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Research paper

Isolation and molecular and phylogenetic analyses of encephalomyocarditis virus from wild boar in central China

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ABSTRACT

Encephalomyocarditis virus (EMCV) can infect many host species and cause acute myocarditis and respiratory failure in piglets, reproductive failure in pregnant sows. In this study, an EMCV strain, designated JZ1202, was isolated from semi-captive wild boars that presented with acute myocarditis and sudden death in central China. It was identified by hemagglutination inhibition (HI) assay, reverse transcription polymerase chain reaction (RT-PCR) and genome sequencing. The subsequent results showed that the virus could produce a specific cytopathic effect on BHK cells and could cause clinical symptoms and pathological changes in mice. Complete genome sequencing and multiple sequence alignment indicated that JZ1202 strain was 81.3%–99.9% identical with other isolates worldwide. Phylogenetic analysis of the whole genome, ORF, VP3/VP1 and 3D genes using neighbor-joining method revealed that JZ1202 isolate was grouped into lineage 1. The results of this study confirmed that an EMCV strain JZ 1202 isolated from wild boar in central China was fatal to mice and provided new epidemiologic data on EMCV in China.

1. Introduction, methods and results

Encephalomyocarditis virus (EMCV; family *Picornaviridae*, genus *Cardioviruses*), is a small, non-enveloped, positive sense single-stranded RNA virus, of a genome size of approximately 7800 bp nucleotides (Palmenberg et al., 1984). It is an important zoonotic disease pathogen that can infect pigs, wild animals, rodents and primates, even humans (Canelli et al., 2010; Jones et al., 2011; LaRue et al., 2003; Oberste et al., 2009). The disease exerts different sequelae in different animals, ranging from asymptomatic persistence in natural reservoirs (rodents) to sudden death in most animal species (Carocci and Bakkali-Kassimi, 2012; Oberste et al., 2009; Philipps et al., 2012). Pigs are considered to be the most commonly and severely infected domestic animal to EMCV (Billinis et al., 1999). EMCV infection can cause severe economic losses on pig farms and is known to be a cause of mortality in young pigs, acute myocarditis, and reproductive failure in sows (Mengeling et al., 2000). Till now, approximately 40 strains of EMCV have been isolated worldwide, and the number of strains discovered in one year is increasing annually. In China, it had been confirmed that EMCV infection occurred in many pig farms, additionally, several EMCV strains have been isolated from exotic pigs (Ge et al., 2010; Zhang et al., 2007).

Wild boar (*Sus scrofa*) may act as a reservoir for many infectious pathogens, such as the causative agents of zoonoses and livestock infectious diseases, and the domestic pig and wild boar share numerous common pathogens (Meng et al., 2009). In Europe, it was reported that wild boar may constitute a reservoir for many diseases that affect domestic pigs (Maurice et al., 2005; Mengeling et al., 2000; Ruiz-Fons et al., 2006; Sedlak et al., 2008). In South Korea, the wild boar population has increased continuously during the last 30 years because of a lack of predators and competitors. This growing population could also increase the risk of Korean wild boar acting as a reservoir for various infectious agents and spreading diseases (Choi et al., 2012). However, few studies have investigated the possible links between EMCV infection and intra-species transmission from wild boar to domestic pigs in China. In the current paper, we described a new strain of EMCV from semi-captive wild boar in central China. The results may examine the possibility of EMCV transmission between domestic pigs and wild boar.

38 clinically ill wild boars were collected in Henan province of China between 2012 and 2015, which exhibited illness symptoms, including anorexia, rapid breathing, staggering and listlessness, treatment with antibiotics was unsuccessful. Post-mortem revealed no obvious lesions except for cerebral hemorrhage, pericardial fluid and myocardial softness (Fig. 1A and B). Fresh tissue samples including brain, heart, liver, spleen, lung and kidney were collected and homogenized with Modified Eagle medium (MEM), frozen and thawed 3 times, and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was passed through a 0.22 μm filter and inoculated into baby hamster kidney 21 (BHK-21)

