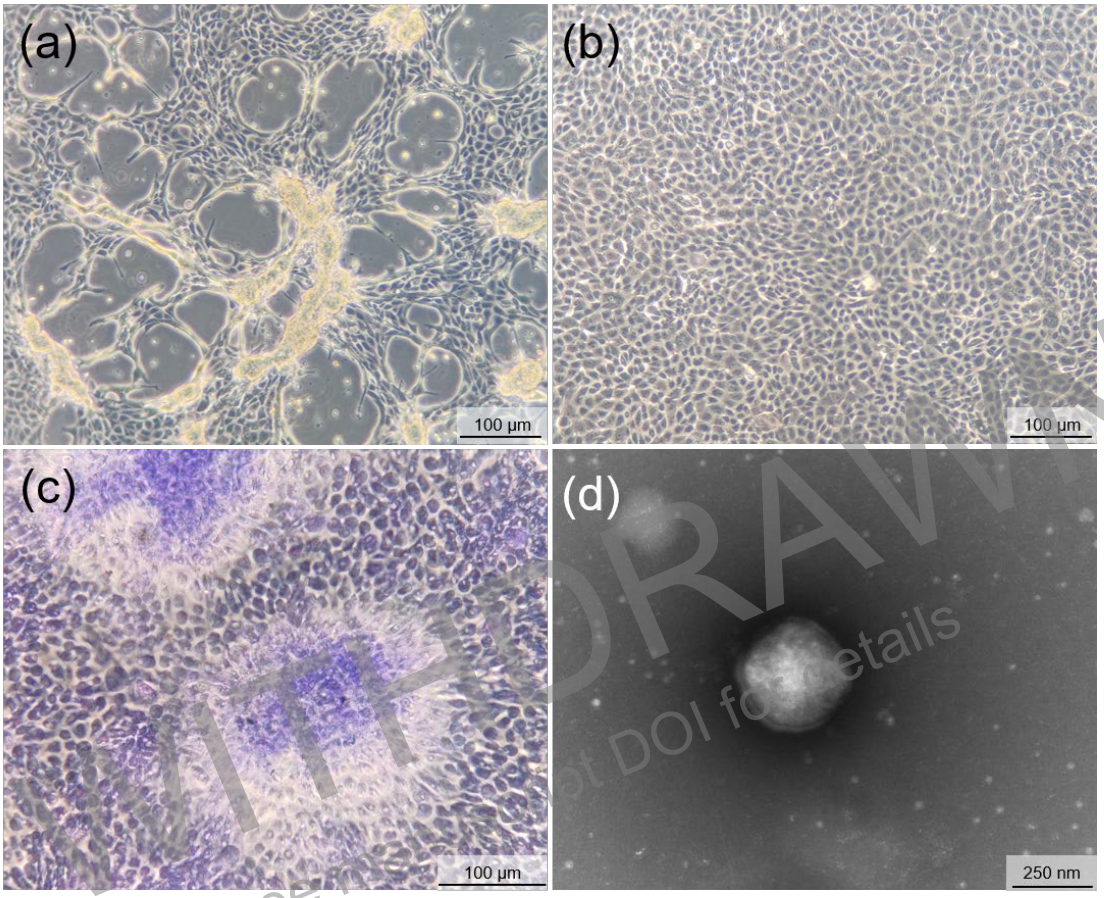

Figure 2. Isolation of LSDV in MDBK cells.

(a) The foci-type plaques in MDBK cells at 4 dpi with inoculated LSDV at a MOI of 1. (b) Uninfected MDBK cells. (c) The foci-type plaques in MDBK cells highlighted by crystal violet staining. (d) Negatively stained virions purified from LSDV-infected MDBK cells.

Phylogenetic analysis

We searched through the BLAST tool of NCBI, and found that GD01/2020 was the most similar to the LSDVs identified in Xinjiang, northwest China in 2019 in the RPO30 and GPCR gene sequences (similarity in the RPO30 gene sequences = 100.0%; similarity in the GPCR gene sequences = 99.9%). Phylogenetic analysis of these two genes showed that GD01/2020 and the LSDVs from Xinjiang, northwest China formed a distinct clade between the vaccine-associated virus group and the field virus group (**Figure 3**), and surprisingly, they were distinct from the LSDVs identified in Kazakhstan, India, Bangladesh, and Russia. This result suggests that GD01/2020 and the LSDVs from Xinjiang/2019, northwest China likely had the same exotic source, and the source remains unknown.

Figure 3 suggested that the nucleotides in the genomes of the LSDVs in the field group or the vaccine-associated group substituted slowly, and hence it should be likely for the LSDVs in

China to form a distinct clade through recombination rather than through nucleotide substitution. This is supported by **Appendix Table 3** which shows that the LSDVs in China shared the same nucleotides with the field virus group in a region of each of the genes and shared the same nucleotides with the vaccine-associated group in other regions of the genes, far from a random distribution, which constituted a marked signal of recombination. **Figure 3** also showed that, as per the sequences of GPCR and RPO30, the LSDVs in China were distinct from the two vaccine-recombinant stains, Saratov/Russia/2017 and Udmurtiya/Russia/2017, causing typical outbreaks of LSD in Russia (Sprygin, Babin, et al., 2018; Sprygin et al., 2020).

