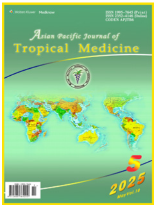

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Tick-borne Wuhan mivirus and Lihan tick virus in *Rhipicephalus microplus* in Guizhou Province, China

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ABSTRACT

Objective: To uncover the characteristics of tick-borne viruses in Guizhou Province.

Methods: A total of 414 *Rhipicephalus microplus* were collected from 5 counties in Guizhou Province, China from August 2022 to October 2023. A group of 12 ticks from each study sites was sequenced by next generation sequencing.

Results: 8 contigs of Wuhan mivirus (Chuviridae, Mivirus) with the length of 2094 bp to 11580 bp and 4 contigs of Lihan tick virus (Phenuiviridae, Uukuvirus) with the length of 1401 bp to 7080 bp were obtained, respectively. The prevalence rate of Wuhan mivirus and Lihan tick virus was 51.98% and 11.30%, respectively. The identities of gene sequences of both Wuhan mivirus and Lihan tick virus were 94%-100% compared with sequences in the National Center for Biotechnology Information. The phylogenetic analysis indicated that the Wuhan mivirus detected in this study was in the same branch with the Wuhan mivirus of Sichuan isolate TIGMIC-27 (NCBI Accession: OP628598) and Zhejiang isolate TIGMIC-45 (NCBI Accession: OP628613). In addition, the Lihan tick virus was in the same branch with the Sichuan Lihan tick virus isolate TIGMIC-46 (NCBI Accession: ON812358).

Conclusions: Both Wuhan mivirus and Lihan tick virus were prevalent in *Rhipicephalus microplus* in Guizhou Province. More studies are needed to understand the pathogenicity and public health threats of these tick-borne viruses.

KEYWORDS: Tick-borne virus; Wuhan mivirus; Lihan tick virus; *Rhipicephalus microplus*; Metagenomics; Guizhou Province

1. Introduction

Tick-borne viruses (TBVs) infect and transmit diseases from vector ticks to vertebrate hosts, causing zoonotic diseases[1,2]. Important zoonotic tick-borne viruses include Crimean-Congo hemorrhagic fever virus, which can cause hemorrhagic fever with clinical symptoms including headache, fever, vomiting, hemorrhagic fever, shock, *etc*. Their hemorrhagic symptoms can be severe and deadly, with a case fatality rate of approximately 30%[3]. Tick-borne encephalitis virus (TBEV) can cause neurological diseases, such as encephalitis and meningitis, in humans and animals[4]. Severe

Summary

Question: What are the characteristics of tick-borne viruses in Guizhou Province?

Findings: Among 414 *Rhipicephalus microplus* collected, Wuhan mivirus and Lihan tick virus were detected, with a prevalence rate of 51.98% and 11.30%, respectively, and were genetically similar to previously reported isolates.

Meaning: Both viruses are prevalent in *Rhipicephalus microplus* in Guizhou, and further studies on their pathogenicity and public health threats are needed.

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fever with thrombocytopenia syndrome virus (SFTSV) can cause acute fever, leukopenia, and can lead to multiple organ failure, with a fatality rate of up to 30%[5]. Emerging TBVs, such as Jingmen tick virus, Alongshan virus, and Heartland virus, can cause febrile diseases[6] and are reportedly associated with human diseases.

The advent of high-throughput transcriptomics has significantly advanced the investigation of tick-borne viruses both known and unknown[7]. Hu *et al.*[8] identified ten RNA viruses from five tick species in Qingdao using metagenomic sequencing, and found Qingdao tick iflavirus was a member of the Flaviviridae family. Qingdao tick iflavirus, Qingdao tick phlebovirus, and Qingdao tick Uukuvirus were new viruses in two families. Ni *et al.*[9] sequenced ticks using the megviriome, identified 724 RNA viruses with unique viral composition, and assembled 1 801 complete or nearly complete viral genomes, revealing the wide diversity of the genome structure of tick-associated viruses, and highlighting the fact that ticks are repositories of RNA viruses. At present, relevant analyses of tick virus groups have been conducted in some areas of China, but the characteristics of tick virus groups in different regions have not been covered in existing studies.

Guizhou Province in the southwestern part of China is a suitable habitat for ticks due to its lush vegetation, abundant rainfall, and diverse wildlife and livestock[10]. Various tick species have been identified in Guizhou Province. *Rhipicephalus (R.) microplus* and *Haemaphysalis (H.) longicornis* ticks are commonly found[11]. Many important viruses have also been detected in these ticks, such as Dabieshan tick virus (Phenuiviridae, Uukuvirus), with an infection rate of 3.28%, which was reported in *R. microplus* and *H. longicornis* in Bijie City[12]. Mini Virus-2 (MIV-2), with a positive rate of 9.52%, has been found in ticks in Guizhou Province and can infect multiple tick species[13]. The whole gene sequence of Jingmen tick virus was obtained in tiny fanephalus ticks in Zunyi City[14]. Overall, these studies indicated the diversity of TBVs in Guizhou and the need for further investigation.

Tick-borne diseases can be prevented and controlled effectively after acquiring information related to TBV diversity in an area. Therefore, tick samples from five areas in Guizhou Province were analyzed in this study using viral metagenomics. Furthermore, the genetic characteristics and prevalence of the Wuhan mivirus from the Chuviridae family and the Lihan tick virus of the Phenuiviridae family were investigated using molecular biological methods.

