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Epidemiological and etiological characteristics of viral meningitis for hospitalized pediatric patients in Yunnan, China

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Abstract

Background: Viral infection is the most common cause of aseptic meningitis. The purpose of this study was to identify the viruses responsible for aseptic meningitis to better understand the clinical presentations of this disease.

Method: Between March 2009 and February 2010, we collected 297 cerebrospinal fluid specimens from children with aseptic meningitis admitted to a pediatric hospital in Yunnan (China). Viruses were detected by using "in house" real-time quantitative polymerase chain reaction or reverse-transcription real-time quantitative polymerase chain reaction from these samples. Phylogenetic analyses were conducted using the Molecular Evolutionary Genetic Analysis version 7.0 software, with the neighbor-joining method.

Results: Viral infection was diagnosed in 35 of the 297 children (11.8%). The causative viruses were identified to be enteroviruses in 25 cases (71.4%), varicella-zoster virus in 5 cases (14.3%), herpes simplex virus 1 in 2 cases (5.7%), and herpes simplex virus 2, Epstein–Barr virus, and human herpesvirus 6 in 1 case each (2.9% each). Of the enteroviruses, coxsackievirus B5 was the most frequently detected serotype (10/25 cases; 40.0%) and all coxsackievirus B5 strains belonged to C group.

Conclusions: In the study, a causative virus was only found in the minority of cases, of them, enteroviruses were the most frequently detected viruses in patients with viral meningitis, followed by varicella-zoster virus and herpes simplex virus. Our findings underscore the need for enhanced surveillance and etiological study of aseptic meningitis.

Abbreviations: CMV = cytomegalovirus, CSF = cerebrospinal fluid, CV = coxsackievirus, CVA = coxsackievirus A, CVB = coxsackievirus B, E = echovirus, EBV = Epstein–Barr virus, EVA = enterovirus A, EVs = enteroviruses, HHV-6 = human herpes virus 6, HSV = herpes simplex virus, VP1 = capsid protein VP1, VZV = varicella zoster virus.

Keywords: central nervous system infection, enteroviruses, etiology, phylogenetic analysis, viral meningitis

1. Introduction

Aseptic meningitis is a syndrome of meningeal inflammation in which the cerebrospinal fluid (CSF) is negative for bacterial agents on routine culture.[1] It is a serious neurological disease with high morbidity and mortality.[2] There are an estimated 200,000 new cases of aseptic meningitis annually around the world. Clinical presentations of aseptic meningitis range from benign and self-limited infection to severe disease. Viral infection is the most common cause of aseptic meningitis.[3] Over 100 viruses can cause aseptic meningitis, including enteroviruses (EVs), herpes simplex virus (HSV), varicella-zoster virus (VZV), human herpesvirus 6 (HHV-6), cytomegalovirus (CMV), and Epstein–Barr virus (EBV). Of these, EVs, HSV, and VZV are among the more common etiologies of this disease.[4,5] Other etiological agents are bacteria that are uncultivable on routine culture (*Mycobacterium tuberculosis* and *Treponema pallidum*), *Chlamydia* species, *Rickettsia* species, *Mycoplasma* species, fungi and other parasites, malignancies, immune diseases, drugs, tumors, chemotherapy, foreign bodies, and cysts.[6]

HL and HZ have contributed equally to this study.

Hongbo Liu and Shaohui Ma conceived the study and drafted the article. Haihao Zhang, Ming Zhang, Sanri Cong, and Danhan Xu performed the experiments. Hao Sun, Changzeng Feng, and Zhaoqing Yang helped to interpret results and contributed to the writing. All authors read and approved the final article.

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All experimental protocols involving human samples were approved by the Human Ethics Commission at the Institute of Medical Biology, Chinese Academy of Medical Sciences on February 19, 2009 (2009-2).

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are publicly available.

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Although aseptic meningitis is recognized as a serious public health concern, there is a lack of surveillance systems for this disease worldwide. In China, several related studies have shown viral infection to be the main cause of aseptic meningitis.[7–10] Because of the diversity of causative pathogens and their clinical presentations, it is imperative to identify the major causative viral agents of aseptic meningitis in time and provide preventative treatment. The aim of this study was to identify the viruses responsible for aseptic meningitis by testing for EVs, HSV, VZV, HHV-6, CMV, and EBV to better understand the clinical presentations of this disease.