Genomic analysis

The genome of GD01/2020 was sequenced using the metagenomic method, which produced 2,957,618 reads assembled into a 150606-nt contig. Phylogenetic analysis based on all available complete genomic sequences of LSDVs showed that GD01/2020 clustered between the vaccine-associated virus group and the field virus group, and between the two recombinant viruses, Saratov/Russia/2017 and Udmurtiya/Russia/2017, reported in Russia (**Figure 3**). Pairwise comparisons showed that GD01/2020 had a higher similarity with these two Russian LSDV recombinant viruses (99.4%) than other viruses (99.2–99.3%). These results indicated that GD01/2020 was likely a recombinant LSDV strain.

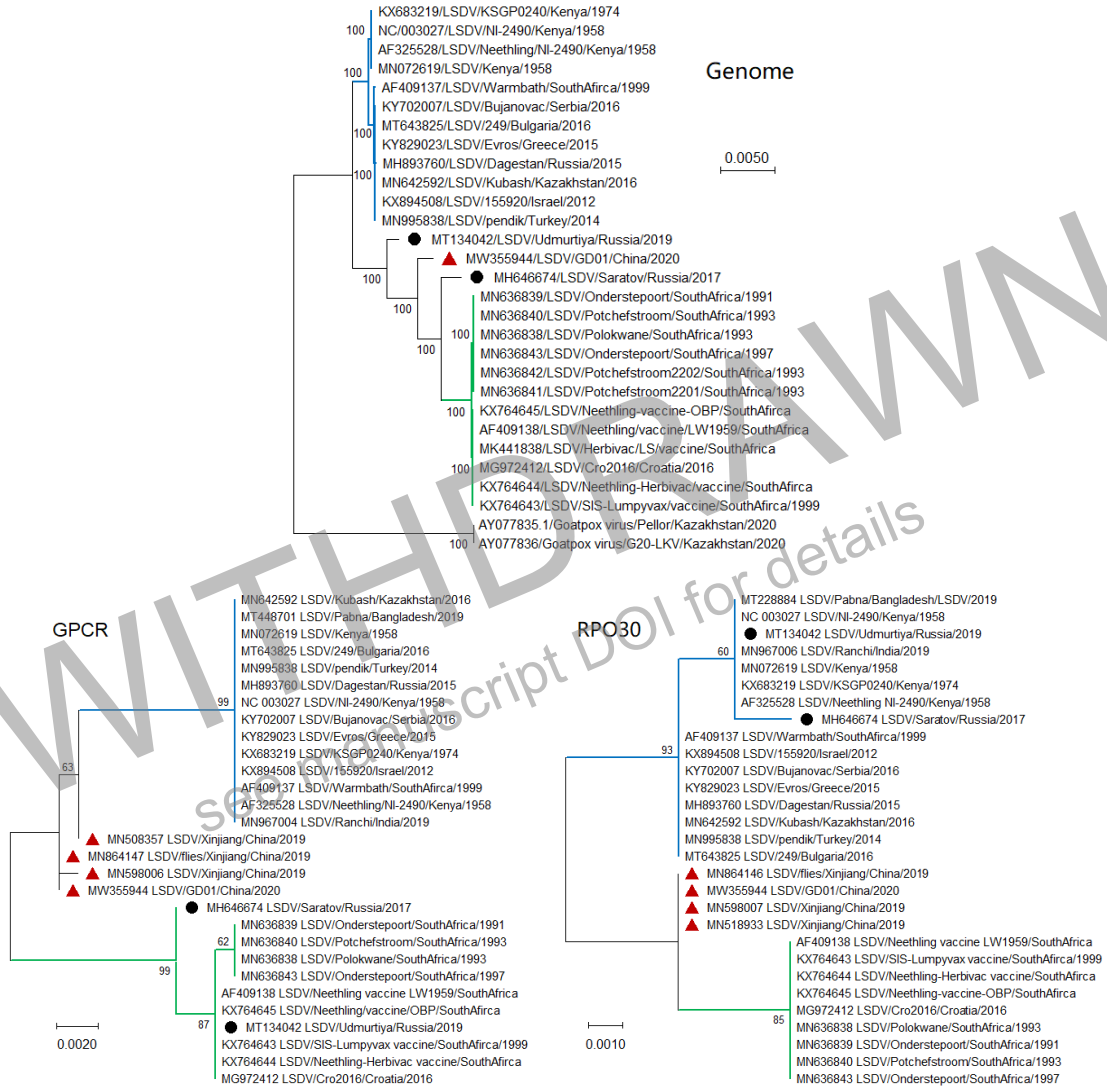

Figure 3. Phylogenetic relationships among some LSDVs based on their whole genomic sequences, GPCR gene sequences, or RPO30 gene sequences. The LSDVs from China are marked with triangles; the field group is marked with blue branches; the vaccine-associated group is marked with green branches. Two Russia isolates are marked with black circles. Scale bars indicate genetic distances.

Recombination analysis

Genomic sequences of 18 LSDVs were analyzed using the RDP program. The results further supported that GD01/2020 was a recombinant virus, with vaccine strains as the putative major parent donors and field strains as the putative minor parent donors (**Appendix Table 4**).