2. Methods

2.1. Sample collection

Between August 2022 and October 2023, 414 live adult ticks were plucked from infested cattle in Qixingguan District, Libo County, Luodian County, Shibing County, and Xishui County in Guizhou Province, and preserved at -80 ℃ temparature (Figure 1).

2.2. Identification of tick species

Tick species were morphologically identified by an experienced technician and confirmed by sequencing the mitochondrial *16S* ribosomal RNA (*16S* rRNA) gene[15].

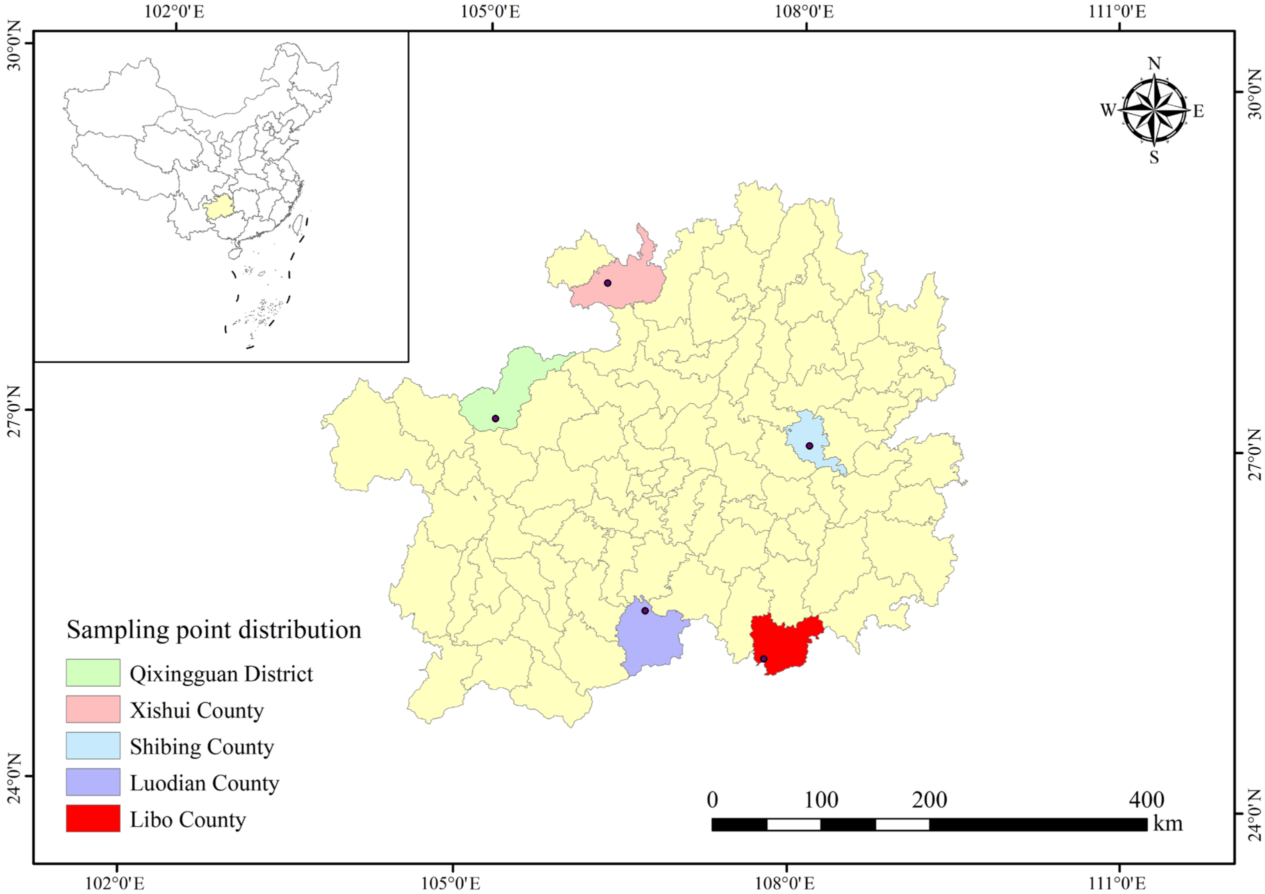

Figure 1. Location of tick collection in five counties/districts of Guizhou Province, China.

2.3. Virus metagenomic sequencing

According to the machine concentration requirements of viral metagenomic sequencing, twelve ticks were randomly selected as sequencing units in each of the five counties/districts. The selection of 12 tick samples was primarily based on meeting the concentration requirements for NGS (next generation sequencing). Preliminary experiments showed that pooling samples from 12 ticks could consistently achieve the optimal DNA/RNA concentration range required for sequencing, ensuring reliable data quality and sufficient sequencing depth. This number balances both experimental efficiency and cost-effectiveness while adhering to standard protocols for high-throughput sequencing. Five sequencing units were established for virus metagenome sequencing. Each tick was placed in a 1.5 mL RNA-free centrifuge tube, to which 250 μL MagZol reagent was added. The mixture was immediately ground with a grinder. Total RNA was extracted using the HiPure Universal RNA Mini Kit (Magen). The RNA quality was analyzed using the NanoDrop One Thermo, Life Technologies Qubit 4.0, and by performing 1% agarose gel electrophoresis. The RNA samples that passed the quality check were depleted of rRNA using the Ribo-off rRNA Depletion Kit (human/mouse/rat) (Vazyme), and the resulting RNA was used to construct the library using several steps, including reverse transcription and double-strand synthesis using the ALFA-SEQ RNA Library Prep Kit (Findrop). The quality of the constructed library was checked using the Qubit® dsDNA HS Assay Kit (Life Technologies) and the Agilent 4200 TapeStation (Agilent). Finally, the libraries were sequenced on the Illumina Novaseq 6000 platform in paired-end mode with a read length of 150 bp.

