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Research Article

Identification and genetic analysis of new ephemeroviruses in wild boars in China

Zhongzhong Tu [a], Tong Wang [a,b], Yu Xu [c], Heting Sun [c], Peng Peng [c], Siyuan Qin [c], Changchun Tu [a,b,*]

[a] Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences, State Key Laboratory of Pathogen and Biosecurity, Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Changchun 130122, PR China
[b] Jiangsu Co-Innovation Center for the Prevention and Control of Important Animal Infectious Disease and Zoonoses, Yangzhou University, Yangzhou 225009, PR China
[c] Biological Disaster Control and Prevention Center, National Forestry and Grassland Administration, Shenyang 110034, PR China

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ABSTRACT

Ephemeroviruses (EVs) are arthropod-borne rhabdoviruses and were isolated exclusively from cattle and haematophagous arthropods until two new ephemeroviruses were first identified from domestic pigs most recently. Here we report the identification of newer EVs in wild boar by meta-transcriptomic (MTT) sequencing. Further screening by specific RT-nPCR of tissue samples of 459 free-ranging wild boars collected between 2018 and 2023 from 26 provinces across China confirmed five positive wild boars in four provinces. Interestingly, two ticks especially collected from two positive wild boars were also EV positive. Finally, four complete genome sequences of wild boar ephemeroviruses (WbEVs) were obtained with two strains belonging to a new EV species, and the rest two falling into porcine ephemerovirus 2 (PoEV2) species identified from domestic pigs. Our study has further extended EV host range and demonstrated natural circulations of divergent EVs in wild boars, in which ticks may play roles. Biological implications of EV infection in wild boars should be interesting topics for future investigations.

INTRODUCTION

The genus *Ephemerovirus* is one of 56 genera in the subfamily *Alpharhabdovirinae*, the family *Rhabdoviridae* (ICTV, 2022). It primarily infects ruminants and is transmitted by haematophagous arthropods (Walker et al., 2022). The ephemerovirus genome consists of a negative-sense, single-strand RNA with approximately 15 kb in size, containing at least 9 open reading frames (ORFs) arranged in the order 3′-N-P-M-G-GNS-α1-α2-β-L-5′ in negative polarity (Walker et al., 2018). The N, P, M, G and L ORFs encode the canonical rhabdovirus structural proteins. In general, accessory ORFs locate in the region between G and L genes, the number of which varies in different ephemerovirus species. The genes are flanked by conserved transcription initiation and transcription termination/polyadenylation (UGAAAAAAA) sequences and are separated by intergenic regions (Walker et al., 2018).

Several ephemeroviruses (EVs) such as Kimberley virus (KIMV), Berrimah virus (BRMV), Adelaide River virus (ARV) and Koolpinyah virus (KOOLV) were detected from healthy cattle in Australia in the 1980s (Cybinski and Zakrzewski, 1983; Gard et al., 1983, 1984, 1992). Bovine ephemeral fever virus (BEFV), Yata virus (YATV), and Kotonkan virus (KOTV) were primarily identified from mosquitoes or biting midges in Africa in the 1960s (Doherty et al., 1969; Kemp et al., 1973; Blasdell et al., 2014). New Kent County virus (NKCV) and Huanggang Rhabd tick virus 2 (HgRTV2) were isolated from ticks (Ixodes scapularis) in North America in 2016 (origin: GenBank, accession number MF615270) and in China in 2019 (origin: GenBank, accession number ON746527), respectively. In addition, BEFV is a type member of the genus and can cause a short-lived disease characterized by a biphasic fever, hypersalivation, ocular and nasal discharge, recumbency, muscle rigidity, lameness, and anorexia. The mortality is very low, although the morbidity is high (Walker and Klement, 2015). Besides BEFV, only KOTV, MVGV and HYV were found to be associated with clinical disease in cattle (Kemp et al., 1973; Dacheux et al., 2019; Blasdell et al., 2020). Pig EV was not reported until the first identification of two new EV species in domestic

* Corresponding author.
E-mail address: changchun_tu@hotmail.com (C. Tu).

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pigs by our team (Wu et al., 2022), although seropositive pigs for EV infection were reported early (Gard et al., 1984; Lim et al., 2007). This finding showed the natural circulation of EVs in pigs, but their transmission source is unknown.

Wild boars are globally distributed wild animals and play important roles in transmission of pathogens to domestic pigs and humans (Meng et al., 2009; Fredriksson-Ahomaa, 2019). Several studies have suggested that wild boars are a likely sentinel animal and can be potential reservoirs of zoonotic tick-borne pathogens (Adjadj et al., 2022; Kirino et al., 2022). Here, our study shows that wild boars harbored new EVs, which are likely transmitted by ticks.