The similarity plot of the full-length genome of GD01/2020 was further analyzed using the LSDV reference sequences of the Neethling vaccine strain OBP (MG972412) and the LSDV field strain Kenya (MN072619). A total of 13 major putative recombination events involving dozens of proteins were found (**Figure 4**, **Appendix Table 5**). All of the putative recombination events had a vaccine virus genome as the major parental donor and a field virus as the minor parental donor. Moreover, the termination codons of five genes of GD01/2020, ORF086, ORF087, ORF131, ORF134, and ORF144, changed as compared with the Neethling vaccine strain OBP. The open reading frames (ORFs) of these genes were extended thereby (Appendix Figure 4).

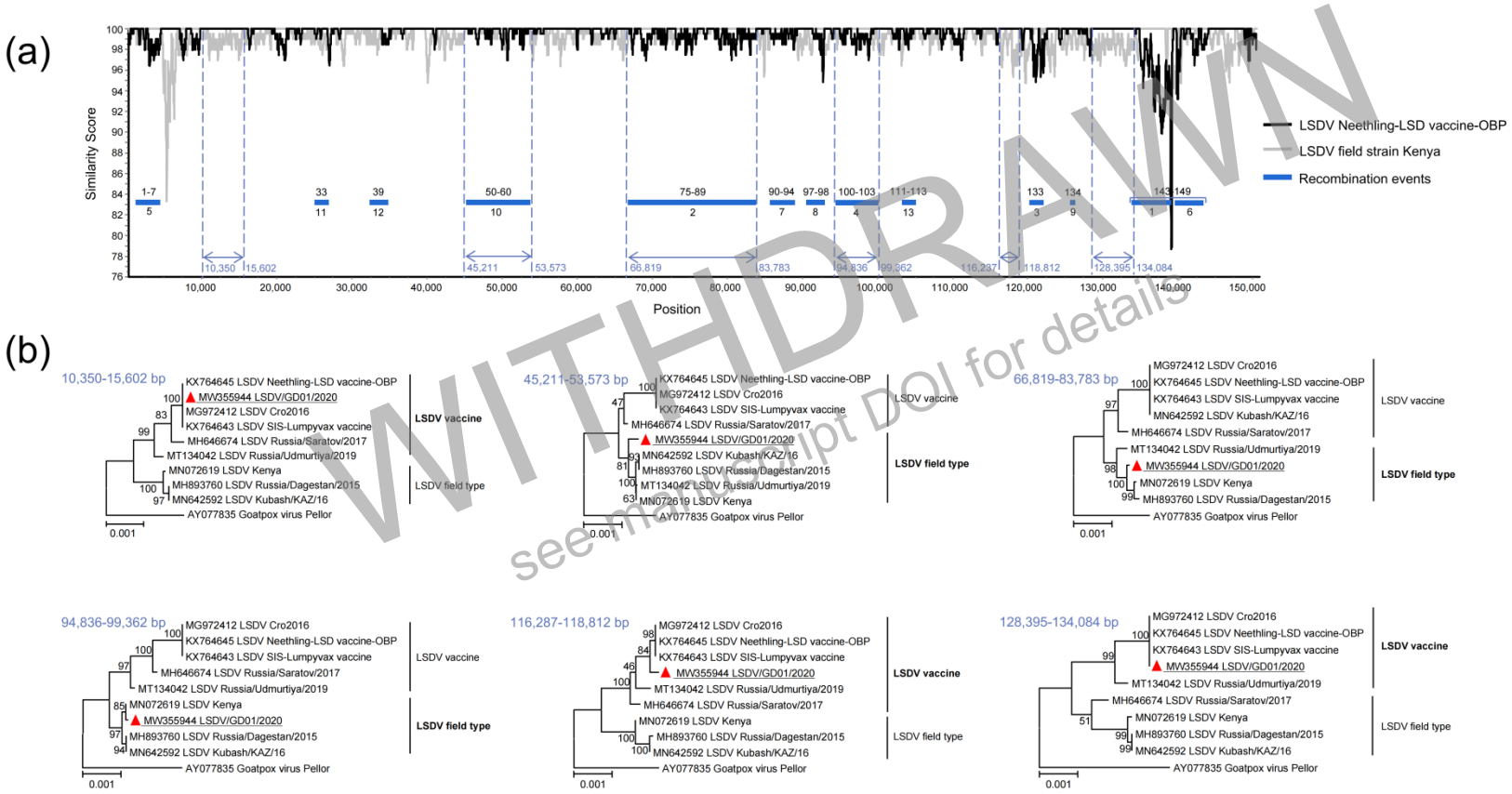

Figure 4. Recombination analysis of the genomes of LSDVs. (a) Similarity plot of the genomic sequence of GD01/2020 compared to the Neethling vaccine strain OBP (in black) and the LSDV field strain Kenya (in gray); the blue boxes show the positions of the predicted recombination events; the numbers on the boxes show the order of the viral proteins affected by the recombination events (e.g., 1–7 means the viral proteins ORF001–ORF007); the numbers below the boxes show the order of the recombination events. The sequences chosen for phylogenetic analysis are indicated by blue arrows; (b) Phylogenic relationships based on the sequences of the selected six regions; sequence positions are marked in blue and GD01/2020 is marked with a triangle.

Discussion

Our field observation in this study suggested that the affected cattle were of typical clinical signs of LSD, and thus the isolated LSDV (GD01/2020) was virulent. Phylogenetic analysis of two genes (GPCR and RPO30) suggested that GD01/2020 and the LSDV caused the LSD outbreaks in Xinjiang, China in 2019 were vaccine-recombinants and likely from the same unknown exotic source. Genomic sequence analysis suggested that GD01/2020 was a vaccine-recombinant similar to the two vaccine-recombinants identified in Russia in 2017 and 2019 (Sprygin, Babin, et al., 2018; Sprygin et al., 2020).