2.4. PCR amplification

The primers of Chuvirus and Uukuvirus were designed using Oligo7 software. The primer reference sequences for the above viruses were selected based on the viral metagenome sequencing (Table 1). PCR amplification was performed in a total volume of 25 μL, which included 2.5 μL of 10 × PCR buffer, 2 μL of 2.5 mM dNTP mixture, 0.5 μL of each primer (10 μM/L), 0.125 μL of Taq polymerase (5 U/μL) (Takara Biotechnology, China), 1 μL of tick cDNA template, and double-distilled water to make up the volume. The tick cDNA templates were prepared as follows: a single tick was placed in a 1.5 mL RNA-free centrifuge tube, and 210 μL sterile phosphate-buffered saline (PBS) was added. The tick was ground with an electric tissue grinder to make a suspension, and the RNA was extracted using the qEx-DNA/RNA Virus Kit (Xi'an Tianlong Science and Technology, Xi'an, China). Finally, RNA was reverse-transcribed using TaKaRa's PrimeScript™ RT reagent Kit with gDNA Eraser (Model: RR047A). The PCR was performed as follows: pre-denaturation and denaturation were performed at 94 ℃ for 3 min and 94 ℃ for 30 s, respectively; annealing temperature and duration for Chuvirus and Uukuvirus were 55 ℃ for 30 s and 59 ℃ for 30 s, respectively; amplification reaction was extended to 72 ℃ for 40 s and a final extension step was conducted at 72 ℃ for 5 min. A total of 35 cycles were performed. Finally, 5 μL PCR products from each sample was analyzed on 1.5% agarose gels treated with 4S green nucleotide stain (Sangon Biotech), and the results were observed using a gel imager (Bio-Rad).

2.5. Sequencing and phylogenetic analysis

The PCR products with amplified target bands were sent to BGI-Chongqing for bi-directional sequencing and sequence splicing. Pearson's *Chi*-square test was performed to evaluate the statistical difference in the positive rate. Differences with $P<0.05$ were considered to be statistically significant. The obtained sequences were aligned with the sequences of registered genes in GenBank using the BLAST tool on the National Center for Biotechnology Information (NCBI) website. Similarly, the classical and most homologous strain sequences of the virus were searched from the GenBank database. MEGA7.0 was used to construct the phylogenetic evolutionary tree, and 1 000 repetitions were tested using the adjacency method to further analyze the genetic evolutionary relationship of the virus obtained in this study.

2.6. Ethics statement

This study was exempt from ethics review. The research focused on the collection and analysis of *R. microplus* ticks from the natural environment in Guizhou Province. It did not involve human subjects, human tissues, or any animal-related experiments that require ethical considerations. As the study posed no ethical risks to humans, animals, or the environment, no formal ethics approval was required.

3. Results

3.1. Tick collection and identification

Between August 2022 and October 2023, 414 ticks were collected from cattle in Qixingguan District (n=79), Libo County (n=82), Luodian County (n=49), Shibing County (n=100), and Xishui County (n=104) of Guizhou Province, China. These ticks were identified as *R. microplus* using morphology and mitochondrial *16S* rRNA gene sequencing. Sequences were deposited in GenBank under the following accession numbers: PQ198481, PQ198483, and PQ198485.

Table 1. PCR primers for virus detection.

Subject	Primer name	Secquence (5'-3')	Product size (bp)
Chuviridae	Chuvirus-F01	5' AGCCTTCATAGACCAGCTCTCTC 3'	315
	Chuvirus-F01	5' GAGTGCTCCAGGATTGCAGTG 3'	
Phenuiviridae	Uukuvirus-F01	5' CTCCTCATACTCCCTGTCCTCTTCC 3'	582
	Uukuvirus-R01	5' TTCAGCACTGCGGTAGATTCTTATTCC 3'	

3.2. Viral metagenomic sequencing and assembly

We obtained approximately 58.9 GB data from tick RNA sequencing libraries. We implemented stringent quality control measures, including the exclusion of low-quality data, such as reads paired with adapters, repetitive sequences, and single-ended sequencing reads containing more than 20% low-quality bases throughout the read counts. Clean tick reads from the five counties/districts ranged from 16 584 927 to 22 743 263. After ribosomal and host contaminations were removed, data assembly yielded 8 889-55 539 contigs with an average N50 of 582 bp.

3.3. Viral metagenomic taxonomic analysis

Ten virus species were detected in the five county/district libraries, belonging to six families namely Nairoviridae, Chuviridae, Rhabdoviridae, Mitoviridae, Retroviridae, and Phenuiviridae, and an unclassified family, with relative abundances ranging from 0.0004% to 8.41%. Wuhan mivirus was detected in all counties/districts except Luodian County, with relative abundances ranging from 0.12% to 6.52%. Lihan tick virus was detected in Xishui County and Qixingguan District, but not in the other three counties/districts, with relative abundances ranging from 0.77% to 2.49%. *Hepelivirales* species and Unuamitovirus fuco1 were only detected in Luodian County, and the Avian sarcoma virus was only detected in Xishui County (Figure 2).

Using viral metagenomic sequencing, eight Wuhan mivirus contigs and four Lihan tick virus contigs were obtained. The gene length is between 2 094 and 11 580 bp (Wuhan mivirus) and 1 401 and 7 080 bp (Lihan tick virus). Using Align X in Vector NTI 11.5 software for multi-sequence analysis, it was found that the sequence consistency of eight Wuhan mivirus contigs was between 26%-98%. The contigs of four Lihan tick viruses were 45%-97% consistent.