RESULTS

Detection of EVs in wild boars

Totally 35 pooled clarified supernatant samples from 459 wild boars were subjected to meta-transcriptomic (MTT) analysis, which produced totally 210 G datasets (to be published elsewhere). Online Blastn/x search of the MTT data identified many wild boar-borne viral reads, in which 5974 and 368 EV reads were respectively identified from pooled Shaanxi and Anhui samples. After *de novo* assembly, a 14,533-nt long EV contig was obtained from pooled Shaanxi sample, which is nearly full EV genome, named WbEV/SN02/2019 strain. While from pooled Anhui sample, 13 contigs of 314–862 nt were obtained, which comprise an incomplete EV genome, named WbEV/AH01/2019 strain. To detect the EV infection rate in these wild boars, a RT-nPCR targeting *L* gene was developed using primers designed based on published porcine ephemerovirus (PoEV) sequences (Wu et al., 2022) and wild boar ephemerovirus (WbEV) sequences in present study (Supplementary Table S1) and used to screen combined tissue samples of each wild boar. As shown in Fig. 1, 5 of 459 wild boars (1.09%) in four provinces (Shaanxi, Anhui, Guangxi, and Hainan) were ephemerovirus-positive, with two positive wild boars in Shaanxi from the same Province, and the detailed information on the characteristics of the WbEV-positive wild boars has been list in Table 1.

Fortunately, four ticks were obtained from positive wild boars in Anhui and Hainan. Of them two from the wild boar in Anhui were identified as *Amblyomma testudinarium* and *Haemaphysalis hystricis*, while other two from the wild boar in Hainan were identified as *H. formosensis* and *H. hystricis* by detection of conserved tick cytochrome c oxidase I gene (Folmer et al., 1994). Interestingly, *A. testudinarium* and *H. formosensis* were EV positive by RT-nPCR detection, while two *H. hystricis* ticks were negative. The amplified partial EV *L* sequence of two ticks shares 100% homology with those of corresponding wild boars, indicating that WbEVs were likely transmitted by ticks.

Comparative genomic analysis of WbEVs

To obtain the complete viral genome, the tissue samples of above four positive wild boars were respectively subjected to MTT analysis again, and four complete WbEV genomes were successfully obtained and named as WbEV/SN02/2019, WbEV/AH01/2019, WbEV/HaiN01/2020, and WbEV/GX05/2020, respectively. The genomes of WbEV strains are 14,503–14,615 nt in length (Table 2), sharing 69.5%–99.6% nt identities with each other, and 66.1%–82.5% nt identities with previous PoEV strains HeN10 (accseeion number: OK086697) and GDMM7 (accseeion number: OK086698) (Fig. 2A). Four WbEVs, like PoEVs, have the same genomic organization, containing 5 structural protein-encoding ORFs (N, P, M, G, and L) and 5 accessory protein-encoding ORFs (GNS, α1, α2, β, and γ) in the order 3′-N-P-M-G-GNS-α1-α2-β-γ-L-5′. Compared to the relative diversity found between WbEV and PoEV strains, the genomic diversity between the strains showed significant differences, except for SN02/2019 and HaiN01/2020 strains (Fig. 2B).

Fig. 1. Geographic distribution of 459 wild boars in the study. Filled circles: five ephemerovirus (WbEV)-positive wild boars; Filled triangles: primary location of two PoEV isolates previously identified (Wu et al., 2022); Parentheses: WbEV detection rates in sampled provinces.

Table 1
Detailed information on the characteristics of the WbEV-positive wild boars.

Sample	Age	Sex	Weight	Physical condition	Collection time	Detection time
19SN02	1-year-old	Female	50 kg	Apparently healthy	August 2019	November 2022
19SN03	5-month-old	ND	20 kg	Apparently healthy	August 2019	November 2022
20HaiN01	4-month-old	ND	10 kg	Apparently healthy	August 2020	November 2022
20GX05	1-year-old	Female	40 kg	Apparently healthy	September 2020	November 2022
19AH01	1-year-old	Female	55 kg	Apparently healthy	July 2019	November 2022

ND: not determined.

Table 2
The information of genome structure of four wild boar ephemeroviruses.

Genomic region[a]	SN02/2019 nt length (location)/aa	HaiN01/2020 nt length (location)/aa	AH01/2019 nt length (location)/aa	GX05/2020 nt length (location)/aa
3′ Leader	73 (1–73)	42 (1–42)	83 (1–83)	83 (1–83)
N	1275 (74–1348)/424	1275 (43–1317)/424	1281 (84–1364)/426	1281 (84–1364)/426
P	930 (1373–2302)/309	930 (1342–2271)/309	930 (1389–2318)/309	930 (1389–2318)/309
M	666 (2321–2986)/221	666 (2290–2955)/221	666 (2360–3025)/221	666 (2360–3025)/221
G	1998 (3011–5008)/665	1998 (2980–4977)/665	1989 (3050–5038)/662	1989 (3050–5038)/662
GNS	1665 (5048–6712)/554	1665 (5017–6681)/554	1689 (5075–6763)/562	1689 (5075–6763)/562
α1	267 (6731–6997)/88	267 (6700–6966)/88	258 (6782–7039)/85	258 (6782–7039)/85
α2	354 (7038–7391)/117	354 (7007–7360)/117	432 (7042–7473)/143	432 (7042–7473)/143
β	288 (7421–7708)/95	288 (7390–7677)/95	288 (7495–7782)/95	288 (7495–7782)/95
γ	336 (7727–8062)/111	336 (7697–8032)/111	336 (7801–8136)/111	336 (7801–8136)/111
L	6378 (8088–14465)/2125	6378 (8058–14435)/2125	6381 (8164–14544)/2126	6381 (8163–14543)/2126
5′ Trailer	68 (14466–14533)	68 (14436–14503)	71 (14545–14615)	71 (14544–14614)

[a] All ORFs contain stop codon.

