Molecular detection and phylogenetic analysis of Lymphatic choriomeningitis virus (LCMV) in ticks in Jilin Province, northeastern China

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Research Article

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Abstract

Background: Lymphatic choriomeningitis virus (LCMV), belongs to the viral family *Arenaviridae*, genus *Mammarenavirus*, can cause aseptic meningitis in humans. Rodents are usually considered as the natural reservoir host of LCMV. But recent study indicates that LCMV may be transmitted by ticks in northeastern (NE) China. To further explore the epidemic distribution of LCMV, we conducted the prevalence and genetic characterization of LCMV in ticks in Jilin Province, NE China.

Methods: Ticks were collected in Huadian, Dunhua, and Jiaohe, and were pooled and detected for LCMV using real-time RT-PCR. LCMV positive pools were used for complete genome amplification and phylogenetic analysis.

Results: A total of 1679 ticks were collected and divided into 170 pools, including *Ixodes persulcatus* (5%), *Dermacentor silvarum* (89%), and *Haemaphysalis japonica* (6%). 24 pools of *D. silvarum* (14.9%, 95% CI: 9.5–22.3) and 3 pools of *H. japonica* (36.3%, 95% CI: 9.8–99.5) were detected LCMV positive, while no *I. persulcatus* pools were identified LCMV positive. Two complete genome sequences (Strain JL-DH01 and JL-DH02) were successfully amplified from LCMV positive pools, showing nucleotide identities of 96.4–99.8 per cent with JX31, JX14, DH46, JX4 strains isolated in ticks from Jilin Province. Phylogenetic analysis further indicated that the two viruses clustered with the Jilin strains in the same branch and belonged to LCMV genotype I.

Conclusions: This study further expands the genetic diversity and the geographical distribution of LCMV in Jilin Province, providing important information for prevalence and genetic characteristics of the virus.

1 Introduction

Lymphatic choriomeningitis virus (LCMV), belongs to the Old World viruses of the family *Arenaviridae*, genus *Mammarenavirus*, is a bi-segmented (L and S) single-stranded RNA virus, which can cause asymptomatic or self-limited infection in humans, while in populations of immunocompromised may lead to aseptic meningitis, or even severe systemic infection and death[1] Most people contract LCMV through direct contact or exposure to the aerosolized droppings or body fluids of infected rodents, organ transplantation, or vertical transmission.

Since first isolated from a patient with meningoencephalitis by Armstrong and Lille in 1933[2], the virus has been proved widely distributed in many countries of Europe, America, Asia, and Africa[3]. Rodents, including Mus musculus and Mus domesticus, are usually considered as the natural reservoir host of LCMV[4]. But latest study has showed that ticks may serve as reservoirs of the virus, indicating that ticks may play an important role in LCMV transmision[5]. In this study, we further investigated the prevalence of LCMV in ticks in NE China, which could further expand the genetic diversity and the geographical distribution of LCMV, and provide more information for prevalence and genetic characteristics of the virus in NE China.

2 Materials And Methods

2.1 Tick collection and identification

In April, 2021, ticks were collected from cattle or by flagging a white cotton cloth over vegetation in three sites of Jilin Province, northeastern China, including Huadian (126°73'E, 42°97'N), Dunhua (128°13'E, 43°21'N), and Jiaohe (127°33'E, 43°72'N) (Figure 1). All the ticks were preliminarily identified by the morphological classification combined with polymerase chain reaction (PCR) assay targeting the 16S rRNA of the ticks as described elsewhere[6].

2.2 DNA and RNA extraction

Every 10 ticks were pooled according to the collection sites and species for molecular detection (Table 1). Pools of ticks in tubes were washed with 75% alcohol and rinsed twice with double distilled water. 500-μL Dulbecco's modified Eagle's minimum (DMEM) and two stainless steel beads (3 mm diameter) were added to the tubes, and crushed using the Tissuelyser (Jingxin, Shanghai, China) at 70Hz for 2min. The lysates were centrifuged at 12 000 rpm for 10min at 4°C, and the supernatant was collected for DNA and RNA extraction. Total DNA of ticks was extracted using a Universal Genomic DNA Kit (Cwbio, Beijing, China) to identify tick species, and viral RNA was extracted using a TIANamp Virus RNA kit (Tiangen, Beijing, China) in accordance with the manufacturer's instructions. The extracted virus RNA was reversed using the PrimeScript 1st Strand cDNA Synthesis kit (TaKaRa, Kyoto, Japan) and used for LCMV detection and whole genome amplification.