3.4. PCR detection

3.4.1. PCR detection of Wuhan mivirus

Wuhan mivirus (Chuviridae, Mivirus) primers were designed according to the obtained contigs (Table 1). Using these primers, we amplified tick samples from five districts/counties. In these five districts/counties, the positive rate for Shibing County was 84.09% (74 of 88). For Xishui County, the Wuhan mivirus positive rate was 65.22% (60 of 92). Notably, no positive samples were detected among the 37 samples tested for Luodian County (Table 2). A significant difference was observed in the prevalence of Wuhan mivirus among ticks collected from the five sampling sites (χ^2=96.724, P=0.000 1).

Wuhan mivirus sequences obtained through Sanger sequencing exhibited genetic identities of 94%, 95%, 98%, 99%, and 100%, as determined using Align X in Vector NTI 11.5 software. Typical Wuhan mivirus gene sequences were selected based on the results of PCR gene analysis and subsequently compared with reference sequences in the GenBank database using BLASTn (https://blast.ncbi.nlm.nih.gov) to determine the genetic identity. Sequences with

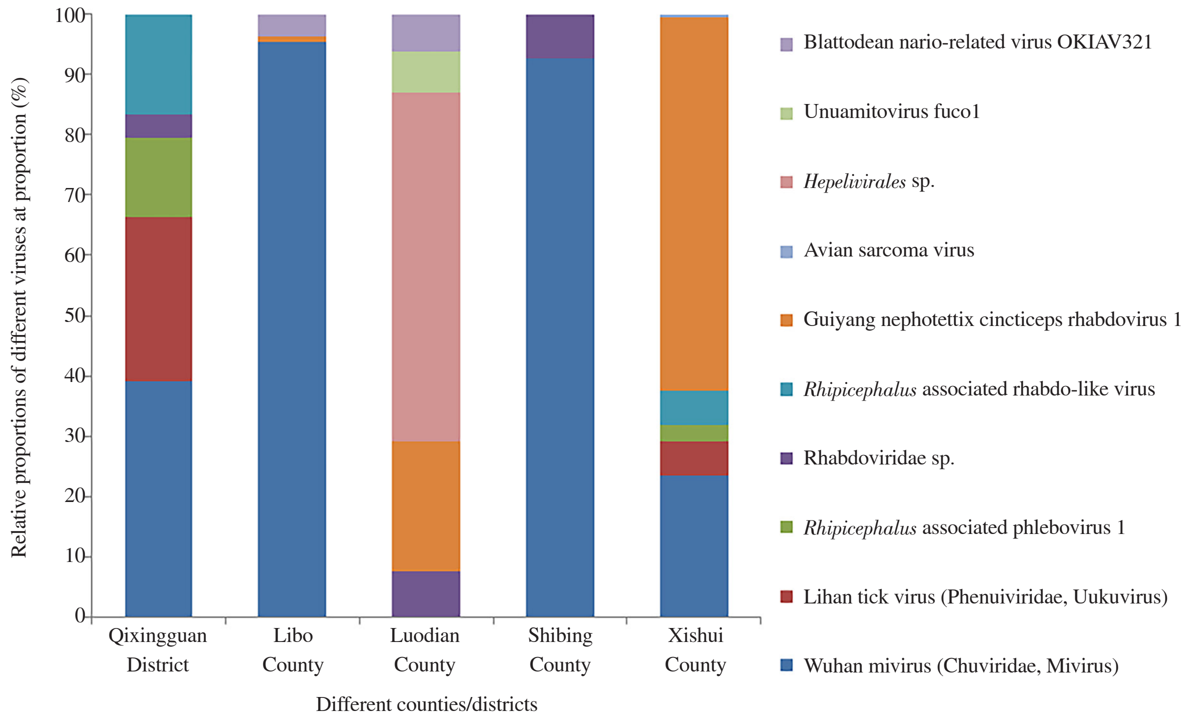

Figure 2. Top 10 viruses species in taxonomic proportion of tick at five counties/districts of Guizhou Province, China. The proportion is derived from the relative abundance of viruses.

100% and 99% genetic identity showed the highest genetic identity with the Wuhan mivirus isolate TIGMIC-30 (NCBI Accession: OP628600), which was found in *R. microplus* in Sichuan in 2018. The genetic identity between them was 98.39% and 98.95%, respectively. The gene sequence demonstrating 98% genetic identity showed the strongest correlation with the Wuhan mivirus (NCBI Accession: OP628558), found in *R. microplus* in Chongqing in 2019, with a sequence consistency of 98.68%. Moreover, the gene sequences with 95% and 94% identity exhibited the closest homology with the Wuhan mivirus isolate TIGMIC-12 (NCBI Accession: OP628565), identified in *H. longicornis* in Guangdong in 2019, with a sequence identity of 99.00%.

3.4.2. PCR detection of Lihan tick virus

Lihan tick virus (Phenuiviridae, Uukuvirus) primers were designed according to the obtained contigs (Table 1) to detect samples from five districts/counties using conventional PCR. The positive rates for Lihan tick virus were 44.78% (30 of 67) in Qixingguan District, 8.7% (8 of 92) in Xishui County, and 2.27% (2 of 88) in Shibing County. Additionally, all tick samples from Luodian County and Libo County tested negative for Lihan tick virus (Table 2). A significant difference was found in the prevalence of Lihan tick virus among ticks collected from the five sampling sites (χ^2=96.32, P<0.001). Moreover, 99% and 100% genetic identity was obtained in Lihan tick virus sequences through Sanger sequencing. The sequences with 100% genetic identity were 99.46% identical to those of Lihan tick virus strains (NCBI Accession: ON812192) detected in *R. microplus* ticks from Chongqing in 2019. Furthermore, other sequences were 99.28% identical to those of Lihan tick virus strains (NCBI Accession: ON812361) identified in Sichuan Province.