Live attenuated LSD vaccines have been in use in Africa for decades. They have also been used in multiple affected countries in the northern hemisphere, including Serbia, Croatia, Kazakhstan, and Armenia (Sprygin, Babin, et al., 2018). Because only heterologous live goatpox or sheeppox vaccines have been permitted for the control of LSD in Russia and China, and live LSD vaccines have not been permitted for use in Russia and China, these vaccine-recombinant LSDVs likely emerged in other countries where live LSD vaccines have been used. Therefore, virulent vaccine-recombinant LSDVs likely caused transboundary transmission to these two countries and had transboundary transmissibility thereby.

It has been found that vaccine-recombinant LSDVs increased the viral virulence as compared with a field LSDV (Kononova et al., 2020), and could be transmitted through direct or indirect contact (Aleksandr et al., 2020). These findings provided the explanation for the potential transboundary transmissibility of vaccine-recombinant LSDVs. They also indicated that more caution should be given in using live LSDV vaccines for control of the disease. This study suggested that virulent vaccine-recombinant LSDVs likely spread from northwest China to southeast China within one year, further supporting that virulent vaccine-recombinant LSDVs can spread rapidly.

Consistent with previous studies (Sprygin, Babin, et al., 2018; Sprygin et al., 2020), the genomic recombination of GD01/2020 involved dozens of proteins of the virus (Appendix Table 4). It is valuable to investigate in the future which proteins are important for the virulence and transmissibility of the virus.

In summary, we isolated a virulent vaccine-recombinant LSDV in southeast China, and

investigated the viral features in replication, phylogenetics, and genomics. The results shed novel insights into the diagnosis, transmission, and control of the disease, and suggested that vaccine-recombinant LSDVs likely caused transboundary transmission to Russia and China. More national and international efforts to investigate and contain virulent vaccine-recombinant LSDVs are desirable.

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Conflict of Interest Statement

The authors have declared no conflict of interest.

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Appendix

Appendix Table 1. The primers used for detection of LSDV in this study.

Target gene	Primer name	Position[a]	Sequence (5′-3′)
GPCR	GPCR-F	7070–7099	TTTTTTTATTTTTTATCCAATGCTAATACT
	GPCR-R	8225–8253	TTAAGTAAAGCATAACTCCAACAAAAATG
RPO30	RPO30-F	27699–27720	ATTCGTTTATCGCAGAACAAGG
	RPO30-R	28910–28935	CACCAACCATAGAATAGTATTGAGAC
GPCR[b]	qGPCR-F	7928–7950	AGTCGAATATAAAGTAATCAGTC
	qGPCR-R	8028–8052	CCGCATATAATACAACTTATTATAG

[a] Numbered according to the reference strain Saratov/Russia/2017 (MH646674).

[b] The pair of primers used in the qPCR.

Appendix Table 2. Detection of LSDV in different clinical samples.

Type of samples	Sample number	qPCR positive rate （%）	Isolation positive rate （%）
Nodules	23	91.3	100.0
Skin wound swabs	19	73.7	94.7
Oral swabs	19	36.8	0.0
Nasal swabs	16	18.8	31.3
Ocular swabs	17	29.4	0.0
Rectal swabs	14	7.1	0.0
Total	108	47.2	42.6

Appendix Table 3. Nucleotide mutations in two genes of the LSDVs in China compared the field virus group and the vaccine-associated group[a].

Gene	Position	LSDVs in China	Field virus group	Vaccine-associated group
GPCR	18	C	T	C
	87	C	T	C
	153	**A**	**A**	**G**
	159	**G**	**G**	**A**
	227	**G**	**G**	**A**
	228	**C**	**C**	**T**
	381	**G**	**G**	**A**
	394	**T**	**T**	**C**
	400	**C**	**C**	**T**
	492	**T**	**T**	**C**
	528	**C**	**C**	**T**
	555	**T**	**T**	**C**
	648	**C**	**C**	**T**
	803	**C**	**C**	**T**
	822	**T**	**T**	**C**
	849	**C**	**A**	**C**
	852	**G**	**A**	**G**
	983	**C**	**T**	**C**
	987	**A**	**G**	**A**
	991	**A**	**C**	**C**
	1050	**T**	**C**	**T**
	1116	**T**	**C**	**T**
RPO30	103	A	A	G
	210	**C**	**C**	**T**
	243	**T**	**C**	**T**
	420	**C**	**T**	**C**
	477	**T**	**C**	**C**
	582	**A**	**G**	**A**

[a] Those lines in blue show the positions where LSDVs in China shared the same nucleotides with the field virus group, and those lines in green show the positions where LSDVs in China shared the same nucleotides with the vaccine-associated group.

Appendix Table 4. Recombination events in the genome of GD01/2020 predicted as compared the genomic sequences of other 18 LSDVs.