2. Materials and methods

2.1. Study design

This retrospective observational descriptive epidemiological study included children who were diagnosed with aseptic meningitis between March 2009 and February 2010 at Kunming Municipal Children's Hospital in Kunming, China. As the only hospital for children in the city, it serves approximately 5 million children, with an outpatient capacity of over 2 million children every year. The study design flow diagram is shown in Figure 1.

2.2. Sample collection

Between March 2009 and February 2010, 297 CSF specimens were collected from children who were diagnosed with aseptic meningitis and admitted to a pediatric hospital in Yunnan (China). The samples were collected within 24 hours after onset of illness. This study was approved by the Human Ethics Commission of the Institute of Medical Biology, Chinese Academy of Medical Sciences on February 19, 2009. The standard case definition was defined as aseptic meningitis on the basis of the following criteria: fever ≥38°C within 72 hours, vomiting, headache, convulsion, lethargy, neck stiffness, and negative CSF bacterial culture results. Patients with other final diagnoses (e.g., tumor and epilepsy) were excluded.

2.3. Viral RNA extraction and real-time quantitative polymerase chain reaction

Viral RNA was extracted from CSF samples using a QIAamp viral RNA Mini Kit (QIAGEN, Valencia, CA) as per the manufacturer's recommended procedure. Then, HSV, VZV, HHV-6, CMV, EBV, and EVs were detected by "in house" real-time quantitative polymerase chain reaction (PCR; qPCR) or reverse-transcription real-time qPCR assay as previously described.[11]

2.4. Nested real-time PCR and sequencing

To identify EV serotypes associated with aseptic meningitis, nested-PCR was performed as previously described.[12] All EV-positive PCR products were sequenced directly using an ABI 3730XL automatic sequencer (Applied Biosystems, Foster City, CA).

2.5. EV typing and phylogenetic analysis

Complete *VP1* sequences obtained by sequencing were compared with *VP1* sequences available in the GenBank database. Strains showing more than 75% nucleotide similarity (>85% amino acid identity) were considered to be the same serotype. Phylogenetic trees were constructed by the neighbor-joining method using MEGA 7.0. The statistical significance of phylogenies was estimated by bootstrap analysis with 1000 replicates.

