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Short Communication

An outbreak of aseptic meningitis caused by a distinct lineage of coxsackievirus B5 in China

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SUMMARY

In 2009, an outbreak of aseptic meningitis caused by coxsackievirus B5 (CVB5) occurred in China. Epidemiological investigations of this outbreak revealed that the proportion of severe cases (14/43, 33%) was higher than in other outbreaks associated with CVB5 in China. Phylogenetic analysis of the entire VP1 sequences demonstrated that the CVB5 isolates from the severe cases form a distinct lineage belonging to genogroup E with the Shandong isolates of 2009. A substitution of serine (S) to asparagine (N) at amino acid 95 in the VP1 region may be a major virulence determinant for the virus. Our findings suggest that this new lineage of CVB5 is circulating in China. Further genetic studies are needed in order to gain a better insight into the genetic variability of CVB5 isolates and the relationship with pathogenicity.

1. Introduction

Coxsackievirus B5 (CVB5) is a member of the genus *Enterovirus* (EV), family *Picornaviridae*. CVB5 is associated with cases of aseptic meningitis, encephalitis, paralysis, and some chronic diseases.[1] CVB5 has been recognized for over 50 years and remains one of the most commonly reported EV serotypes. Outbreaks due to CVB5 have been reported worldwide, including three occurring in the USA in 1961, 1972, and 1983, respectively.[2] Several outbreaks of aseptic meningitis caused by mixed EV serotypes have been noted in Greece in 1999 and 2001, and in Belgium in 2000.[3,4] In China, CVB5 is frequently associated with sporadic cases, although outbreaks, including ones in Shandong Province in 2005 and 2009, are also evident.[1,5] Here we report an outbreak of aseptic meningitis caused by CVB5 in Henan Province, China.

2. Methods

During July 2009, 43 pediatric patients with aseptic meningitis were admitted to the 153rd Hospital of Zhengzhou in Henan Province, China. Clinical and epidemiological investigations were carried out in the hospital using a standard questionnaire. Cases typically presented with fever, headache, nausea, vomiting, and some neurological manifestations. Severe cases were defined in accordance with the case definition described previously.[6]

Cerebrospinal fluid (CSF) samples were collected from each case and were inoculated into human rhabdomyosarcoma (RD) cell lines for EV culture. Viral RNA was extracted directly from CSF specimens and cell cultures that exhibited a typical EV cytopathic effect (CPE). RT-PCR assays, described previously,[7,8] were used to detect the conserved 5′-untranslated region (5′UTR) and the viral capsid protein 1 (VP1) gene. The sequences reported here were deposited in GenBank (accession numbers **HQ830207**–**HQ830209**, **HQ830211**–**HQ830223**). Additional CVB5 sequences were retrieved from GenBank to construct a phylogeny using Mega 4.0. The study protocol was approved by the Medical Ethics Committee of the Academy of Military Medical Sciences.

3. Results

An outbreak of aseptic meningitis occurred between July 8 and July 29, 2009 in Zhongyuan District of Zhengzhou City, Henan Province, China (**Supplementary Material**, Figure S1). Forty-three cases (including 14 severe cases) were documented. The median

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Table 1
Summary of clinical and laboratory characteristics associated with an outbreak of aseptic meningitis due to coxsackievirus B5 in Henan Province, China, 2009 (N = 43)

Characteristics	Result
Sex	
Male	27 (63%)
Female	16 (37%)
Age	
≤5 years	18 (42%)
≥6 years	25 (58%)
Median (range), years	6.5 (1–13)
Symptoms	
Fever	39 (91%)
Meningeal irritation	27 (63%)
Vomiting	25 (58%)
Nausea	19 (44%)
Myoclonus	12 (28%)
Cough	13 (30%)
Lethargy	16 (37%)
Positive test (No.)	16 (37%)
Median WBC in blood ($\times 10^9$/l)	9.33
Median WBC in CSF ($\times 10^6$/l)	234
Median NEUT-R in blood (%)	60.2
Median protein in CSF (g/l)	0.313

WBC, white blood cells; CSF, cerebrospinal fluid; NEUT-R, neutrophile ratio.

age of cases was 6.5 years (range 1–13 years) and cases aged >5 years accounted for 58% of all cases. Twenty-seven cases (63%) were male (Table 1).