3.5. Genetic evolutionary analysis

3.5.1. Genetic evolutionary analysis of Wuhan mivirus

A phylogenetic tree was constructed with the five sequences and 12 reference sequences available from GenBank. The five sequences

Table 2. Infection situation of tick Wuhan mivirus/Lihan tick virus in five counties/districts of Guizhou Province, China.

Sampling location	No. of ticks	Wuhan mivirus		Lihan tick virus	
		Positive number	Positive rate (%)	Positive number	Positive rate (%)
Qixingguan District	67	22	32.83	30	44.78
Libo County	70	28	40.00	0	0.00
Luodian County	37	0	0.00	0	0.00
Shibing County	88	74	84.09	2	2.27
Xishui County	92	60	65.22	8	8.70
Total	354	184	51.98	40	11.30

Note: A significant difference was found in the prevalence of Wuhan mivirus (χ^2=96.323, P=0.000 1) and Lihan tick virusd (χ^2=96.32, P<0.001) among ticks collected from the five sampling sites. Statistical analyses were performed using Pearson's *Chi*-square test to determine significant differences in positive rates among groups. Differences with P<0.05 were considered statistically significant.

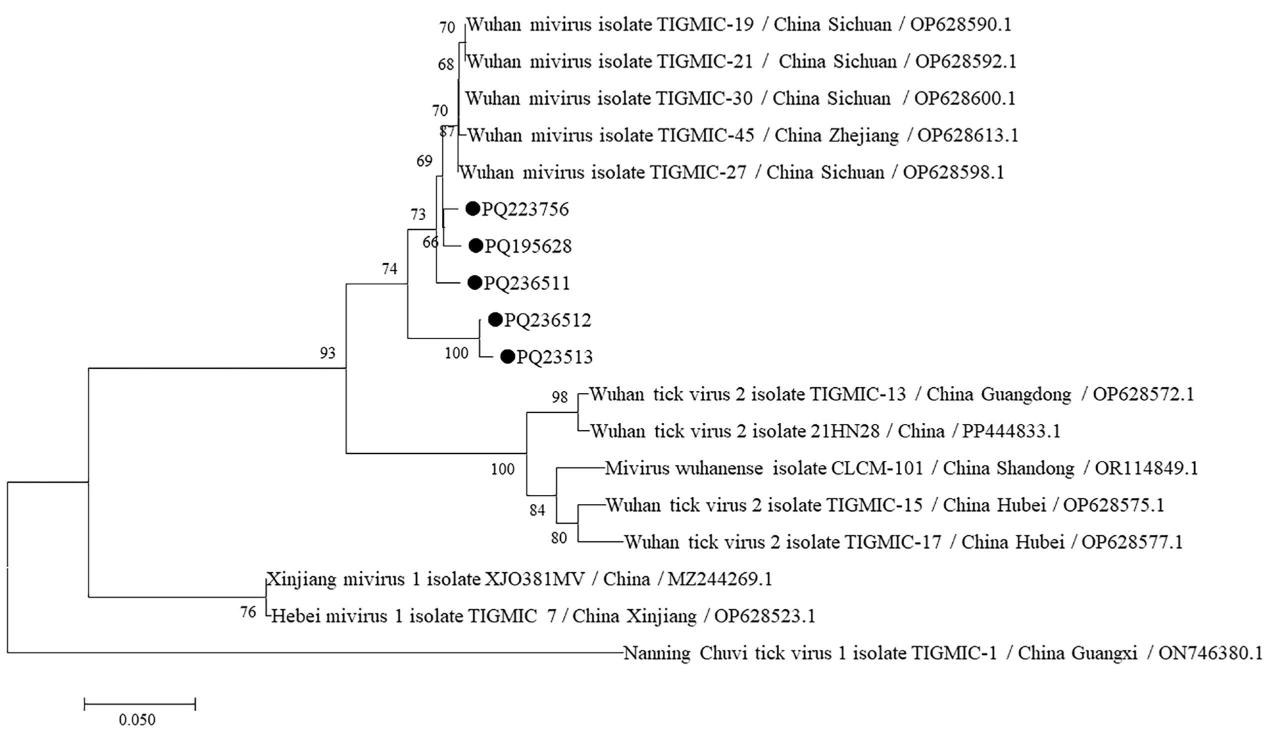

Figure 3. Phylogenetic analysis of Wuhan mivirus sequences (315 bp) carried by *Rhipicephalus microplus* in five counties/districts of Guizhou Province, China. The sequences determined in this study are shown in •. Numbers at the nodes represent the percentage of occurrence of clades in 1000 bootstrap replicates of the taxa.

had 94%-100% gene identities from different locations. The Nanning Chuvi tick virus (NCBI Accession: ON746380) formed an outgroup in the phylogenetic tree. Wuhan mivirus sequences (NCBI Accession: PQ223756, PQ195628, PQ236511, PQ236512, and PQ23513) determined using Sanger sequencing formed a clade with Wuhan mivirus from Sichuan (NCBI Accession: OP628598) and Zhejiang (NCBI Accession: OP628613). These viruses are distant from Hebei and Xinjiang mivirus 1 (NCBI Accession: OP628523 and MZ244269) (Figure 3).

