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Letter

First complete genomic sequence analysis of canine distemper virus in wild boar

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Dear Editor,

Canine distemper virus (CDV) is the causal agent of a highly contagious viral infectious disease that affects domestic and wild carnivores globally. It is an enveloped, non-segmented negative sense RNA virus that belongs to the *Morbillivirus* genus in *Paramyxoviridae* family, which contains viruses of epidemiological relevance to humans and animals. Based on the variability of the hemagglutinin gene (*H*), CDV strains have been at least 21 major genetic lineages: America-1 to America-5, Canada-1 and -2, Asia-1 to Asia-6, Europe wild-life, Arctic, Africa-1/South Africa, Africa-2, South America-1 to South America-3 and Rockborn-like (Giacinti et al., 2022). Mutations in the binding sites of the *H* protein, which interact with viral entry receptors such as signaling lymphocytic activation molecule (SLAM) and nectin-4, are associated with the emergence of the disease in new host species (Beineke et al., 2015).

Canine distemper disease is characterized by strong immunosuppression and lesions in the gastrointestinal, respiratory and neurological systems (Rendon-Marin et al., 2019), leading to high fatality in many mammalian species. Currently, CDV has been discovered to infect hosts from over 20 families of carnivores and non-carnivores (Duque-Valencia et al., 2019), including Canidae, Procyonidae, Mustelidae, Ursidae, Ailuridae, Felidae, Viverridae, Hyaenidae, Tayassuidae, Cercopithecidae, Artiodactyla, Rodentia, Proboscidea, and Primates. CDV has been isolated from javelinas (*Pecari tajacu*, family: Tayassuidae; the New World *Suidae* relatives) suffering severe encephalitis in the deserts of southwest Arizona in 1989 (Appel et al., 1991). Subsequently, about 58% of the serum samples (212/364) collected from javelinas during 1993–1996 had anti-CDV neutralizing antibody, suggesting natural infections with CDV in javelinas in Arizona (Noon et al., 2003). Appel et al. (1974) also reported experimental CDV infection of pigs. In Japan, epizootic infections of CDV in wild boars (26.8%, 11/41) were identified by virus-neutralization (VN) test (Kameo et al., 2012). In China, CDV is a recognized threat to animals in fur farm (raccoon dogs, foxes, minks) and wild animals (such as Siberian tigers, giant pandas, and red pandas) (Wang et al., 2022). A recent study investigated serological surveillance for CDV in hybrid wild boar (0.56%, 2/358) in Heilongjiang Province using a commercial ELISA kit (Wang et al., 2023). However, there are no reports about CDV prevalence in free-ranging wild boars from northeast China.

An epizootiological survey in free-ranging wild boars has been conducted between August 2018 and November 2020 (Gong et al., 2023). During the survey, we collected 582 tissue samples from 97 sick and apparently healthy wild boars in Heilongjiang, Jilin, and Liaoning provinces. Samples (tonsil, spleen, liver, kidney, lung and lymph node) from each boar were mixed and homogenized for RNA extraction and then subjected to viral metagenomic analysis using *meta*-transcriptomic (MTT) protocol as previously described (Sun et al., 2022). Online Blastn/x search based on the high-throughput sequence data revealed that 115 viral reads were annotated to CDV. To further determine the CDV infection rate, we employed the published CDV-specific RT-qPCR method (amplification of a conserved 83-bp fragment of the CDV N protein gene, Methods in Supplementary Materials) to analyze the organ samples from these wild boars. Results showed that the spleen sample of a wild boar collected from Dunhua City in 2019 was CDV RNA positive (Fig. 1A).