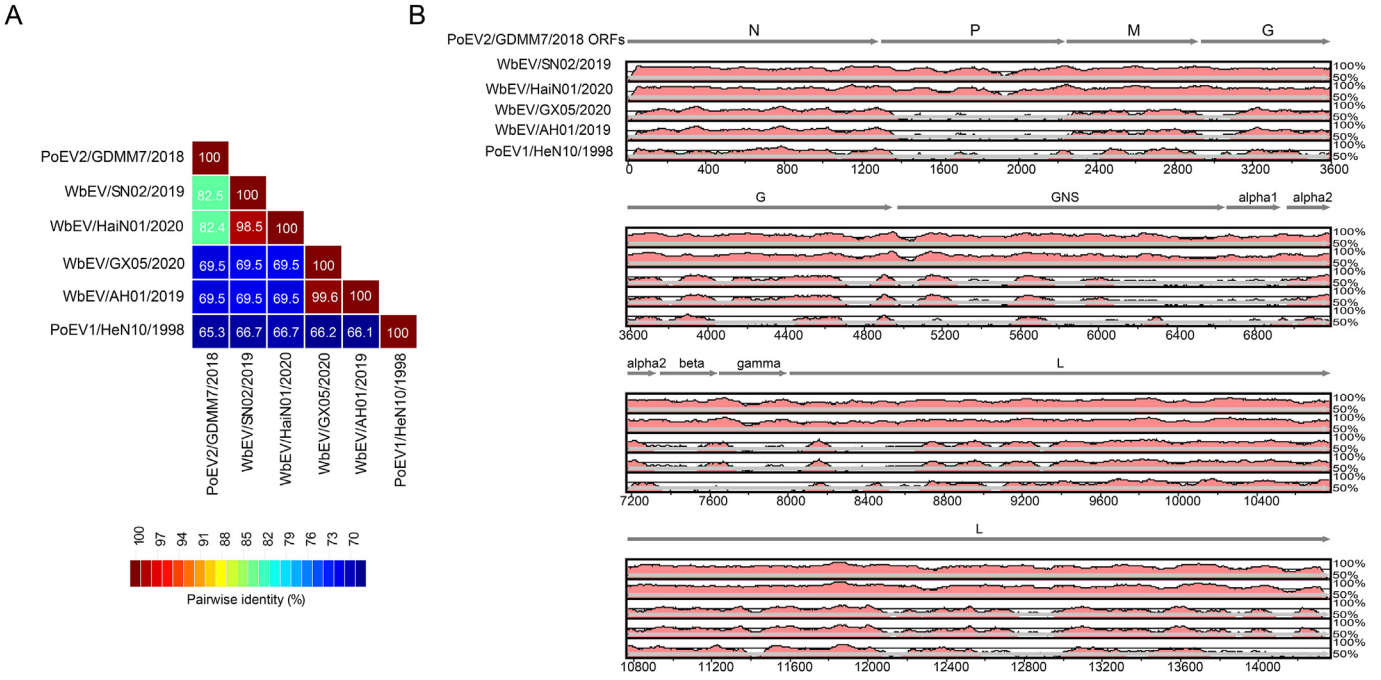

Fig. 2. Genetic diversity of WbEVs. **A** Pairwise sequence identity matrices of WbEV and PoEV strains complete genome by Sequence Demarcation Tool (SDT). **B** The mVISTA similarity plot showed sequence conservation among WbEV and PoEV strains. Sequence conservation was determined from a multiple sequence alignment, and the conservation score was plotted in a sliding 100-bp window.

The classification of WbEV within the genus *Ephemerovirus*

Comparison of N and L protein sequences revealed that the WbEV strains respectively shared 86.8%–100% and 75.1%–99.8% amnio acid (aa) identity with each other, and 44.3%–98.1% and 43.9%–93.9% aa identity with other 14 representative EV species (Supplementary Table S2). Phylogenetic tree based on N and L protein sequences showed that four WbEV strains are clustered together with two PoEV species within the genus *ephemerovirus* (Fig. 3). According to the ICTV standard, the genetic distance between 8% and 15% of the full protein sequence of the N and L is used to demarcate EV species (Walker et al., 2022; Wu et al., 2022). WbEV strains AH01/2019 and GX05/2020 belong to a new species since the genetic distances of their N and L protein sequences are more than 12.9% and 25.2%, while strains SN02/2019 and HaiN01/2020 belong to the species of PoEV2 since the genetic distances of their N and L protein sequences are only 1.9% and 6.1% (Table 3).