3.5.2. Genetic evolutionary analysis of Lihan tick virus

A phylogenetic tree was constructed with two sequences and 13 reference sequences available in the GenBank. The two Lihan tick virus gene sequences had 99% and 100% gene identity. Heartland viruses (NCBI Accession: PP869891 and KC466562) formed an outgroup in the phylogenetic tree. Our virus sequences (NCBI Accession: PQ195629 and PQ236510) obtained using Sanger sequencing, and those of the Lihan tick virus from Sichuan province (NCBI Accession: ON812358) were in the same branch. In addition, the sequences formed sister branches with Lihan tick virus isolate *R. microplus* (NCBI Accession: ON812190) obtained from Chongqing. These Lihan tick virus gene sequences were distant from those of SFTSV in China (NCBI Accession: JQ733567), Heartland virus in the United States, and other viruses pathogenic to humans (Figure 4).

4. Discussion

Ticks are crucial vectors for various pathogens, including viruses, bacteria, and protozoa. Studies have revealed that the incidence of TBV infection is increasing globally, which poses a significant threat to public health[16]. Understanding TBVs will enable the accurate prediction of potential virus outbreaks by comprehensively monitoring viruses carried by ticks.

In this study, we conducted a metagenomic analysis of RNA viruses harbored by ticks in Guizhou, southwest China. Our results revealed a high viral diversity in the collected ticks. We identified ten viruses belonging to six families, namely Nairoviridae, Chuviridae, Rhabdoviridae, Mitoviridae, Retroviridae, and Phenuiviridae, and an unclassified family. Some of these viruses are important for public health security, such as Nairovirus, Rhabdovirus, and Phenuivirus, which suggests that long-term and extensive collection of tick samples in Guizhou should be conducted for virus diversity analysis and epidemiological monitoring.

We selected Wuhan mivirus and Lihan tick virus design primers for PCR detection and analysis of their genetic evolution relationship based on the results of viral metagenomic analysis.

Chuvirus was first identified in 2015 in *R. microplus* in Wuhan, Hubei Province, using metatenomics technology. Its genome structure includes the ring and segmental and double-segmental structures of negative-sense RNA viruses[17]. Arthropods reportedly carry these viruses; however, their pathogenicity remains unclear[18]. Wuhan mivirus belongs to the Chuviridae family and harbors a circular RNA as genome. Similar viruses have recently been reported in many regions worldwide. However, studies on the vector, host, and pathogenicity of these viruses are lacking[19,20]. By conducting metagenomic sequencing and PCR, we investigated the prevalence of Wuhan mivirus in tick samples obtained from Qixingguan District, Libo County, Luodian County, Shibing County, and Xishui County in Guizhou Province. The total positive rate of samples was 51.98%,

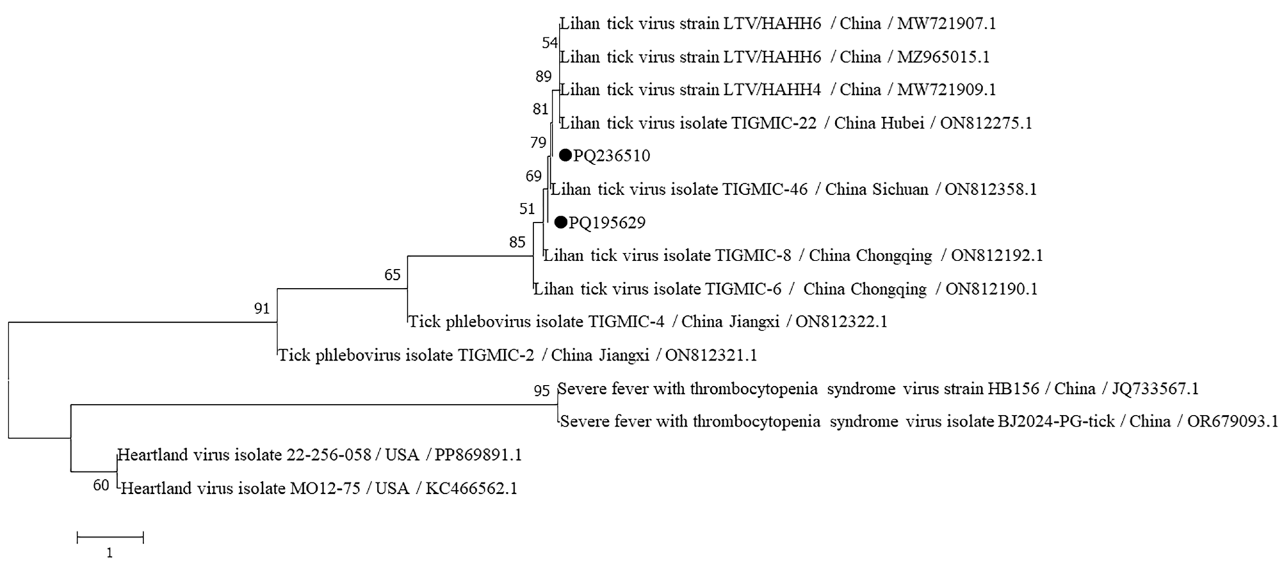

Figure 4. Phylogenetic analysis of Lihan tick virus sequences (582 bp) carried by *Rhipicephalus microplus* in five counties/districts of Guizhou Province, China. The sequences determined in this study are shown in •. Numbers at the nodes represent the percentage of occurrence of clades in 1000 bootstrap replicates of the taxa.