Sixteen CVB isolates were recovered from CSF after cell culture; 14 of these were from severe cases and two were from mild cases. Blast analysis of the partial VP1 sequences showed that all 16 isolates were CVB5 with >93.7% nucleotide identity. The data strongly suggest that CVB5 was responsible for the observed outbreak. Comparison of the translated amino acid sequence of the 16 partial VP1 sequences representing CVB5 isolates detected in this study and other outbreak-associated CVB5 isolates, indicated that a serine (S) to asparagine (N) substitution at amino acid 95 in VP1 was present in all cases of severe aseptic meningitis in the Henan outbreak, but not in either of the two mild cases (represented by HN24 and HN28; Figure 1). Phylogenetic analysis of CVB5 VP1 sequences clustered all 16 isolates into two distinct sublineages (I and II) in genogroup E (Figure 2). The mean p-distance between these two sublineages is 6.8%. Of note, the two Henan isolates from mild cases (HN24 and HN28) in this outbreak belong to sublineage I, while all the severe cases cluster to sublineage II. These results suggest that the severe cases form a distinct lineage in genogroup E.

4. Discussion

Concerns regarding an apparent increased virulence of CVB5-associated infections prompted this investigation. In 2005, during a CVB5 outbreak in Shandong Province, all patients presented with mild symptoms and no severe or fatal cases occurred.[9] However, in less than 5 years, two outbreaks with severe cases, one in Shandong[1] and this outbreak in Henan, occurred. Phylogenetic analyses clearly demonstrated that the Henan outbreak was probably caused by two distinct viral lines. Strains from severe cases in sublineage II had not been observed before 2009 when viruses from this sublineage were observed in both Shandong and Henan provinces. These results indicate that there are two major genetic lineages of CVB5 existing in China. Sublineage I has been the predominant circulating lineage since 1998, while sublineage II is a new viral line first observed in Shandong and Henan provinces in 2009. In the new lineage, S at amino acid 95 in the VP1 region is replaced by an N among the present strains in sublineage II. Al-Hello et al.[10] characterized an alteration of amino acid 95 in VP1 that played a role in mice infected with CVB5. Amino acid 95 is located at the outer surface of the virion, suggesting that it might be involved in virus–cell interactions, possibly augmenting the initiation of virus infection.[11] We hypothesize that the severity of clinical disease is associated with this substitution. Hence, further genetic studies are needed in order to gain a better insight into the genetic variability of CVB5 isolates and any relationship with pathogenicity.

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#CVB5-Zhejiang-CHN-2002(AY695108.1) NHGTDGDNFG YWVISTRQVA QLRRKLEMFT YARFDLELTF VITSTQEQST IQGQDSPVLT HQIMYVPPGG PVPTKVNSYS  [160]
#CVB5-Zhejiang-CHN-2002(AY695109.1) .......... .......... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-SD-CHN-2002(GQ329770.1)       .......... .......... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-SD-CHN-2005(GQ246506.1)       .......... .......... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-SD-CHN-2005(GQ246515.1)       .......... .......... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-SD-CHN-2005(GQ246516.1)       .......... .......... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-CS26-SD-CHN-2009_(JN712675.1) .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-CS35-SD-CHN-2009_(JN712670.1) .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-Full-SD-CHN-2009_(JX276378.1) .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN24-CHN-2009_(HQ830220)      .......... .......... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN28-CHN-2009_(HQ830221)      .......... .......... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN5-CHN-2009_(HQ830207)       .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN6-CHN-2009_(HQ830208)       .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN7-CHN-2009_(HQ830209)       .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN9-CHN-2009_(HQ830211)       .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN10-CHN-2009_(HQ830212)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN11-CHN-2009_(HQ830213)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN12-CHN-2009_(HQ830214)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN13-CHN-2009_(HQ830215)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN14-CHN-2009_(HQ830216)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN15-CHN-2009_(HQ830217)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN21-CHN-2009_(HQ830218)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN22-CHN-2009_(HQ830219)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN32-CHN-2009_(HQ830222)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
#CVB5-HN33-CHN-2009_(HQ830223)      .......... ....N..... .......... .......... .......... .......... .......... ..........  [160]
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Figure 1. Sequence alignment of the VP1 region of the strains in China during 2002, 2005, and 2009. The amino acid sequences are numbered according to the sequence of CVB5-Zhejiang-CHN-2002 (**AY695108.1**). Identical residues are indicated with dots. Amino acids that differ from the consensus sequence are shaded.