Event	Positions	Size (bp)	The most likely minor parent contributing less nucleotides	The most likely major parent contributing more nucleotides	Affected proteins	P-value calculated using different methods in the RDP software package					
						RDP	GENECONV	Bootscan	Maxchi	Chimaera	SISscan
1	134908–139307	4399	MN072619/Kenya/1958	MN636839/Onderstepoort/SouthAfrica/1991	ORF143–ORF145	6.24E-47	3.79E-52	1.48E-52	5.83E-27	3.33E-26	4.20E-39
2	66880–83858	16978	MN072619/Kenya/1958	KX764645/Neethling-vaccine-OBP/SouthAfirca	ORF075–ORF089	7.54E-33	7.29E-33	4.98E-17	5.30E-13	5.27E-13	3.90E-11
3	120459–122549	2090	MN072619/Kenya/1958	KX764645/Neethling-vaccine-OBP/SouthAfirca	ORF133	1.22E-11	1.17E-08	1.35E-11	4.88E-09	4.85E-09	4.85E-04
4	94912–99362	4450	MN072619/Kenya/1958	KX764645/Neethling-vaccine-OBP/SouthAfirca	ORF100–ORF103	9.88E-13	4.12E-12	2.33E-12	1.06E-06	1.05E-06	NS
5	343–4411	4069	MN072619/Kenya/1958	KX764645/Neethling-vaccine-OBP/SouthAfirca	ORF001–ORF007	NS	2.85E-20	2.00E-14	9.50E-07	1.72E-06	NS
6	139929–144054	4125	MN072619/Kenya/1958	MN636839/Onderstepoort/SouthAfrica/1991	ORF146–ORF149	4.84E-13	1.18E-08	2.11E-05	1.64E-11	1.62E-11	NS
7	85731–89270	3539	KX683219/KSGP0240/Kenya/1974	AF409138/Neethling/vaccine/ORF1959/SouthAfrica	ORF090–ORF094	8.68E-07	1.59E-05	6.76E-05	1.29E-05	8.53E-06	NS
8	91420–92899	1479	MN072619/Kenya/1958	KX764645/Neethling-vaccine-OBP/SouthAfirca	ORF097–ORF098	NS[a]	9.68E-07	3.34E-08	3.38E-05	3.38E-05	NS
9	127999–128410	411	MH893760/Dagestan/Russia/2015	KX764644/Neethling-Herbivac/vaccine/SouthAfirca	ORF134	NS	1.30E-06	3.75E-03	NS	NS	2.92E-27
10	45262–53624	8362	KY702007/Bujanovac/Serbia/2016	AF409138/Neethling/vaccine/ORF1959/SouthAfrica	ORF050–ORF060	NS	1.78E-03	NS	1.95E-06	1.93E-06	4.54E-12
11	26759–27542	783	MN642592/Kubash/Kazakhstan/2016	KX764645/Neethling-vaccine-OBP/SouthAfirca	ORF033	NS	0.0004025	2.81E-04	NS	NS	NS
12	34010–35138	1128	MN072619/Kenya/1958	KX764645/Neethling-vaccine-OBP/SouthAfirca	ORF039	NS	1.37E-04	1.65E-05	NS	NS	NS
13	103506–104806	1300	MN072619/Kenya/1958	MK441838/Herbivac/LS/vaccine/SouthAfirca	ORF111–ORF113	NS	1.42E-04	1.56E-04	NS	NS	NS