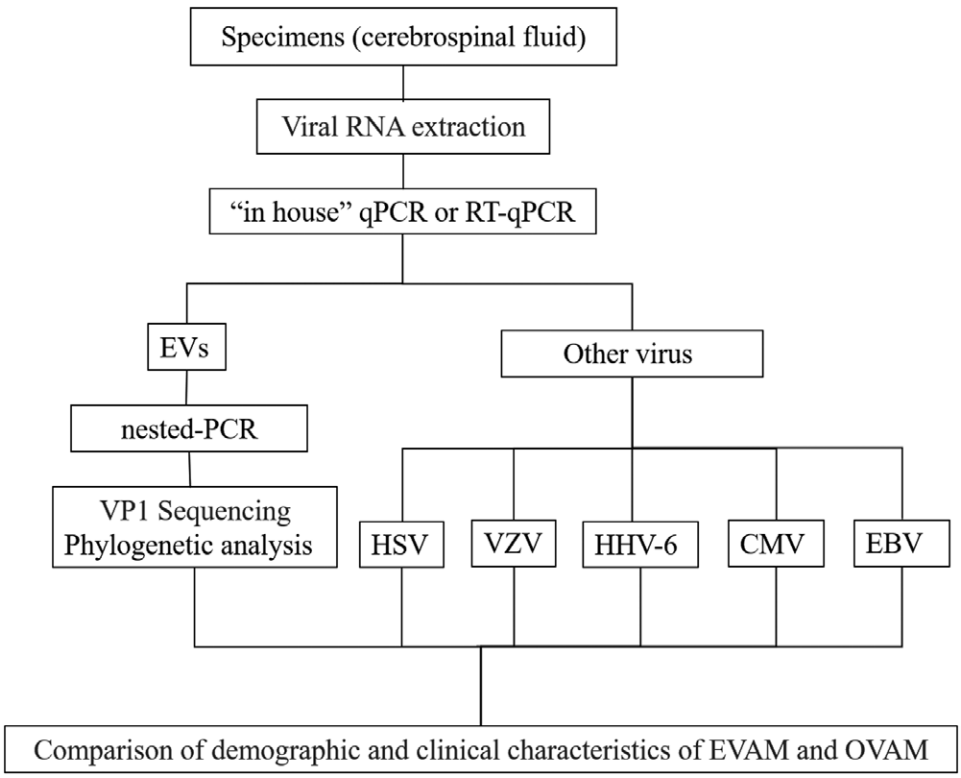

Figure 1. The study design flow diagram. EV = enterovirus, HSV = herpes simplex virus, PCR = polymerase chain reaction, qPCR = quantitative PCR, RT qPCR = real-time qPCR, VP1 = capsid protein VP1.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 12.0 software (Chicago, IL). Statistical significance was defined as $P < .05$ using a chi-square test or t test. We did not calculate the power of the sample because the statistical analysis was performed on unrelated datasets in the study.

2.7. Nucleotide sequence accession numbers

Sequences described in this study have been deposited in GenBank (accession numbers MF098517–MF098531).

3. Results

3.1. Cases and epidemiology

The clinical study group consisted of 297 patients (male, 176; female, 121) with a mean age of 5.5 years (range, 4 months to 14 years). Overall, 35 samples exhibited positive results upon real-time PCR (Table 1), of which 25 were positive for EVs (71.4%), 5 for VZV (14.3%), 3 for HSV (8.6%; HSV-1, 2 cases; HSV-2, 1 case), and 1 each for EBV and HHV-6 (2.9% each). The most common symptoms observed in this cohort were fever (92.9%), headache (81.8%), neck stiffness (72.4%), and vomiting (65.7%). Other symptoms, such as sleepiness, twitching, hyperarousal, limb jitters, dizziness, nervous disorder, cough, paralysis, febrile convulsion, obnubilation, and hallucination, were also observed (Table 2). Children with EV-associated infection were more likely to present with fever and abdominal pain ($P < .001$ and $P = .026$, respectively). And these symptoms were not observed any more frequently in EV-associated aseptic meningitis than in aseptic meningitis resulting from other viral infections (Table 3).

3.2. Enterovirus typing

Among the 25 EV-positive real-time PCR products analyzed by further *VP1* sequencing and molecular typing for EVs, 21 sequences were assigned to 5 different EVs serotypes (Table 4). All clinical isolates from Yunnan belonged to the EVB species, of which coxsackievirus (CV) B5 was the most frequently detected serotype (10/25 cases; 40.0%), followed by echovirus (E) 30 (E30; 4/25; 16.0%), coxsackievirus B1 (CVB1) (3/25; 12.0%), coxsackievirus A9 (CVA9) (2/25; 8.0%), and E9 (2/25, 8.0%).

3.3. Phylogenetic analysis and homologous comparison

Phylogenetic analyses showed that Yunnan CVB5, E30, E9, CVA9, and CVB1 isolates clustered with other corresponding Chinese strains (Fig. 2). The nucleotide and amino acid similarities between these Yunnan CVB5, E30, E9, CVA9, and CVB1 isolates and other corresponding Chinese strains within the complete VP1 sequence were 89.9% to 99.6% and 93.6% to 100%, 92.0% to 95.1% and 97.9% to 99.3%, 89.0%% to 98.5% and 95.4%% to 98.0%, 92.9% to 96.0% and 80.6% to 99.2%, and 95.7% to 99.3% and 88.4% to 99.6%, respectively.

4. Discussion

In the 297 CSF samples collected from patients with aseptic meningitis from 2009 to 2010, 35 (11.78%) positive cases were caused by viral agents. This is in line with a previous study (732/6,705, 10.9%, 2015–2019), which was confirmed using the FTD-viral meningitis diagnostic kit.[13] Compared to our study, authors from other countries found higher rates of viral agents, including Brazil (44/70, 62.9%, 2010 and 2013), which was confirmed using the SYBR qPCR,[14] Lebanon (205/252, 82.7%, 2008–2016)[15] and the United Arab Emirates (34/92, 37%; 2000–2005),[16] which were confirmed using laboratory tests (clinical grounds and by exclusion of bacterial meningitis). The differences in prevalence between the different studies could be attributed to the test method and population of patients, and factors such as age group, sampling time, sample storage and transport, season, sporadic cases or an outbreak, and the high variability of the viral genes or other viral

Table 1

Disease etiology and demographic characteristics of patients with aseptic meningitis diagnosed by CSF qPCR.