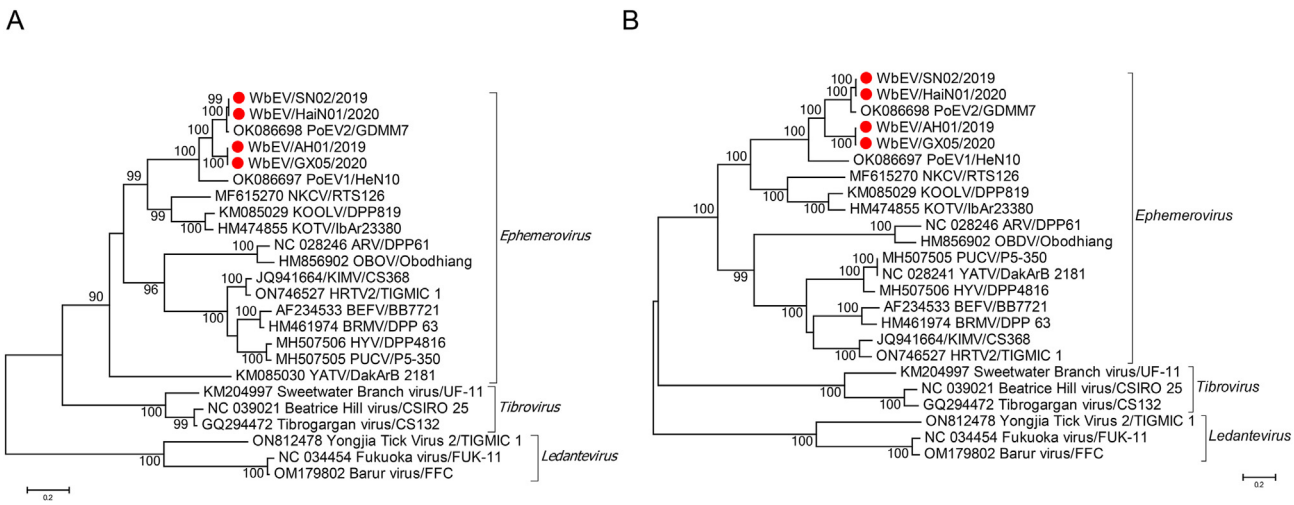

Fig. 3. Phylogenetic trees based on full length of N (**A**) and L (**B**) protein sequences of representative species of 3 genera within the *Rhabdoviridae* family. Four WbEV strains are marked with red dots. The phylogenetic tree was constructed by MEGA v7.0 using the maximum likelihood method with 1000 bootstrap replicates, and the best fitting substitution models was LG + G + I and LG + G + I + F, respectively. The bootstrap support values greater than 70% are shown at relevant nodes.

Table 3
Homology of amino acid of ephemerovirus N and L protein.

N protein amino acid sequence identity (%)																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
WbEV/SN02/2019		86.8	100.0	86.8	78.4	98.1	46.9	49.5	49.3	48.8	51.4	58.1	58.1	59.5	46.5	48.3	47.5	50.9
WbEV/AH01/2019	74.3		86.8	100.0	78.8	87.1	46.9	49.8	50.7	50.2	51.7	58.1	59.3	60.2	44.3	50.2	50.1	52.4
WbEV/HaiN01/2020	99.7	74.2		86.8	78.4	98.1	46.9	49.5	49.3	48.8	51.4	58.1	58.1	59.5	46.5	48.3	47.5	50.9
WbEV/GX05/2020	74.3	99.8	74.2		78.8	87.1	46.9	49.8	50.7	50.2	51.7	58.1	59.3	60.2	44.3	50.2	50.1	52.4
OK086697/PoEV1	69.7	70.0	69.6	70.0		78.1	43.2	47.2	48.3	49.5	49.1	59.3	60.0	60.5	43.4	50.2	49.4	50.0
OK086698/PoEV2	93.9	74.8	93.9	74.8	70.1		47.2	49.5	48.8	48.8	51.4	58.8	58.8	59.5	46.9	48.3	48.0	50.9
NC_028246/ARV	45.5	44.1	45.4	44.1	45.2	45.8		48.8	48.6	50.5	50.5	46.0	46.9	46.2	87.0	50.5	40.5	50.5
AF234533/BEFV	46.9	47.2	46.9	47.1	47.7	47.1	47.2		91.7	75.7	76.2	52.1	51.4	50.0	49.8	75.7	45.2	75.9
HM461974/BRMV	47.2	47.1	47.1	47.0	47.7	47.6	46.7	83.9		77.1	77.1	52.6	52.3	51.6	49.5	77.5	46.1	77.1
MH507506/HYV	47.2	47.4	47.2	47.3	47.3	47.0	46.2	63.0	63.8		74.4	51.9	53.1	53.1	51.6	95.2	45.6	75.5
JQ941664/KIMV	48.1	48.7	48.2	48.7	47.6	48.0	46.4	63.5	63.8	62.8		52.1	52.6	52.3	50.9	74.1	47.3	93.6
KM085029/KOOLV	54.6	55.4	54.6	55.4	54.9	55.1	46.2	48.7	48.3	48.4	48.7		92.7	71.4	46.5	52.6	50.9	52.8
HM474855/KOTV	54.7	55.0	54.7	55.0	54.8	55.1	45.9	48.0	48.8	48.7	48.3	84.0		71.4	46.5	53.3	51.4	53.8
MF615270/NKCV	54.3	55.2	54.2	55.2	54.9	54.6	44.9	48.4	48.7	48.9	49.0	64.8	64.0		45.8	53.3	50.9	52.8
HM856902/OBOV	44.9	43.9	45.0	43.9	45.6	45.6	77.9	47.3	46.7	46.6	46.8	46.6	45.7	45.2		51.9	38.4	50.5
MH507505/PUCV	47.1	46.8	47.3	46.8	47.3	47.1	45.4	62.8	63.0	86.7	62.3	48.0	48.2	48.3	46.1		45.6	75.5
KM085030/YATV	53.7	53.5	53.7	53.5	54.1	54.0	45.3	48.6	48.9	48.5	49.3	57.4	57.3	58.1	45.1	49.2		48.0
ON746527/HRTV2	48.0	48.5	48.0	48.5	47.4	47.7	45.5	63.6	64.0	62.5	86.4	49.2	49.1	49.0	46.4	61.8	49.1	
L Protein amino acid sequence identity (%)																		