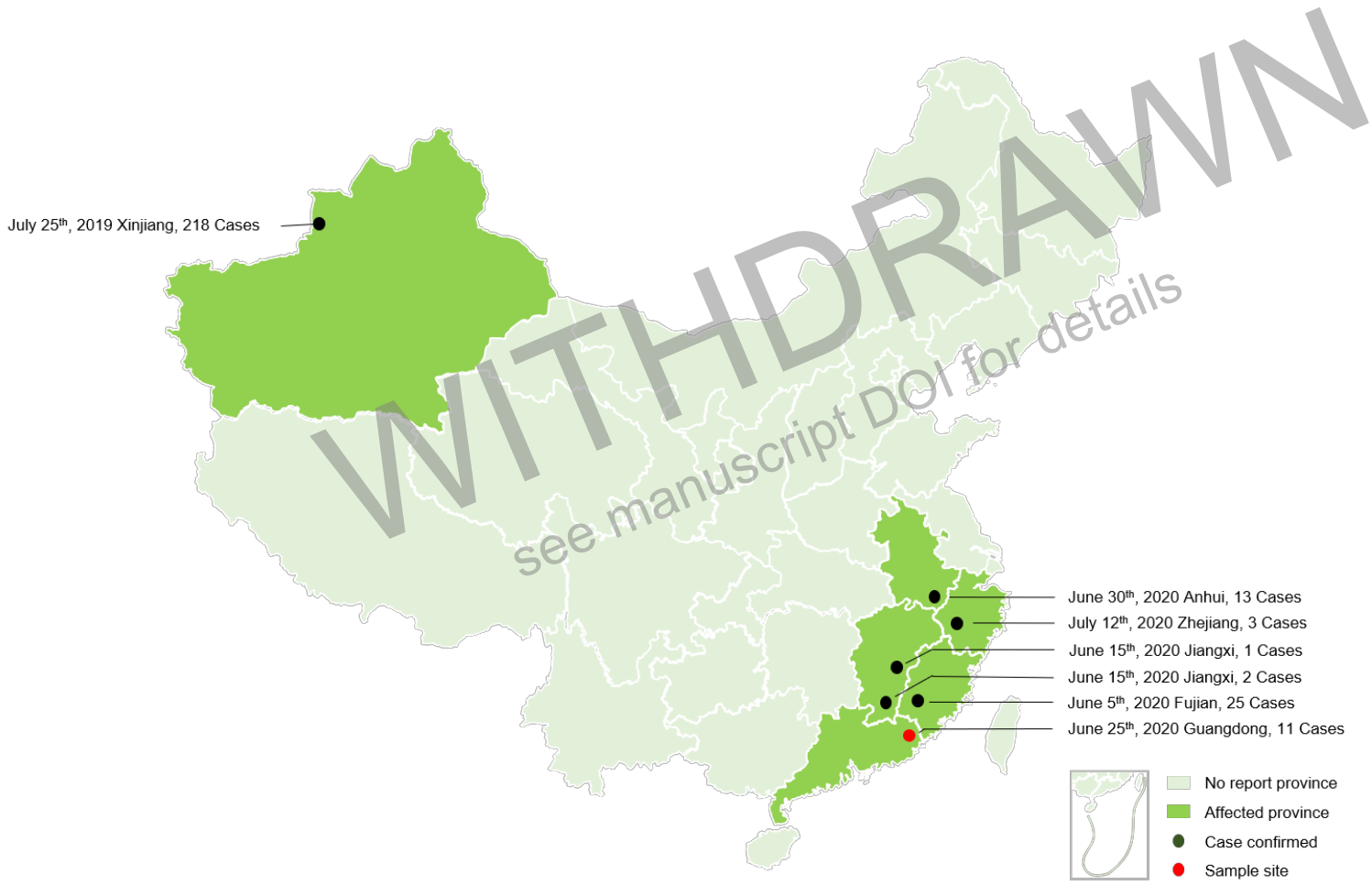
[a] These 18 strains included 6 strains predicted as the major parents donating more nucleotides to GD01/2020 (they were all in the vaccine-associated group):
KX764643/LSDV/SIS-Lumpyvax/vaccine/SouthAfirca/1999, MG972412/LSDV/Cro2016/Croatia/2016, KX764644/LSDV/Neethling-Herbivac/vaccine/SouthAfirca, AF409138/LSDV/Neethling/vaccine/ORF1959/SouthAfrica, KX764645/LSDV/Neethling-vaccine-OBP/SouthAfirca, MK441838/LSDV/Herbivac/LS/vaccine/SouthAfrica.
These 18 strains also include 12 strains predicted as the minor parents donating less nucleotides to GD01/2020 (they were all in the field virus group):
MN072619/LSDV/Kenya/1958, KX683219/LSDV/KSGP0240/Kenya/1974, NC/003027/LSDV/NI-2490/Kenya/1958, AF325528/LSDV/Neethling/NI-2490/Kenya/1958, AF409137/LSDV/Warmbath/SouthAfirca/1999, KY702007/LSDV/Bujanovac/Serbia/2016, MH893760/LSDV/Dagestan/Russia/2015, MT643825/LSDV/249/Bulgaria/2016, KY829023/LSDV/Evros/Greece/2015, MN642592/LSDV/Kubash/Kazakhstan/2016, MN995838/LSDV/pendik/Turkey/2014, KX894508/LSDV/155920/Israel/2012.

[b] NS, not statistically significant.

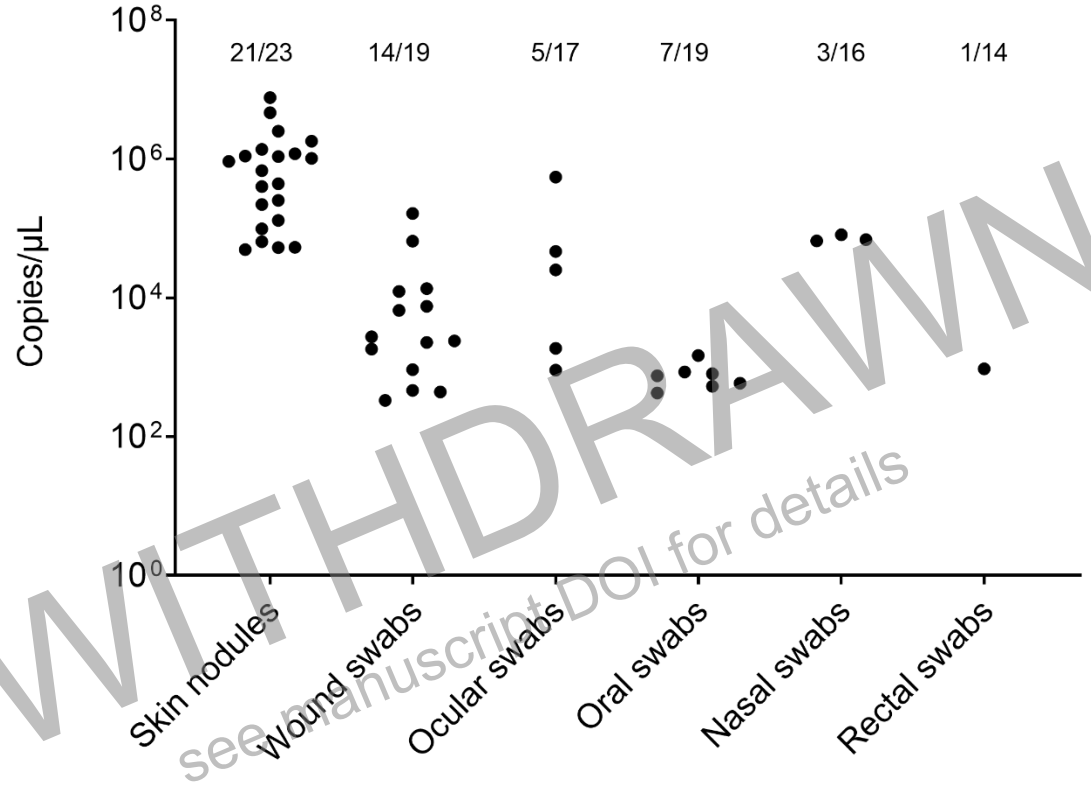
Appendix Table 5. The proteins of GD01/2020 affected by 13 major predicted genomic recombination events[a].