	EV	VZV	HSV-1	HSV-2	EBV	HHV-6	Undetermined	Total
Number of patients (%)	25	5	2	1	1	1	262	297
Mean age, years (range)	5.8 (3–9)	3 (2–3)	3.5 (1–7)	8	7	13	5.5 (1–14)	5.5 (1–14)
Number of female patiens (%)	11 −44	2 −4	0 0	1 −100	1 −100	0 0	106 −42.1	121 −45.3

CSF = cerebrospinal fluid, EBV = Epstein–Barr virus, EV = enterovirus, HHV = human herpes virus, HSV = herpes simplex virus, qPCR = quantitative polymerase chain reaction, VZV = varicella zoster virus.

Table 2

Clinical characteristics of patients with aseptic meningitis.

	Number of patients (%)							
Symptoms	**EV**	**VZV**	**HSV-1**	**HSV-2**	**EBV**	**HHV-6**	**Undetermined**	**Total**
Headache	19 (76.0)	2 (40.0)	1 (50.0)	1 (100)	1 (100)	1 (100)	212 (80.9)	243 (81.8)
Fever	17 (68.0)	5 (100)	2 (100)	1 (100)	1 (100)	1 (100)	249 (95.0)	276 (92.9)
Vomiting	16 (64.0)	2 (40.0)	1 (50.0)	1 (100)	0 (0)	0 (0)	175 (66.8)	195 (65.7)
Neck stiffness	18 (72.0)	4 (80.0)	1 (50.0)	0 (0)	0 (0)	1 (100)	191 (72.9)	215 (72.4)
Sleepiness	2 (8.0)	3 (60.0)	0 (0)	0 (0)	0 (0)	0 (0)	52 (19.9)	57 (19.2)
Dizziness	3 (12.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (5.0)	16 (5.4)
Cough	3 (12.0)	1 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	24 (9.1)	28 (9.4)
Abdominal pain	2 (8.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (1.5)	6 (2.0)
Others	0 (0)	1 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	91 (34.6)	171 (57.4)

Others symptoms: hyperarousal, limb jitter, diarrhea, nerve malaise, paralysis, febrile convulsion, obnubilation, hallucination and twitch.
EBV = Epstein–Barr viru, EV = enterovirus, HHV = human herpes virus, HSV = herpes simplex virus, VZV = varicella zoster virus.

Table 3

Comparison of demographic and clinical features of patients with aseptic meningitis associated with enteroviruses and other viruses.

Variable	EVAM (n = 25)	OVAM (n = 272)	*P* value
Demographics			
Mean age, years	5.8 ± 3.4	5.1 ± 3.5	–
Sex(no. [%])			
Male	14 (56.0)	162 (59.6)	.729
Female	11 (44.0)	110 (40.4)	
Clinical symptoms [no.(%)]			
Fever	17 (68.0)	259 (95.2)	<.001
Headache	19 (76.0)	218 (80.2)	.621
Vomiting	16 (64.0)	179 (65.8)	.855
Neck stiffness	18 (72.0)	197 (72.4)	.964
Sleepiness	2 (8.0)	55 (20.2	.138
Dizziness	3 (12.0)	13 (4.8)	.126
Abdominal pain	2 (8.0)	4 (1.5)	.026
Cough	3 (12.0)	24 (8.8)	.597

EVAM = enterovirus-associated aseptic meningitis, OVAM = other-virus-associated aseptic meningitis.

The significance of bold values: children with EV-related infections are statistically more likely to have fever and abdominal pain ($P < .001$ and $P = .026$, respectively). When $P < .05$, the difference was statistically significant.

Table 4

Enterovirus serotypes identified in 25 CSF samples in Yunnan, China.