1: WbEV/SN02/2019, 2: WbEV/AH01/2019, 3: WbEV/HaiN01/2020, 4: WbEV/GX05/2020, 5: OK086697/PoEV1.
6: OK086698/PoEV2, 7: NC_028246/ARV, 8: AF234533/BEFV, 9: HM461974/BRMV, 10: MH507506/HYV.
11: JQ941664/KIMV, 12: KM085029/KOOLV, 13: HM474855/KOTV, 14: MF615270/NKCV, 15: HM856902/OBOV.
16: MH507505/PUCV, 17: KM085030/YATV, 18: ON746527/HRTV2.

WbEV viral RNA loads in the different tissues of wild boars

To determine tissue distribution of WbEV, various tissues and blood of positive wild boars were analyzed by TaqMan RT-qPCR, developed on the basis of the standard curve generated. As shown in Fig. 4, WbEV was detected in various tissues and blood with viral loads ranging from 5.84×10^2 to 3.66×10^5 gene copies per gram of tissue or per mL of blood, showing that the virus has a broad tissue tropism and can cause viremia.

DISCUSSION

EVs are typical arboviruses transmitted by blood-sucking insects, and some of them are frequently detected in bovines. Recent identification of porcine EVs confirmed that domestic pigs are new hosts of EVs (Wu et al., 2022), and present study has further extended EV host range to wild boars, resulting in identification of another new EV species. EVs from pigs and wild boar are clustered together with significant virus diversity (Figs. 2 and 3). These researches indicated: 1, EVs from pigs and wild boars were not derived from other animals and have their own evolutionary pathways to form a rich diversity; 2, Suidae comprises important hosts in maintaining EV circulation in nature; 3, Current sequence identities do not show existence of cross-species EV transmission between domestic pigs and wild boars (Fig. 2).

In this study, only 5 of 459 wild boars (1.09%) were EV positive in four provinces, indicating that the pathogen prevalence is very low (Fig. 1). But WbEV could be detected in a broad range of tissues and blood samples (Fig. 4), which suggest that infection sometimes may be systematic and cause viremia. However, BEFV, the type member of the genus *Ephemerovirus*, is an important pathogen of cattle and water buffalo (Lavon et al., 2023), and has viral neurotropism in the infected cattle, in which many tissues and blood samples were detected positive for viral RNA (Barigye et al., 2016). BEFV can induce viremia, which often