Event	Amino acid differences between the predicted major parent KX764645/Neethling-vaccine-OBP/SouthAfirca and the predicted minor parent of MN072619/Kenya/1958
1	ORF143 (hypothetical protein): T267S; ORF144 (kelch-like protein)**:** K51R, G78R, F119L, R139K, E195A, I218D, S234N, F252L, D257I, extension of the protein with 281 amino acid residues due to a change in the termination codon, 338Edel, Y374H, P384S, S422T, E455A, S518A, C538S, F548L ORF145 (ankyrin repeat protein): I8V, K22L, S121G, I139V, V144I, T181I, G196N, S202R, N205D, T210S, C216S, K241Q, M261V, V264I, L269I, N273S, S292N, S309N, V336I, I340V, H342N, D349E, Y353H, K378R, S385F, V416A, I457V, S461N, L472I, N481D, D502E, F511S, S512L, S516G, K561R, K564R, V575I, H599N, D611N, N615S, V624I
2	ORF075 (RNA polymerase-associated protein): V324A; ORF076 (late transcription factor VLTF-4): V64A, 98DNdel, 103Ndel, D151G; ORF079 (mRNA capping enzyme large subunit): I206T, D295N, T374P; ORF080 (hypothetical protein): I26M, T93I; ORF081 (putative virion protein): H17N, S227N; ORF082 (uracil DNA glycosylase): R54Q; ORF083 (putative NTPase): S3G, S49T, G106D, I135M, I253L, I708T; ORF084 (putative early transcription factor small subunit): L353V, D581N; ORF085 (RNA polymerase subunit): M136T; ORF086 (mutT motif): E121D, L191F, extension of the protein with 3 amino acid residues due to a change in the termination codon,; Translation similar to minor parent, thus 207–213 NTLVNSK; ORF087 (mutT motif putative gene expression regulator): V46I, extension of the protein with53 amino acid residues due to a change in the termination codon; ORF088 (putative transcription termination factor): I24V; ORF089 (mRNA capping enzyme small subunit): V171I
3	ORF133: V165I, D200E, S275N, L312S, I344T, D347N, S514A, ORF134: G2R
4	ORF101**:** E223D; ORF102: A61N, L115S, T162A; ORF103: T50N, P72T, S89G
5	ORF001: V42E, D129V, I144M; ORF003**:** S93T, A100S; ORF005: 15Fdel, A23V, I24V; ORF006: F13L, S61L, 95Sdel, I111S, S216N; ORF007: S200T
6	ORF146: T285S; ORF147: I487M; ORF148: G40S, G51D, I102L, N167D, E169D, V351M, K361Q, C397Y, K413E, A418T, N439S.
7	ORF093: D60N; ORF094: D93N, F607L
8	ORF098: I355V, R404H, I505V, D553G, T652I
9	ORF134: L1973I, 2007Ndel, extension of the protein with 59 amino acid residues due to a change in the termination codon
10	ORF050: 376Ndel; ORF054: 6 LP; ORF055: M184I; ORF056: K171R; N174D; ORF057: V372I; ORF059: Q125K
11	ORF033: I37S, R88K, A117T
12	ORF039: C144F, A150S, D215E, Y222F
13	ORF112**:** V22I, 93Ndel, 94Ddel, N95D; ORF113: A53T, F283S

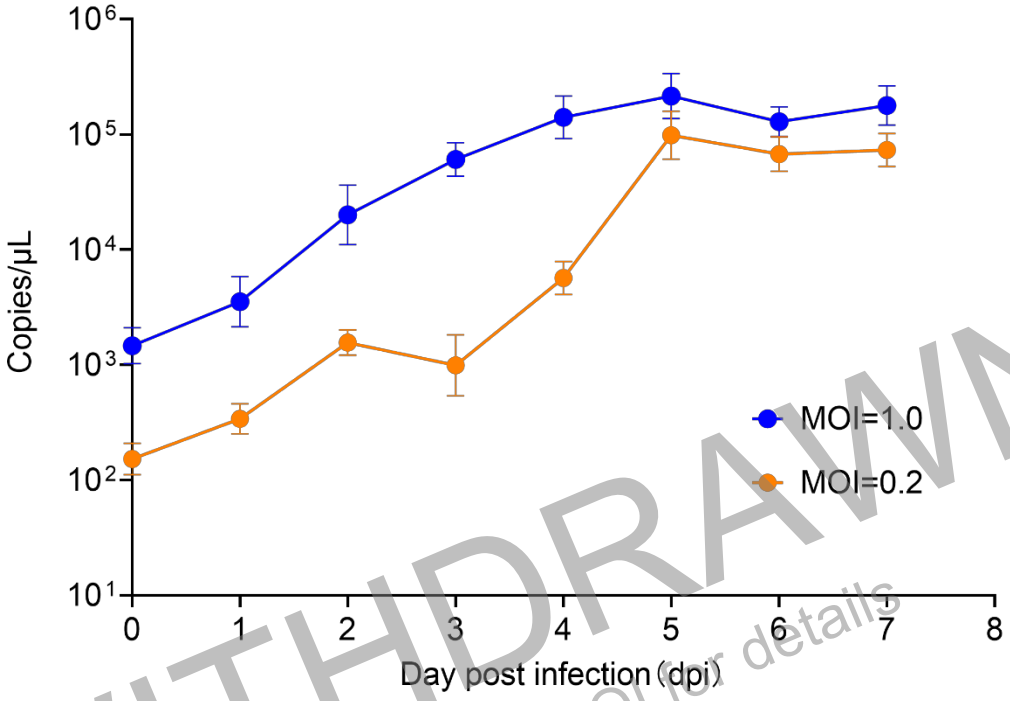
[a] Some minor recombination events in the genome of GD01/2020 could be not predicted; the amino acid changes also existing in the genome of Russia/Saratov/2017 (MH646674) are underlined; other over 16 proteins involved in these major predicted recombination events without amino acid sequence difference between the two parents are not listed in this table.