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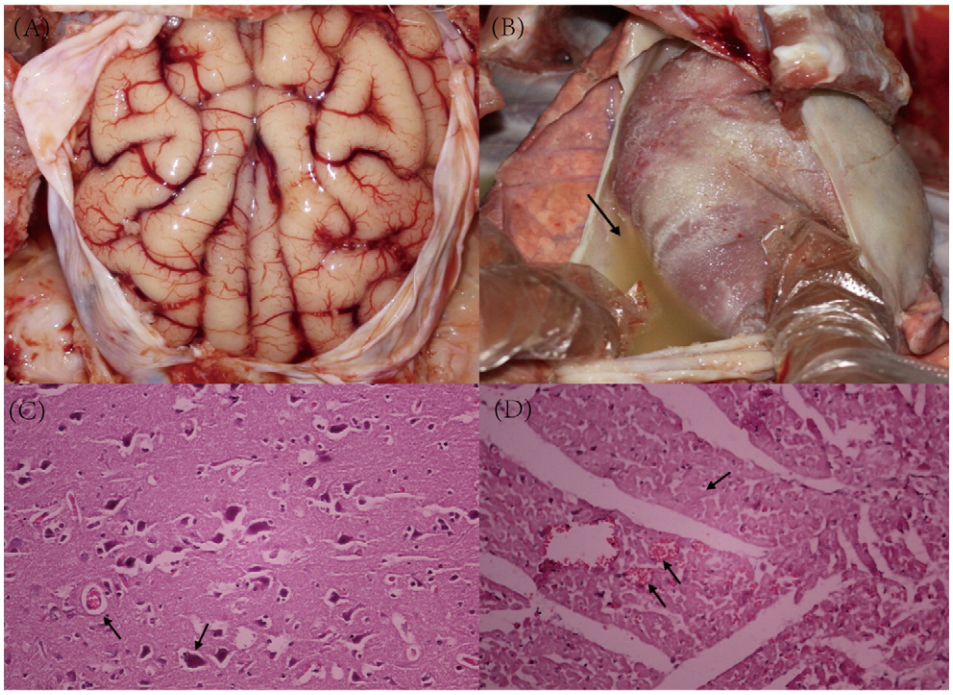

Fig. 1. Pathological and histopathological changes of brain and heart tissues of wild boars. (A) Cerebral hemorrhage; (B) pericardial fluid; (C) hyperemia in myocardium; (D) microglial nodule. Arrows pointed at the pathologic phenomenon.

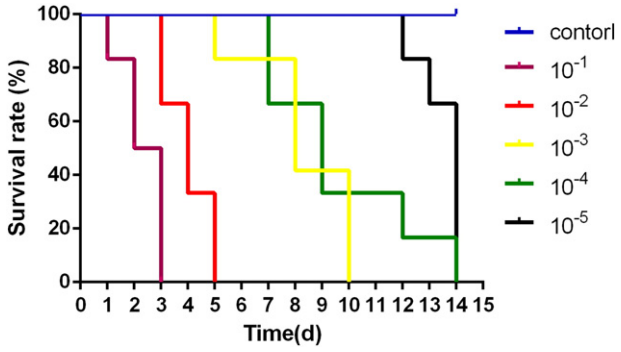

Fig. 2. Survival curve of mice after infected with different dilutions of EMCV JZ1202. All dilutions were made with physiological saline, and physiological saline as a negative control.

cells. After three passages on BHK-21 cells, a distinct CPE was observed in samples from brains and hearts, characterized by cell rounding, pyknosis, and degeneration of the cell monolayer.

The tissue samples of brain and heart were collected for bacteriological, virological and histopathological examinations. Genomic RNA and DNA were extracted and screened against a panel of potential pathogens including EMCV, porcine parvovirus (PPV), classical swine fever virus (CSFV), porcine circovirus (PCV2), pseudorabies virus (PRV) and porcine reproductive and respiratory syndrome virus (PRRS). The results of PCR indicated that the tested specimens were positive for EMCV but negative for other potential pathogens tested, moreover, no pathogenic bacteria were isolated (data not shown). Histopathology studies on brain tissue showed conspicuous microglial nodule, and satellite phenomenon (Fig. 1C), and the heart tissue showed different degrees of lymphocytes and red cell infiltration in myocardium (Fig. 1D). The purified PCR product was sequenced by a commercial corporation

Table 1
Oligonucleotide primers used for the amplification of the complete genome of the JZ1202 strain of encephalomyocarditis (EMCV).

Primers	Sequence 5′–3′	Positions[a]	Amplified fragment
E-1F	ATTGTATGGGATCTGATCTGGGG	605–625	1513 bp
E-1R	GTGTTGGATGCCTCAATGTAGGG	2093–2115	
E-2F	GTATTCTACTCTGCCAGACAGCAC	1948–1971	1575 bp
E-2R	CTAAACAATCTAACCTCCAAACCTC	3498–3522	
E-3F	CTGATTTCGGCACTCTGTTCTTT	3252–3280	2097 bp
E-3R	CATCTGTCGCTTCCTGTCTTGTT	5332–5354	
E-4F	TGGCTAGGATTGAAAGGAAGAAG	5226–5248	1050 bp
E-4R	TTTTACGTGGTACGTGAATACGG	6253–6275	
E-5F	GTAGTGAATGCCTTTGAGCCACA	6200–6222	1314 bp
E-5R	TTCCTGCTTACCAGAATGAACGG	7491–7513	
5'GSP1	GAGGAGGAGTTATTCTTGTCTGAGG	942–966	5′-UTR
5'GSP2	GGGGCCTAGACGTTTTTTAACCTC	659–682	(708 bp)
5′-RACE outer primer	CATGGCTACATGCTGACAGCCTA		
5′-RACE inner primer	CGCGGATCCACAGCCTACTGATCAGTCGATG		
3′-GSP1	ATGTTGTCATACTATCGTCCAGG	7430–7452	3′-UTR
3′-RACE outer primer	TACCGTCGTTCCACTAGTGATTT		(138 bp)

[a] Nucleotide positions were indicated based on the position of EMCV BJC3 (DQ464062).