and the sequence consistency was between 94% and 100%. The positive rate of Wuhan mivirus was 84.09% and 65.22% in Shibing County and Xishui County, respectively. Wuhan mivirus discovered in this study had a closer relationship with the strains discovered in Sichuan Province than those identified in Xinjiang Province. Li *et al.*[13] discovered MIV-2 in *R. microplus*; Wuhan mivirus and MIV-2 are different strains of the same virus. In addition, Chuvirus was detected in Hunan, Henan, Gansu, Qinghai, Xinjiang, and other areas. Its hosts include *R. microplus*, *H. longicornis*, *Hyalomma asiaticum*, *Haemaphysalis montgomeryi*, *Dermacentor silvarum*, and *Rhipicephalus sanguineus*, *etc*[21]. It was shown that MIV-2 can infect multiple tick species; however, whether these tick species can transmit truovirus and their pathogenicity to humans require further investigation.

Phenuiviridae, a TBV family to which Uukuvirus and Phlebovirus belong[22], can transmit pathogens to humans and animals through tick bites, causing severe diseases such as hemorrhagic fever with renal syndrome[23]. Infection with SFTSV causes febrile disease, multiple organ damage, hemorrhagic disease, and death[24]. Infection of humans with Uukuvirus Tacheng Tick Virus 2 can cause symptoms such as headache, myalgia, rash, and meningitis[25]. Lihan tick virus, belonging to Uukuvirus, was first reported in Chinese *R. microplus* in 2015[26] and Colombian Dermacentor nitens ticks[27]. In our study, the infection rate of the Lihan tick virus was the highest in Qixingguan District, followed by that in Xishui County and Shibing County. The total positive rate of the samples was 11.30%, and the sequence consistency was between 99% and 100%. The obtained Lihan tick virus sequence was 99.46% consistent with that of the Lihan tick virus detected in *R. microplus* in Chongqing in 2019. The consistency with the Lihan tick virus detected in *R. microplus* in Sichuan in 2018 was 99.28%. The Lihan tick virus has been found in *R. microplus* in Fujian, Hubei, Guangdong, Yunnan, Chongqing, and Sichuan provinces[28–30], suggesting that *R. microplus* may be an essential carrier of Lihan tick virus. The pathogenicity of Lihan tick virus remains unknown as there are no reports of human and animal infection locally and internationally. However, its genomic sequence is 30.00%-35.00% homologous to that of SFTSV, which was first discovered in Hubei Province in 2009[31]. In our study, the Lihan tick virus sequence and the SFTSV reference sequence was 37.00%-49.00% homologous, suggesting that there may be more similarities, including similar biological characteristics and pathogenicity between these two viruses. Moreover, the Lihan tick virus possesses a single-stranded RNA, has an unstable structure, and is easy to mutate. Hence, whether this virus can mutate into a pathogenic virus needs to be studied.

This study has several limitations: firstly, all ticks were collected from the cattle and most were engorged suggesting that the virome in the ticks might predominantly originate from their animal hosts. Secondly, The research was conducted at only five sampling sites, which limits our understanding of the overall prevalences of tick-borne viruses in Guizhou Province.

In conclusion, we provide a comprehensive investigation on virome profiles of *R. microplus* tick in Guizhou. Although viruses pathogenic to humans and animals were not detected, our results confirmed the presence of Nairovirus, Rhabdovirus, Chuvius, Phenuivirus, *etc.*, in *R. microplus* in Guizhou. The evolution data of Wuhan mivirus and Lihan tick virus provides crucial preliminary data to understand the diversity of tick viruses in this region. Our results provide data that can be used to prevent and control tick-borne diseases in Guizhou Province.

Conflict of interest statement

The authors declare that they have no competing interests.

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Authors' contributions

TJX conceptualized the study, designed the methodology, and wrote the original draft. CQ and MJ performed formal analysis and software-related work. WSC and ZCM were responsible for data curation and investigation. DYS and CXX conducted investigation tasks. JJF contributed to the conceptualization of the study. SY and CWC participated in writing the review and editing process, and supervised the project. YFX, WJH, and ZL acquired the funding for the research.

Availability of data and materials

The data presented in the study are deposited in the NCBI repository, accession numbers PQ198481, PQ198483, PQ198485, PQ223756, PQ195628, PQ236511, PQ236512, PQ23513, PQ195629, and PQ236510.

References

[1] Shi J, Hu Z, Deng F, Shen S. Tick-borne viruses. *Virol Sin* 2018; **33**(1): 21-43.

[2] Schembri E, Campbell AJD, Villanueva-Cabezas JP. South Asian dairy smallholders: A scoping review of practices and zoonoses. *Asian Pac J Trop Med* 2023; **16**(10): 446-452.

[3] Hawman DW, Feldmann H. Crimean-Congo haemorrhagic fever virus. *Nat Rev Microbiol* 2023; **21**(7): 463-477.

[4] Johnson N, Migné CV, Gonzalez G. Tick-borne encephalitis. *Curr Opin Infect Dis* 2023; **36**(3): 198-202.

[5] Cai X, Cai X, Xu Y, Shao Y, Fu L, Men X, et al. Virome analysis of ticks and tick-borne viruses in Heilongjiang and Jilin Provinces, China. *Virus Res* 2023; **323**: 199006.

[6] Ma JG, Liu N, Liu ZY, Liu Q, Wei F, Wang ZD. Epidemiology of pathogenic tick-borne viruses in China: A review. *Chin J Schistosom Control* 2023; **35**(4): 325-330+348.