To determine the complete genome sequence of the wild boar CDV strain, the positive spleen sample was subjected to MTT sequencing. This resulted in the generation of 1230 CDV reads, which were subsequently assembled into a nearly full CDV genome (named CDV/JLwb/2019). The rapid amplification of cDNA ends (RACE) (TaKaRa, Dalian, China) and specific RT-PCR was used to complete the genome sequence, which has been deposited in GenBank under accession number OR506461. The complete genome contains 15,690 base pairs consisting of 6 genes in the

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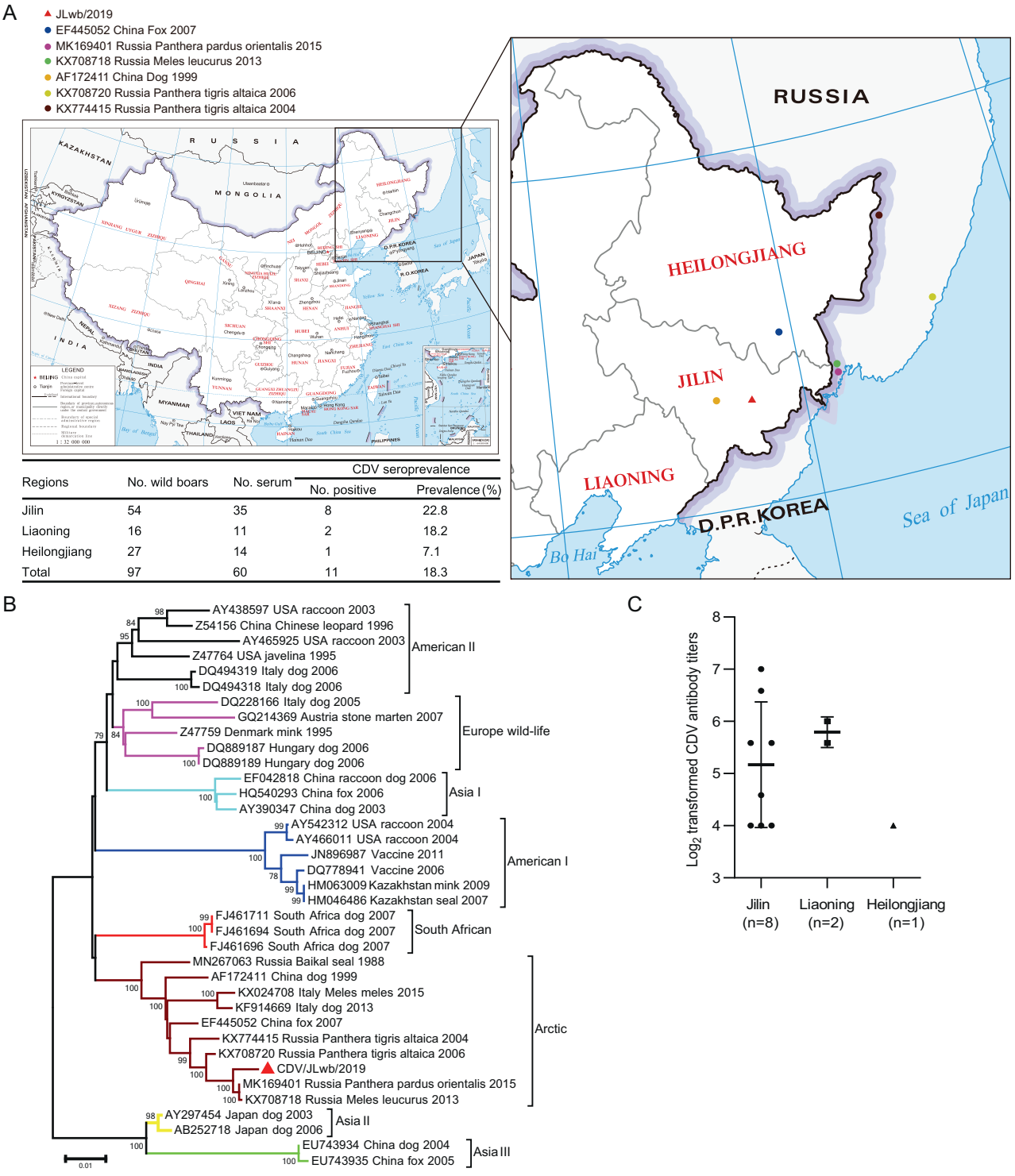