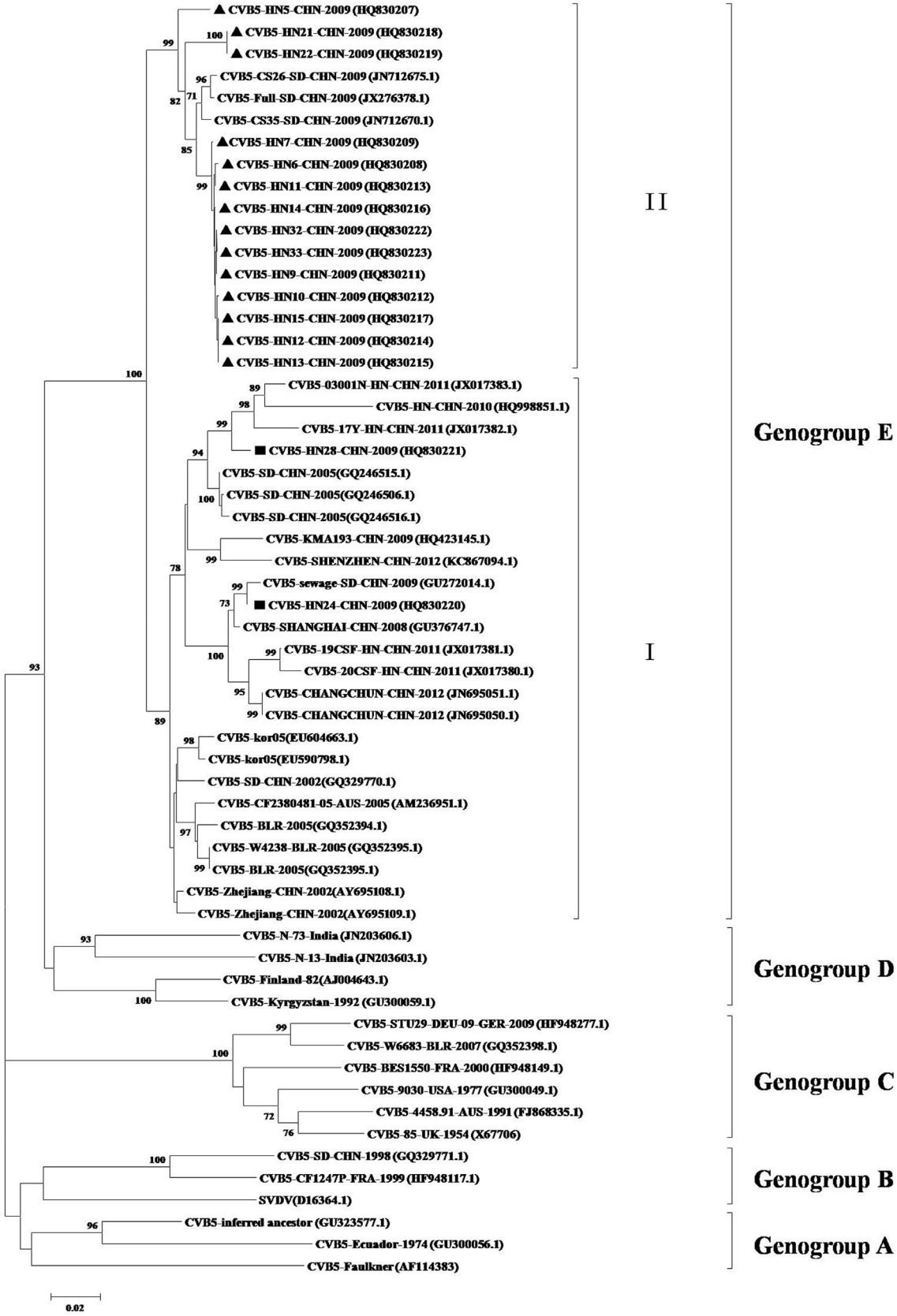

Figure 2. Dendrogram showing the phylogenetic relationships of the coxsackievirus B5 (CVB5) isolates in this outbreak and the reference strains from GenBank based on VP1 sequence alignment; this was constructed using Mega software version 4.0. The isolates analyzed in this outbreak are indicated with black dots. ▲, CVB5 strains from severe cases detected in the present study. ■, CVB5 strains from mild cases detected in the present study. SD, Shandong Province; HN, Henan Province.

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Conflict of interest: All authors report no conflicts.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2014.02.005.

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