[7] Chandra S, Harvey E, Emery D, Holmes EC, Šlapeta J. Unbiased characterization of the microbiome and virome of questing ticks. *Front Microbiol* 2021; **12**: 627327.

[8] Hu G, Jiang F, Luo Q, Zong K, Dong L, Mei G, et al. Diversity analysis of tick-borne viruses from hedgehogs and hares in Qingdao, China. *Microbiol Spectr* 2023; **11**(3): e0534022.

[9] Ni XB, Cui XM, Liu JY, Ye RZ, Wu YQ, Jiang JF, et al. Metavirome of 31 tick species provides a compendium of 1 801 RNA virus genomes. *Nat Microbiol* 2023; **8**(1): 162-173.

[10]Xiang Y, Zhou J, Yu F, Zhang Y, Li S, Hu Y, et al. Characterization of bacterial communities in ticks parasitizing cattle in a touristic location in southwestern China. *Exp Appl Acarol* 2023; **90**(1-2): 119-135.

[11]Li RT, Li WY, Liu ZH. Survey and genetic evolutionary analysis of tick species in Guizhou Province. *Heilongjiang Anim Sci Vet Med* 2024; **52**(11): 72-77.

[12]Wang A, Pang Z, Liu L, Ma Q, Han Y, Guan Z, et al. Detection and phylogenetic analysis of a novel tick-borne virus in Yunnan and Guizhou Provinces, Southwestern China. *Pathogens* 2021; **10**(9): 1143.

[13]Li J, Wang Q, Xia LY, Pan YS, Jia N, Cao WC. Natural infection of MIV-2 virus in ticks of different regions of China. *Acta Parasitol Med Entomol Sin* 2021; **28**(2): 99-103.

[14]Li W, Li R, Tang X, Cheng J, Zhan L, Shang Z, et al. Genomics evolution of Jingmen viruses associated with ticks and vertebrates. *Genomics* 2023; **115**(6): 110734.

[15]Liu H, Li Q, Zhang X. Characterization of rickettsiae in ticks in northeastern China. *Parasit Vectors* 2016; **9**(1): 498.

[16]Brinkmann A, Dinçer E, Polat C, Hekimoğlu O, Hacıoğlu S, Földes K, et al. A metagenomic survey identifies Tamdy orthonairovirus as well as divergent phlebo-, rhabdo-, chu- and flavi-like viruses in Anatolia, Turkey. *Ticks Tick Borne Dis* 2018; **9**(5): 1173-1183.

[17]Yang Z, Wang H, Yang S, Wang X, Shen Q, Ji L, et al. Virome diversity of ticks feeding on domestic mammals in China. V*irol Sin* 2023; **38**(2): 208-221.

[18]Wang H, Liu Y, Liu W, Cao M, Wang X. Sequence analysis and genomic organization of a novel chuvirus, Tàiyuán leafhopper virus. *Arch Virol* 2019; **164**(2): 617-620.

[19]Li CX, Shi M, Tian JH, Lin XD, Kang YJ, Chen LJ, et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Elife* 2015; **4**: e05378.

[20]Gondard M, Temmam S, Devillers E, Pinarello V, Bigot T, Chrétien D, et al. RNA Viruses of *Amblyomma variegatum* and *Rhipicephalus microplus* and cattle susceptibility in the French antilles. *Viruses* 2020; **12**(2): 144.

[21]Yang ZJ. *Analysis of tick virus community and genetic characteristics of emerging viruses*. [MSc Thesis]. Zhenjiang: Jiangsu University; 2022.

[22]Han HM, Zhang YL, Peng J. Overview of medical vector tick organisms and viral infectious diseases. *China Port Sci Technol* 2021; **3**(10): 4-11.

[23]Rodino KG, Pritt BS. Novel applications of metagenomics for detection of tickborne pathogens. *Clin Chem* 2021; **68**(1): 69-74.

[24]Fang L, Yu S, Tian X, Fu W, Su L, Chen Z, et al. Severe fever with thrombocytopenia syndrome virus replicates in platelets and enhances platelet activation. *J Thromb Haemost* 2023; **21**(5): 1336-1351.

[25]Jia Y, Zhang Y, Wu X, Dong Z, Xie S, Li W, et al. Clinical and historical infection of Tacheng tick virus 2: A retrospective investigation. *PLoS Negl Trop Dis* 2024; **18**(6): e0012168.

[26]Chandra S, Harvey E, Emery D, Holmes EC, Šlapeta J. Unbiased characterization of the microbiome and virome of questing ticks. *Front Microbiol* 2021; **12**: 627327.

[27]López Y. First report of Lihan tick virus (Phlebovirus, Phenuiviridae) in ticks, Colombia. *Virol J* 2020; **17**(1): 63.

[28]Teng AY. S*patial distribution and transmission risk prediction of the viral pathogens belonging to the order Bunyavirales in China*. Beijing: The Academy of Military Sciences; 2022.

[29]Guo L, Ma J, Lin J, Chen M, Liu W, Zha J, et al. Virome of *Rhipicephalus* ticks by metagenomic analysis in Guangdong, southern China. *Front Microbiol* 2022; **13**: 966735.

[30]Wang J. *Investigation of tick–borne pathogens in Nujiang, Yunnan Province* [Dissertation]. Dali: Dali University; 2023.

[31]Wang W, Shin WJ, Zhang B, Choi Y, Yoo JS, Zimmerman MI, et al. The cap-snatching SFTSV endonuclease domain is an antiviral target. *Cell Rep* 2020; **30**(1): 153-163.e5.

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