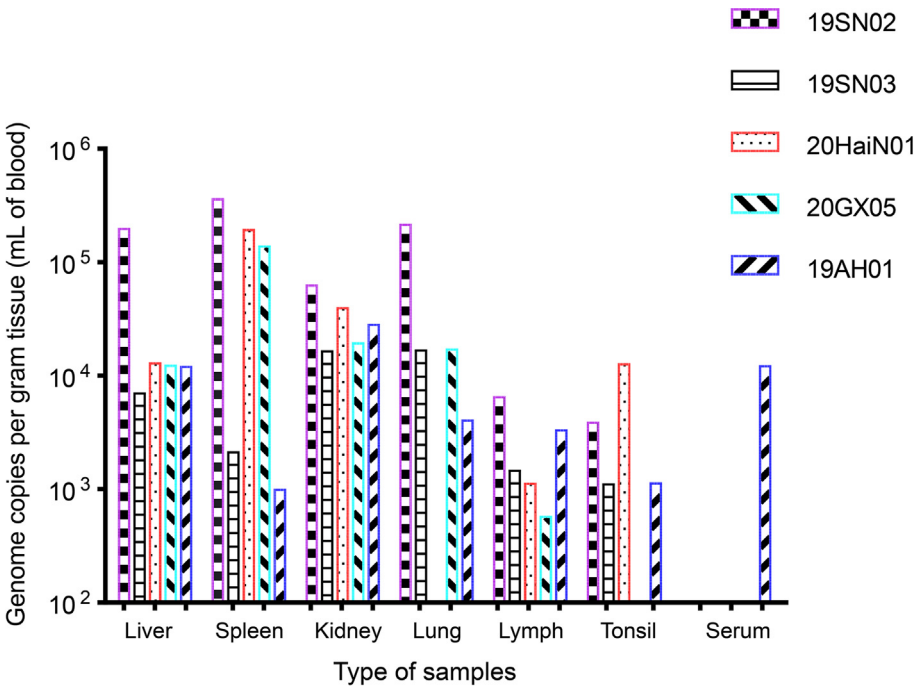

Fig. 4. Viral loads of WbEV in organs and blood of wild boar. WbEV RNA loads of 5 positive wild boars were tested by the RT-qPCR assay. Blood sample was only available from WbEV/AH01/2019-positive wild boar. Lung was not available from WbEV/HaiN01/2020, and tonsil was not available from WbEV/GX05/2020.

persists no more than one or two days in natural cases and three days in experimental cases (St George, 1988; Young and Spradbrow, 1990). In addition, BEFV has preferential tropism for bovine lymphoid tissues, and the spleen and haemal node may be potential sites for the virus after 6 days cessation of viremia (Barigye et al., 2017). WbEVs in present study were from health wild boars and their pathogenicity is unknown. Furthermore, WbEV loads in different wild boar individuals and tissues varied significantly, along with low detection rates, indicating that WbEV infection in wild boars may persist shortly. But their pathogenicity and transmissibility in wild boars need further studies in future. BEFV is an arthropod-borne virus transmitted by mosquitoes and *Culicoides* midges (Venter et al., 2003; Walker, 2005; Chizov-Ginzburg et al., 2023). However, how PoEV is transmitted in pigs is unknown. Interestingly, WbEV was detected in two ticks collected from two positive wild boars, suggesting that ticks may play a role in the transmission of EVs in wild boars, but more studies are needed to verify this in future.

Taken together, the present study provides solid evidence showing natural circulation of divergent EVs in wild boars, and its biological implications should be interesting topics for future investigations.

CONCLUSIONS

In this study, the genetic analysis of WbEV from China showed that there is another new species and an extended host range of EVs, which have their own evolutionary pathways to form a rich diversity. In addition, our results suggest that ticks may play an important role in the transmission of EVs in wild boars.

MATERIALS AND METHODS

Samples and high throughput sequencing

Archived tissue samples of 459 healthy wild boars (tonsils, spleen, liver, kidney, lung, lymph nodes, and serum) were collected between 2018 and 2023 from 26 provinces in China (Fig. 1) for disease surveillance, ranging in age from 3 months to 6 years old and in weight from 10 kg to 150 kg (Gong et al., 2023). To further understand the viruses harbored by the wild boars, these samples were subjected to MTT analysis using previously described method (Sun et al., 2022). Briefly, all samples were ground separately with Minimal Essential Medium (MEM; Corning, USA) to prepare 10% homogenates, followed by centrifugation. The obtained supernatants were pooled (10–20 wild boars per pool) and filtered through 0.45 μm filters. A 400 μL filtrate of each pool were processed for total RNA extraction by using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. The RNA was then used for construction of a long noncoding RNA library after removal of rRNA and precipitation of the remaining RNA by ethanol, followed by high throughput sequencing using the Illumina Novaseq 6000 platform, and the resulted 150 bp paired-end viral reads were *de novo* assembled into viral contigs or whole-genomic sequences by using MEGAHIT v1.1.3, which were then aligned using BLASTn/x.

Virus detection, quantification and sequencing

Clarified supernatants of various tissues of each wild boar were subjected to total RNA extraction using the TIANamp Virus RNA Kit (TIANGEN, China) according to the manufacturer's instructions. The obtained RNA of each wild boar was reverse transcribed into cDNA using random primers and Moloney murine leukemia virus reverse transcriptase (TaKaRa, China), with the resulting cDNA serving as a template for the reverse transcription nested PCR (RT-nPCR). The primers and probe used for detection of WbEV are listed in Supplementary Table S1. The amplified PCR products were directly sent to commercial sequencing (Comate Bioscience, China) with ABI 3700. To further determine the tissue distribution of WbEV, the different tissues of positive wild boars were detected by RT-qPCR (amplification of a conserved 97-bp or 154-bp fragment of the WbEV *N* gene). To quantify the viral loads in the tissues, a plasmid standard was constructed from the prototype strain of PoEV2/GDMM7 (GenBank accession number NC_077154) and synthesized by Comate Bioscience (Jilin, China). The genome equivalent titers of WbEV were determined based on the standard curves.