2.3 Detection of LCMV and whole genome amplification

The prevalence of LCMV in ticks were detected by real-time RT-PCR and primers were designed based on the S sequences of strain DH46 (MG554177), JX14 (MG554175), JX4 (MG554174), and JX31 (MG554176). The following components: 10.0 μL of Probe Premix Ex Taq (TaKaRa), 7.4 μL of double distilled water, 0.4 μL of forward primer (LCMV-NP F:5'-TGATGAGTCYTTCACGTCCCA-3') and reverse primer (LCMV-NP R:5'-GACAACACAGCAGCTTGACC-3') for each, 0.8 μL of probe (LCMV-NP P: FAM-5'-TCACCACACCAGTTGCACCCT-3'-BHQ1) and 1 μL of cDNA template were added to a total reaction volume of 20 μL. The PCR reaction parameters were 95℃ for 30s, followed by 50 circles at 95℃ for 5s, 60℃ for 30s. The positive pools were used for whole genome amplification by nested PCR assay with the primers listed in (Table S1 and S2). All the PCR products were separated by using 1% agarose gel electrophoresis for sanger sequencing, and the acquired sequences were assembled using DNAMAN software.

2.4 Phylogenetic analysis

Nucleotide sequences of the whole genome of LCMV were aligned using ClustalW and calculated for identities by MegAlign program available within DNAstar V7.1. The phylogenetic relationships were estimated using the maximum-likelihood method with the Tamura-Nei model conducted in MEGA software version 7.0. A bootstrapping analysis of 1000 replicates was conducted, and the bootstrap values more than 60 were shown in the phylogenetic trees.

2.5 Recombination analysis

To find the potential recombination events in the evolutionary history of LCMV, the full length of L and S segment sequences of LCMV strains were concatenated in order, and then analyzed using RDP, geneconv, bootscan, maxchi, chimera, siscan, and 3seq prediction methods in RDP4 software[7]. The reorganization event should meet the following two conditions: ①, the event should be verified by at least two methods with $P < 0.05$, ②, and the RDP recombination consensus score should be > 0.6[8] . Additionally, if the recombination event met the first condition, but the RDP recombination consensus score was between 0.4 and 0.6, a possible recombination event can be considered. Otherwise, the event was considered untenable. Moreover, the aligned LCMV sequences were also analyzed using Simplot version 3.5.1 to confirm the recombination events[9].

2.6 Statistical analyses

The prevalence of LCMV infection in ticks was calculated by using the program PooledInfRate Excel Add-In version 4.0[10]. Maximum likelihood estimation (MLE) method with 95% confidence intervals (CIs) and one-sample analysis for unequal pool sizes were used to analyze the infection rates for pooled ticks. Significant difference ($p < 0.05$) between the tick species or collection regions were performed by Fisher's exact test using SAS statistical software, version 9.3 (SAS Institute, Inc., Cary, NC).

3 Results

3.1 Tick collection and identification

A total of 1679 ticks, including *I. persulcatus* (n=84, 5%), *H. japonica* (n=93, 6%), and *D. silvarum* (n=1502, 89%), were collected in Dunhua, Jiaohe, and Huadian in Jilin Province, NE China (Fig 1, Table 1). Of the three collection sites, ticks of Dunhua and Jiaohe were collected from cattle, while ticks in Huadian were collected in the field. Based on species and sampling sites, ticks were divided into 170 pools. Of them, 1, 3, and 90 pools of *I. persulcatus*, *D. silvarum*, and *H. japonica* ticks were from Dunhua, respectively. Similarly, 6, 6, and 43 pools were from Jiaohe, and 2, 1, and 18 pools were from Huadian (Table1).