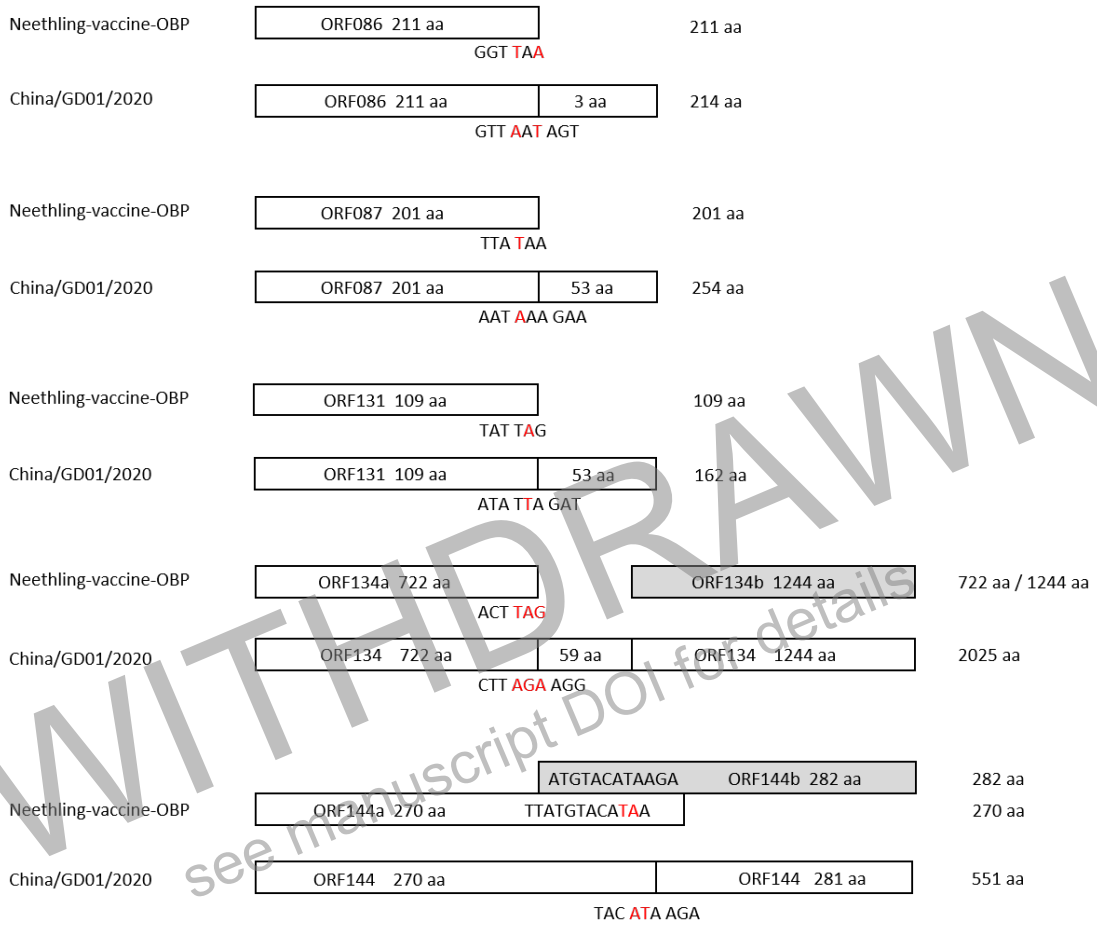
Appendix Figure 1. Dates and affected provinces of the LSDV outbreaks in China in 2019 and 2020. All data were from the Ministry of Agriculture and Rural Affairs of China (http://www.xmsyj.moa.gov.cn/yqfb/202007/t20200715_6348686.htm).



Appendix Figure 2. Detection of the GD01/2020 in clinical samples by the qPCR. Numbers above each group represent the numbers of positive samples versus total samples.



Appendix Figure 3. Growth kinetics of GD01/2020. Each point is shown as the mean ± SD of three independent experiments.



Appendix Figure 4. Termination codons of five genes of GD01/2020 changed as compared with the Neethling vaccine strain OBP.