Alignment and phylogenetic analysis

The Sequence Demarcation Tool (SDT) version 1.3 was used to assess the pairwise sequence identity among the viral sequences (Muhire et al., 2014). Phylogenetic analyses were performed with Clustal W and MEGA 7.0 (Center for Evolutionary Functional Genomics, Tempe, AZ). For sequence analysis, phylogenetic trees based on the complete N and L protein sequences were constructed using the maximum-likelihood (ML) method with 1000 bootstrap replicates and the best fit substitution models. Moreover, the complete genomic sequences of obtained WbEV strains in this study are submitted to the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) using the Web-based submission tool BankIt.

DATA AVAILABILITY

The genome sequence of four WbEVs obtained in this study have been deposited in the GenBank database (accession numbers: PP975073–PP975076) and Science Data Bank (https://doi.org/10.57760/sciencedb.18664).

ETHICS STATEMENT

The sample collection from wild boars was approved by Biological Disaster Control and Prevention Center, National Forestry and Grassland Administration, China. The procedures for sampling and processing wild boars were reviewed and approved by the Administrative Committee on Animal Welfare of Changchun Veterinary Research Institute (Institutional Animal Care and Use Committee Authorization, permit numbers IACUC of AMMS-11-2018-021 and IACUC of AMMS-11-2023-010).

AUTHOR CONTRIBUTIONS

Zhongzhong Tu: methodology, data curation, writing-original draft preparation, writing-reviewing and editing. Tong Wang: methodology, software, data curation. Yu Xu: visualization, investigation, resources. Heting Sun: visualization, investigation, resources. Peng Peng: visualization, investigation. Siyuan Qin: visualization, investigation. Changchun Tu: funding acquisition, writing-reviewing and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2025.02.002.

REFERENCES

Adjadj, N.R., Vervaeke, M., Sohier, C., Cargnel, M., De Regge, N., 2022. Tick-borne encephalitis virus prevalence in sheep, wild boar and ticks in Belgium. Viruses 14, 2362.

Barigye, R., Davis, S., Hunt, R., Hunt, N., Walsh, S., Elliott, N., Dyrting, K., Weir, R., Melville, L.F., 2017. Post-viraemic detection of bovine ephemeral fever virus by use of autogenous lymphoid tissue-derived bovine primary cell cultures. Aust. Vet. J. 95, 49–52.

Barigye, R., Davis, S., Hunt, R., Hunt, N., Walsh, S., Elliott, N., Burnup, C., Aumann, S., Day, C., Dyrting, K., Weir, R., Melville, L.F., 2016. Viral neurotropism, peripheral neuropathy and other morphological abnormalities in bovine ephemeral fever virus-infected downer cattle. Aust. Vet. J. 94, 362–370.

Blasdell, K.R., Widen, S.G., Diviney, S.M., Firth, C., Wood, T.G., Guzman, H., Holmes, E.C., Tesh, R.B., Vasilakis, N., Walker, P.J., 2014. Koolpinyah and yata viruses: two newly recognised ephemeroviruses from tropical regions of Australia and africa. Vet. Microbiol. 174, 547–553.

Blasdell, K.R., Davis, S.S., Voysey, R., Bulach, D.M., Middleton, D., Williams, S., Harmsen, M.B., Weir, R.P., Crameri, S., Walsh, S.J., Peck, G.R., Tesh, R.B., Boyle, D.B., Melville, L.F., Walker, P.J., 2020. Hayes yard virus: a novel ephemerovirus isolated from a bull with severe clinical signs of bovine ephemeral fever is most closely related to puchong virus. Vet. Res. 51, 58.

Chizov-Ginzburg, A., Stram, Y., Rot, A., Taub-Umansky, L., Izhaki, O., Behar, A., 2023. Stretching the wings further- susceptibility of culex pipiens linnaeus to bovine ephemeral fever virus infection under experimental conditions. Acta Trop. 246, 106995.

Cybinski, D.H., Zakrzewski, H., 1983. The isolation and preliminary characterization of a rhabdovirus in Australia related to bovine ephemeral fever virus. Vet. Microbiol. 8, 221–235.

Dacheux, L., Dommergues, L., Chouanibou, Y., Doméon, L., Schuler, C., Bonas, S., Luo, D., Maufrais, C., Cetre-Sossah, C., Cardinale, E., Bourhy, H., Métras, R., 2019. Co-circulation and characterization of novel african arboviruses (genus ephemerovirus) in cattle, Mayotte island, indian ocean, 2017. Transbound Emerg. Dis. 66, 2601–2604.