3.2 LCMV detection and whole genome amplification

In total, 22 tick pools from Dunhua and 5 pools from Jiaohe were detected LCMV positive, showing prevalence of 26.7% (95% CI: 17.3–39.6) and 9.4% (95% CI: 3.5–20.8), respectively, while no positive pools from Huadian were detected (Table 1). The overall prevalence of LCMV infection in *D. silvarum* in Jiaohe was 7.1% (95% CI: 1.9–19.1), which was lower than that in *D. silvarum* from Dunhua (26.3%, 95% CI: 16.8–39.4). The prevalence of LCMV in *H. japonica* from Dunhua (44.8%, 95% CI: 2.9–100.0) was higher than that from Jiaohe (35.8%, 95% CI: 6.7–100.0). No significant difference was detected between the positive tick species, or the positive regions ($p > 0.05$).

The whole genome sequence of LCMV from two positive samples of JL-DH01, JL-DH02 were successfully amplified with nucleotide length of 7241 in L and 3375 in S, showing nucleotide identities of

96.2–99.7% (segment L) and 97.7–99.8% (segment S) with JX31, JX14, DH46, and JX4 LCMV strains that were previously found in Jilin Province[5], respectively (Table 2). The sequences had been uploaded to GenBank with accession numbers of ON236642–ON236645.

3.3 Phylogenetic and recombination analyses

In the phylogenetic trees based on the sequences of L and S segments of LCMV, the virus strains identified in this study clustered with JX31, JX14, DH46, and JX4, and showed close relationship with other members including 810885, Pasteur, Marseille*12 strains belonging to genotype I (Figure 2). Moreover, no recombination event was found among LCMV strains. SimPlot analysis showed the changing trend of polyprotein amino acid similarity among the four lineages. Notably, the identified JL-DH01and JL-DH02 strains showed a low amino acid similarity of 92%–98% with JX31 (Figure 3).

4 Discussion

In recent years, many novel tick-borne viruses have been found in NE China, including Severe fever with thrombocytopenia syndrome virus, Jingmen tick virus, Alongshan virus, Songling virus, and Beiji nairovirus, which together with Tick-borne encephalitis virus pose serious threats to public health security of the endemic areas [9, 11-14]. It is generally believed that LCMV is a rodent-borne arenavirus which can cause aseptic meningitis, or even severe systemic infection and death in humans[15].

Recently, LCMV has been detected in *Haemaphysalis longicornis*, *Dermacentor nuttalli*, *D. silvarum*, and *I. persulcatus* ticks in NE China[5]. In this study, LCMV was detected in *H. japonica* for the first time with a total prevalence of 36.3% (95% CI: 9.8–99.5). No LCMV positive samples were identified in *I. persulcatus* ticks from any of the collection sites. Notably, same phenomenon was reported in Jingxin *I. persulcatus* ticks in Jilin Province[5], indicating the low proportion of LCMV infection in *I. persulcatus*. Moreover, compared with the prevalence of LCMV in *D. silvarum* (4.4, 95% CI: 2.0–9.0) in the previous study[5], a higher prevalence was detected in the same tick species (17.2, 95% CI: 11.3–25.1).

It is remarkably, however, that the viral copy numbers of all the positive pools detected in this study were low with Ct values various from 35–40 (data not show). The results indicated that the ticks may not serve as reservoirs of the virus, but instead obtained the virus by ingesting the blood from LCMV infected rodents. Due to the low titer of virus, we only obtained the full genome sequence from 2 positive samples.

There are some shortcomings in this study. Firstly, only three tick species were collected, and the majority of the ticks was *D. silvarum* tick species (89%), which may not reflect the true prevalence of the other tick species objectively. Moreover, although high prevalence of LCMV was detected in the *D. silvarum* and *H. japonica* ticks, only two complete genome sequences were amplified, which may limit the genetic diversity analyses of the virus. Lastly, our study further confirmed the presence of LCMV in ticks in NE China, but the role of ticks in the transmission of LCMV remains unclear. Further studies should be focus on the vector competence of the various species of ticks for transmission of LCMV, and the epidemiology

studies on tick-bites patients, livestock, and even wild animals (especially wild rodents) should also be conducted.

Conclusions

In conclusion, LCMV RNA was detected the *D. silvarum* and *H. japonica* ticks in Jilin Province, NE China, showing prevalence of 14.9% and 36.27%, respectively. The findings further expand the genetic diversity and the geographical distribution of LCMV in Jilin Province, providing useful information for the prevalence and genetic characteristics of the virus.