Regions	No. wild boars	No. serum	CDV seroprevalence	
			No. positive	Prevalence (%)
Jilin	54	35	8	22.8
Liaoning	16	11	2	18.2
Heilongjiang	27	14	1	7.1
Total	97	60	11	18.3

Fig. 1. Canine distemper virus (CDV) in wild boars in northeast China provinces. **A** Location of the CDV-positive wild boar in northeast China (red triangle). Colored dots: locations of reference CDV strains. The detailed CDV seroprevalence in serum samples from three provinces are listed. **B** Phylogenetic analysis based on CDV *H* gene sequences was performed by MEGA v7.0 using the neighbor-joining (NJ) method with 1000 bootstrap replicates. Red triangle: CDV strain identified in this study. **C** Titers of neutralizing anti against CDV in serum samples. The virus-neutralization titers were calculated using the method of Reed and body Münch Titers≥1:16 were considered positive.

order 3′-N-P-M-F-H-L-5′, which is typical of CDV genomic structure. The CDV/JLwb/2019 strain exhibits high genome identity (97.3%) with Panthera tigris altaica (CDV/PT61/Pt, 2004) CDV strain, which was isolated in Khabarovskii, Russia (Fig. 1A). Phylogenetic analysis based on the hemagglutinin (*H*) gene showed that the CDV/JLwb/2019 strain belongs to the Arctic lineage (Fig. 1B). A similar phylogenic relationship was obtained using the complete genome (Supplementary Fig. S1). The *H* gene (nt/aa) identities with reference strains ranged from 88.9% to 99.3%, and 83.2%–99.3% respectively, with the closest relationship (99.3% sequence identity) to Panthera pardus orientalis

(FELeopard2015H) and Meles leucurus (FUR0309H) CDV strains collected in Primorye, Russia (Fig. 1A).

Furthermore, the *H* gene-based multiple sequence alignment showed a mutation (C to A) at the 1814 nt position, leading to an amino acid (aa) change from S to a stop codon at aa 605. Four additional amino acid substitutions were identified at aa positions 2 (L to F), 373 (E to D), 594 (N to K) and 571 (D to E) (Supplementary Table S1), compared to CDV Arctic lineage reference strains. Further study is needed to determine whether these aa substitutions in the H protein are associated with cross-species transmission of CDV. However, these results suggest that the CDV/JLwb/2019 strain is closely related to CDV circulating among wild carnivores in the nearby Primorye region of Russia, and wild boar infections may contribute to the transmission of CDV to endangered tigers and leopards (Gilbert et al., 2020).

Virus-neutralization tests were used to investigate the seroprevalence of CDV in wild boars in northeast China (Methods in Supplementary Materials). Results showed that 11 of 60 wild boar serum samples (18.3%) had detectable neutralizing antibodies against CDV, with the neutralizilng antibody titers ranging from 1:16 to 1:128 (Fig. 1C). Among the positive sera, 22.8% (8/35) were from Jilin Province, 18.2% (2/11) were from Liaoning Province, and 7.1% (1/14), were from Heilongjiang Province (Fig. 1A). This result is consistent with previous results from Japan, which reported a CDV seropositivity rates of 18.0% (13/71) in wild boars from Wakayama prefecture (Kameo et al., 2012; Suzuki et al., 2015).

The wild boar from Dunhua City, in which CDV/JLwb/2019 was identified, was 2 years-old and tested positive for both CDV nucleic acid and antibody. However, attempts to isolate CDV using mammalian cell lines Vero and Vero/dog SLAM and Vero/pig SLAM failed, and therefore we were unable to study the infectivity and pathogenicity of the CDV strain in pigs or wild boars.

To our knowledge, this is the first genome sequence analysis of CDV infection of free-ranging wild boar. The results indicate that the natural circulation of CDV in ecosystem may pose a significant threat to the endangered wild carnivores in northeast China. Future surveillance of CDV in forest animals should therefore be considered.

Footnotes

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