Enterovirus serotypes	Number detected	Percentage of total (%)
Coxsackievirus B5	10	40
Echovirus 30	4	16
Coxsackievirus B1	3	12
Coxsackievirus A9	2	8
Echovirus 9	2	8
Not detected	4	16

CSF = cerebrospinal fluid.

infections.[17,18] In this study, the most frequent cause of viral meningitis was EVs infection, followed by VZV and HSV infection. In America, in addition to EVs and various arboviruses, HSV and VZV have also been frequently isolated in patients with aseptic meningitis.[19] In Poland, the most common cause of this disease among adult patients has been reported to be HHV-1 infection, followed by infection with EVs, VZV, and tick-borne encephalitis virus.[20] In Georgia, EVs, VZV, and HSV-1 have been reported to be the more frequent pathogens isolated in this disease.[21] In Guangxi, China, the 3 most commonly identified viral etiological agents have been reported to be EVs, mumps virus, and Japanese encephalitis virus.[9] However, in other provinces of China, EVs, VZV, and HSV-1 have been reported to be the main viral etiological agents of aseptic meningitis.[22,23] Thus, the prevalence and distribution of viral etiological agents that cause aseptic meningitis vary among different parts of the world.[24,25] Given that more than 100 viruses can cause this disease, the etiologic diagnosis of aseptic meningitis is challenging. This was true in our study as well.[26,27] Generally, several main viruses are detected in cases of aseptic meningitis.

However, early etiological diagnosis helps improve disease prognosis. Therefore, it is necessary to improve the process of etiological diagnosis of aseptic meningitis.

In children, EVs have been reported to be the predominant pathogen of viral meningitis,[28] with the E30, E6, CVA9, CVB3, and CVB5 serotypes being frequently associated with outbreaks.[24,29] In the present study, the most commonly occurring EV serotype was CVB5, followed by E30, CVB1, CVA9, and CVB3. However, in Spain, the predominant serotype detected between 1998 and 2007 was reported to be E30, followed by E6 and E13[30]; in 2008, E4 was the most frequently identified serotype, followed by E30 and E9.[31] In 2013 and 2015, E30 was found to be the most and second-most frequently detected serotype in adult and pediatric populations in Switzerland, respectively; however, in 2014, E30 was not detected in either population.[32] In Hangzhou, China, E30 was found to be the predominant serotype in 2105, followed by E5 and E16.[33] Thus, a previously rare serotype might become the predominant strain over time, changing the etiology of the disease.

Since 2008, hand-foot-and-mouth disease caused by EVs has emerged as a common EV-associated disease, especially in China. Cases of EV-associated hand-foot-and-mouth disease with aseptic meningitis have been reported, with the identified isolates from these cases including enterovirus A (EVA) (EVA71, CVA16, CVA6, CVA10, and CVA4) and EVB (E9, E30, CVB5, CVA9, and CVB3); in some of these cases, EV has been characterized as the major causative pathogen.[25,34] Thus, EVs could continue to remain important causative pathogens of aseptic meningitis in China. Etiological survey of aseptic meningitis might reveal new and reemerging pathogens.

All CVB5, E30, CVB1, CVA9, and E9 strains detected in the present study belonged to a single genogroup and were closely related to strains from geographically nearby areas as well as strains that occurred during the same period. This indicates that there might be no obvious variation of prevalent serotypes in China at present. This finding is helpful for developing an appropriate vaccine and vaccination strategy.

There are some limitations to this study. First, the study data were collected in 2009 and 2010, which was a major limitation because the epidemiological and etiological characteristics may have changed. Second, the study was performed in a single-center, and may not be representative of the full region. Third, the findings are limited by the absence of testing for more viral infections. The viruses detected in the study are not fully representative of the outbreak.

5. Conclusion

In the present study, EVs were the most frequently detected viruses in patients with aseptic meningitis, followed by VZV and HSV. Of the EVs, CVB5 was the most frequently detected serotype. Molecular diagnosis is essential for management of patients with aseptic meningitis. Our findings underscore the need for enhanced surveillance and etiological study of aseptic meningitis, and this should help clinicians to treat related diseases better.