Declarations

Acknowledgments

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Author contributions

ZYL, FW designed the research. FW collected the samples. ZYL, LL, LZ, DW, SYF, XJL performed the experiments. ZYL and ZDW analyzed the data. ZYL and ZDW wrote the original manuscript. FW reviewed and edited the manuscript. All authors gave final approval for publication.

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Availability of data and materials

Viral genomes have been submitted to GenBank with Accession Nos: ON236642–ON236645. The datasets supporting the conclusions of this article are included within the article.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Tables 1 to 2 are available in the Supplementary Files section

Figures

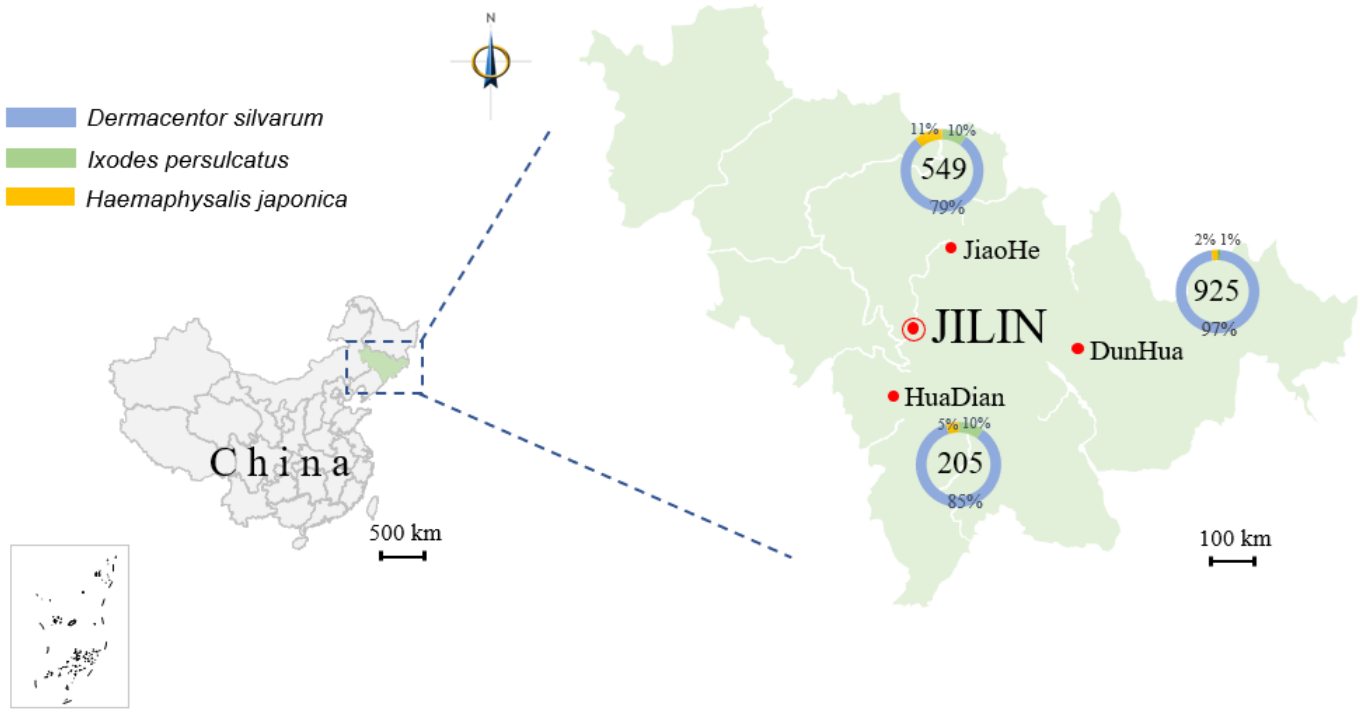

Figure 1

Ticks were collected from Huadian, Dunhua, Jiaohe region in Jilin provinces of China (Green shadowed areas). The proportion of *Ixodes persulcatus*, *Dermacentor silvarum, Haemaphysalis japonica in* three regions is displayed in the doughnut chart.