Doherty, R.L., Standfast, H.A., Clark, I.A., 1969. Adaptation to mice of the causative virus of ephemeral fever of cattle from an epizootic in queensland, 1968. Aust. J. Sci. 31, 365–366.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit i from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Fredriksson-Ahomaa, M., 2019. Wild boar: a reservoir of foodborne zoonoses. Foodb. Pathog. Dis. 16, 153–165.

Gard, G.P., Cybinski, D.H., St George, T.D., 1983. The isolation in Australia of a new virus related to bovine ephemeral fever virus. Aust. Vet. J. 60, 89–90.

Gard, G.P., Cybinski, D.H., Zakrzewski, H., 1984. The isolation of a fourth bovine ephemeral fever group virus. Aust. Vet. J. 61, 332.

Gard, G.P., Melville, L.F., Calisher, C.H., Karabatsos, N., 1992. Koolpinyah: a virus related to kotonkan from cattle in northern Australia. Intervirology 34, 142–145.

Gong, W., Du, H., Wang, T., Sun, H., Peng, P., Qin, S., Geng, H., Zeng, Z., Liang, W., Ling, H., Tu, C., Tu, Z., 2023. Epizootiological surveillance of porcine circoviruses in free-ranging wild boars in China. Virol. Sin. 38, 663–670.

Kemp, G.E., Lee, V.H., Moore, D.L., Shope, R.E., Causey, O.R., Murphy, F.A., 1973. Kotonkan, a new rhabdovirus related to mokola virus of the rabies serogroup. Am. J. Epidemiol. 98, 43–49.

Kirino, Y., Yamamoto, S., Nomachi, T., Mai, T.N., Sato, Y., Sudaryatma, P.E., Norimine, J., Fujii, Y., Ando, S., Okabayashi, T., 2022. Serological and molecular survey of tick-borne zoonotic pathogens including severe fever with thrombocytopenia syndrome virus in wild boars in miyazaki prefecture, Japan. Vet. Med. Sci. 8, 877–885.

Lavon, Y., Ezra, E., Friedgut, O., Behar, A., 2023. Economic aspects of bovine ephemeral fever (bef) outbreaks in dairy cattle herds. Vet. Sci. 10, 645.

Lim, S.I., Kweon, C.H., Tark, D.S., Kim, S.H., Yang, D.K., 2007. Sero-survey on aino, akabane, chuzan, bovine ephemeral fever and Japanese encephalitis virus of cattle and swine in korea. J. Vet. Sci. 8, 45–49.

Meng, X.J., Lindsay, D.S., Sriranganathan, N., 2009. Wild boars as sources for infectious diseases in livestock and humans. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364, 2697–2707.

Muhire, B.M., Varsani, A., Martin, D.P., 2014. Sdt: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9, e108277.

St George, T.D., 1988. Bovine ephemeral fever: a review. Trop. Anim. Health Prod. 20, 194–202.

Sun, Y., Qu, Y., Yan, X., Yan, G., Chen, J., Wang, G., Zhao, Z., Liu, Y., Tu, C., He, B., 2022. Comprehensive evaluation of rna and DNA viromic methods based on species richness and abundance analyses using marmot rectal samples. mSystems 7, e0043022.

Venter, G.J., Hamblin, C., Paweska, J.T., 2003. Determination of the oral susceptibility of south african livestock-associated biting midges, culicoides species, to bovine ephemeral fever virus. Med. Vet. Entomol. 17, 133–137.

Walker, P.J., 2005. Bovine ephemeral fever in Australia and the world. Curr. Top. Microbiol. Immunol. 292, 57–80.

Walker, P.J., Klement, E., 2015. Epidemiology and control of bovine ephemeral fever. Vet. Res. 46, 124.

Walker, P.J., Blasdell, K.R., Calisher, C.H., Dietzgen, R.G., Kondo, H., Kurath, G., Longdon, B., Stone, D.M., Tesh, R.B., Tordo, N., Vasilakis, N., Whitfield, A.E., Ictv Report C, 2018. Ictv virus taxonomy profile: Rhabdoviridae. J. Gen. Virol. 99, 447–448.

Walker, P.J., Freitas-Astúa, J., Bejerman, N., Blasdell, K.R., Breyta, R., Dietzgen, R.G., Fooks, A.R., Kondo, H., Kurath, G., Kuzmin, I.V., Ramos-González, P.L., Shi, M., Stone, D.M., Tesh, R.B., Tordo, N., Vasilakis, N., Whitfield, A.E., Ictv Report C, 2022. Ictv virus taxonomy profile: Rhabdoviridae 2022. J. Gen. Virol. 103. https://doi.org/10.1099/jgv.0.001689.

Wu, Q., Yang, Z., Lu, Z., Mi, S., Feng, Y., He, B., Zhu, G., Gong, W., Tu, C., 2022. Identification of two novel ephemeroviruses in pigs infected by classical swine fever virus. Infect. Genet. Evol. 100, 105273.

Young, P.L., Spradbrow, P.B., 1990. Clinical response of cattle to experimental infection with bovine ephemeral fever virus. Vet. Rec. 126, 86–88.