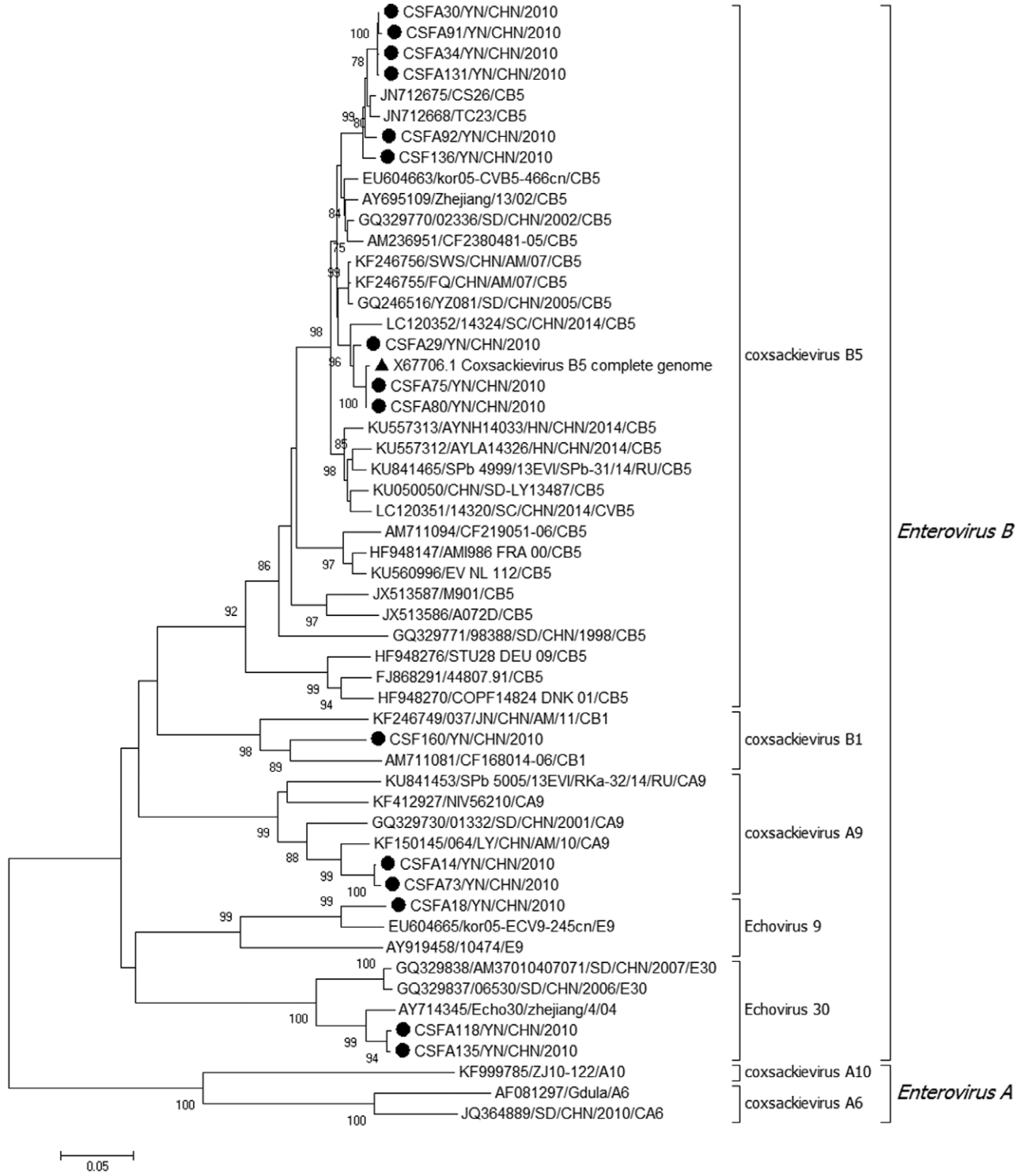

Figure 2. Phylogenetic analysis of the complete *VP1* region of the enterovirus genome. The tree was constructed using the neighbor-joining method and Kimura 2-parameter model. The significance of phylogenies was investigated by bootstrap analysis with 1000 pseudoreplicate datasets. Bootstrap values >70% are indicated on the tree. ▲ indicates prototype strains, VP1 = capsid protein VP1.

Author contributions

Hongbo Liu and Shaohui Ma conceived the study and drafted the article. Haihao Zhang, Ming Zhang, Sanri Cong, and Danhan Xu performed the experiments. Hao Sun, Changzeng Feng, and Zhaoqing Yang helped to interpret results and contributed to the writing. All authors read and approved the final article.

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