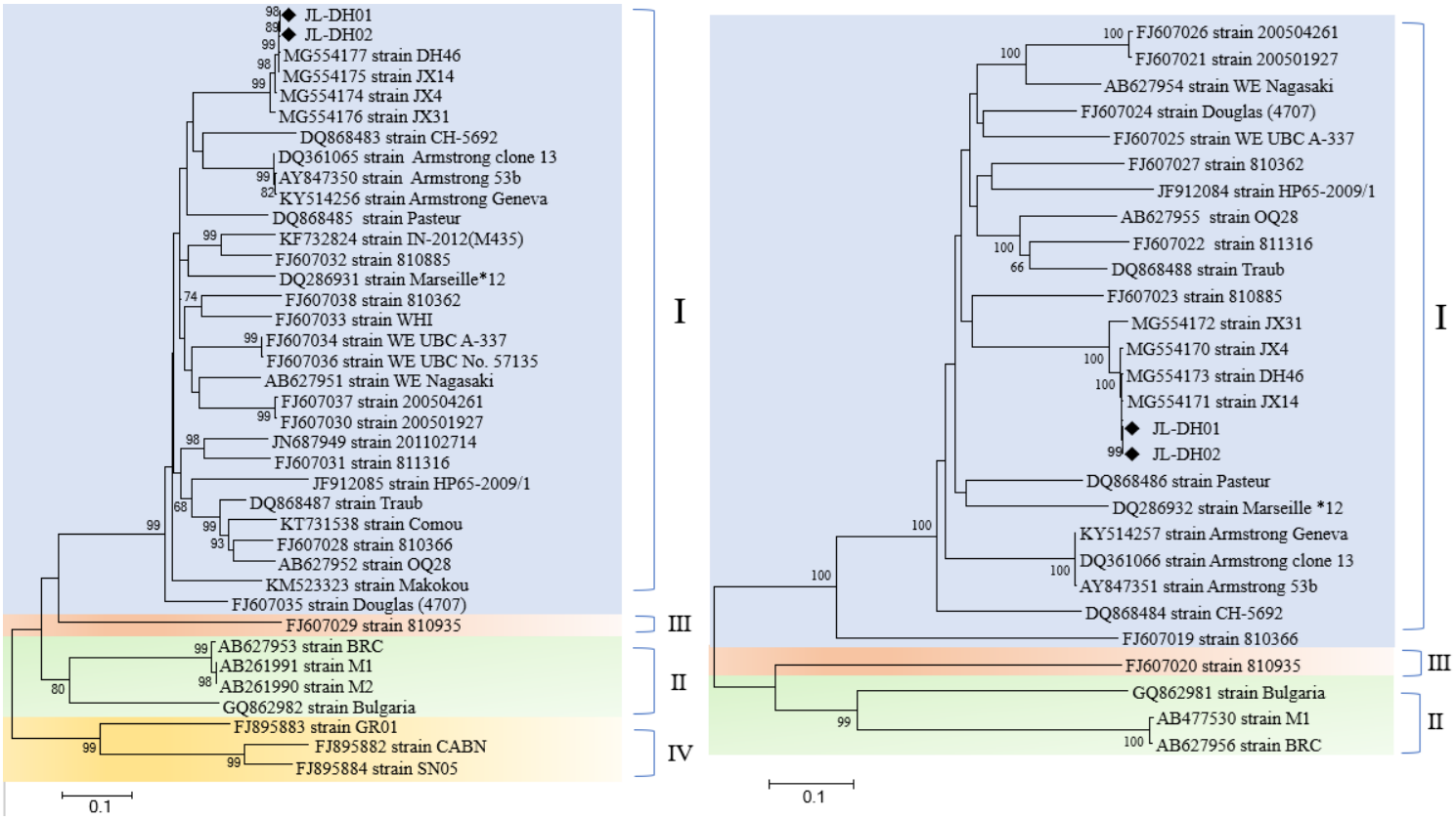

Figure 2

Phylogenetic trees based on LCMV segment S (left) and L (right). The two LCMV sequences detected in this study were aligned with those retrieved from the GenBank database. Phylogenetic analysis was performed using the Maximum Likelihood method and trees were tested by bootstrapping (1000 pseudoreplicates). The scale-bar indicates the number of substitutions (Tamara-Nei model) per site. The scale bars in segment L ans segment S panel indicate 0.1 substitutions per site.

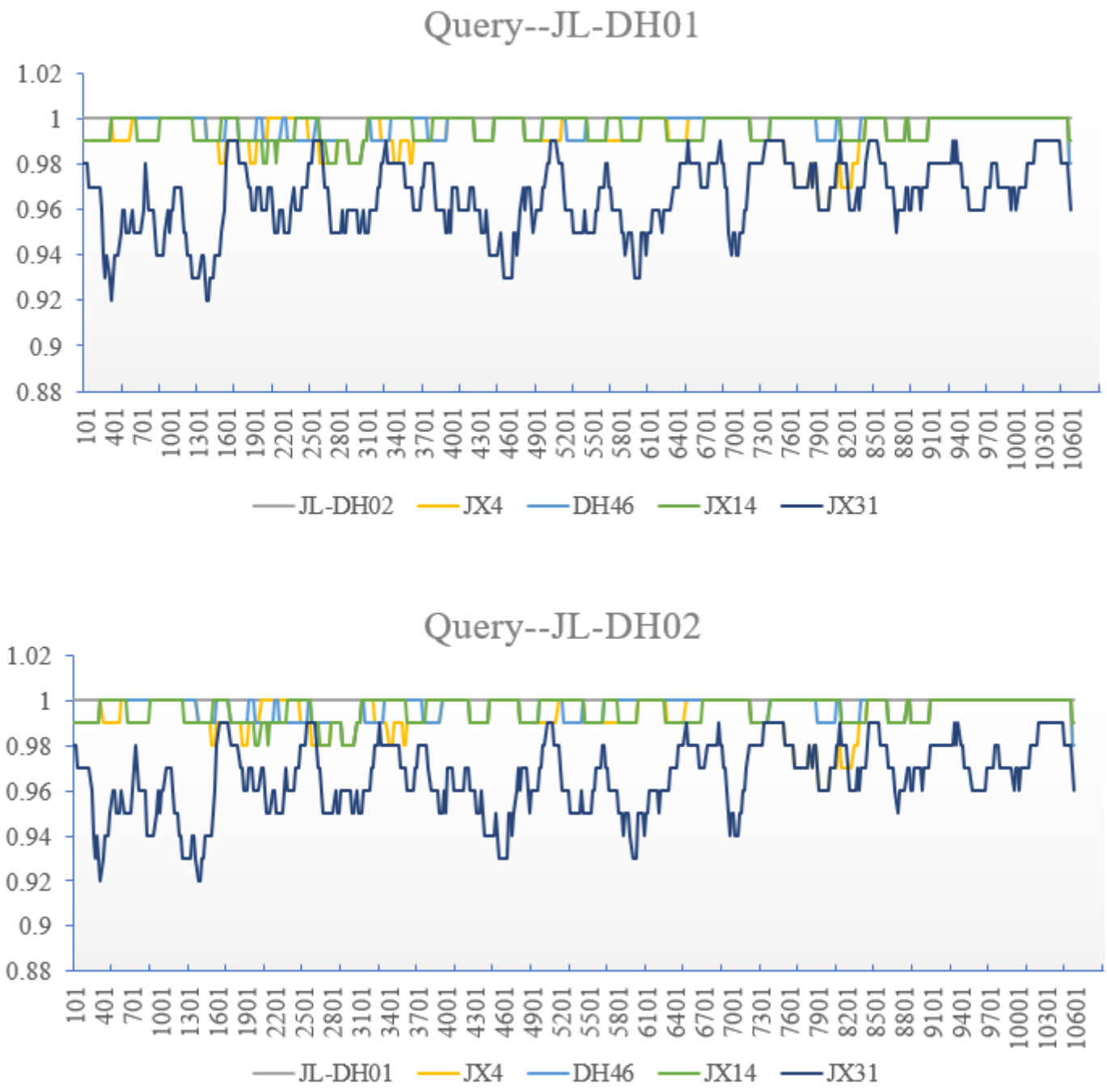

Figure 3

Simplot analysis of LCMV based on nucleic acid sequence. The identified strains LCMV S1/S2 were used as the query strains, and the analysis was calculated using Simplotversion 3.5.1 with a sliding window of 200 and a step size of 20 residues.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1S2.doc
- Table1.docx
- Table2.xlsx