

# Clinical Epidemiological and Pathogenic Analyses for Acute Viral Infections of Central Nervous System in Hospitalized Children of Hunan Province, China

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## Abstract

**Background:** Virus infection of the central nervous system is a common disease in children. The major causes of ineffective therapy are misdiagnosis and delayed interventions. Based upon our pilot study, consecutive specimens of cerebrospinal fluid were collected and detected.

**Methods:** From October 2012 to July 2014, a total of 161 cerebrospinal fluids samples were collected from the patients with a clinical diagnosis of viral encephalitis at our hospital. The technique of real-time fluorescence quantitative-polymerase chain reaction was employed.

**Results:** Viruses were detected in 71 cases with an overall detection rate of 44.10%. In our cohort, the average viral load was  $198.24 \pm 993.61$  copies/ $\mu$ L. And the viral loads had no obvious correlations with hospitalization duration. Enterovirus had the highest detection rate of 18.01% and occurred predominantly in spring and summer. The highest detection rate was found in the 3-6 year age group. Most cases of Human herpesviruses-6 were detected in autumn. And Adenovirus positive cases were largely restricted for rural children. Only 1 Mumps virus positive case had an enlargement of parotid gland. None of 12 Measles virus positive cases had the classic manifestations of measles.

**Conclusions:** During the study period, 11 common viruses in cerebrospinal fluids were detected from hospitalized children of viral encephalitis. The detection rates have slightly increased as compared with earlier reports. And Measles virus and Mumps virus were increasingly detected. For positive and negative viral cases, no differences existed in gender, age, urban-rural region or clinical characteristics. And the positive cases of Enterovirus, Measles virus and Mumps virus have their unique profiles.

**Keywords:** Virus; Encephalitis; Hospitalized children; Fluorescence quantitative-polymerase chain reaction; Cerebrospinal fluid

## Introduction

According to the statistical figures of World Health Organization (WHO), the global mortality toll was estimated at 7.6 million for children aged under 5 years in 2010. And 64% was related with infectious diseases [1]. As one of the most common infection sites for children, central nervous system (CNS) was predominantly attacked by viruses [2]. As revealed by a survey report of 103 encephalitis patients at Johns Hopkins University Hospital in the US, the causes were viral (27.18%), autoimmune (16.5%) and non-specific (46.6%) [3].

The clinical manifestations of acute viral encephalitis (AVE) and cerebropathy are somewhat similar and partially overlapping. According to the latest British guideline, the criteria of encephalitis (VE) included encephalitis (>24 h mental disturbance of varying extents) plus at least two of the following accompanying conditions: fever, convulsion, nervous system impairment, an elevated cell count in CSF, abnormal electroencephalography (EEG) and neuroimaging changes [4]. Currently there are over 100 viruses causing VE [5]. And

new viruses have continuously emerged. In cases with a definite etiological diagnosis, clinical manifestations and axillary examination results have not shown any specificity. According to the literature report, among 75 children positive for virus in CSF, there were fever (n=68), mental disturbance (n=52), vomiting (n=51), headache (n=41) and convulsion (n=33). And auxiliary examinations revealed elevated leucocyte count in CSF (n=43), elevated protein in CSF (n=37), abnormal EEG findings (n=38) and neuroimaging changes (n=34) [6]. It suggested that encephalitis due to different pathogenic viruses may manifest similar symptoms and signs. And some clinical manifestations are also similar to those of other CNS infectious and non-infectious illnesses. Therefore an etiological diagnosis is of vital importance. And an incorrect diagnosis and delayed treatment are often two major causes of therapeutic failure [7]. At present, detecting viruses in CSF samples through molecular biological methods has become a golden standard for an etiological diagnosis of viral infection in CNS [8]. It is reported in the literature that the magnitude of viral load was closely correlated with disease severity [9]. And early diagnosis and early treatment could lower the viral load and boost the therapeutic efficacies [10]. In recent years, the arrival of fluorescence quantitative-polymerase chain reaction (FQ-PCR) has marked a great

transformation from qualitative to quantitative analyses of viruses. And it is possible to make accurate measurements of viral load [11]. Globally the most common viruses are Herpes Simplex Virus type (HSV), Varicella-Zoster virus (VZV), Enterovirus (EV), Measles virus (MV), Mumps virus (MuV), Japanese Encephalitis virus (JEV), Adenovirus (ADV) and etc. [12].

From October 2012 to July 2014, based upon earlier studies, CSF samples were collected consecutively from the hospitalized children of acute CNS infectious illnesses at Center of Pediatric Medicine (CPM), Hunan Provincial People's Hospital (HPPH). The technique of FQ-PCR was employed for detecting the following 11 viruses of EV, MV, MuV, JEV, ADV, VZV, Human parainfluenza virus (HPIV), Herpes simplex virus type 1 (HSV-1), Herpes simplex virus type 2 (HSV-2), Epstein-Barr virus (EBV) and Human herpesviruses-6 (HHV-6). In conjunction with the relevant clinical profiles, analyses were performed for providing the latest reference rationales for a local control of this disease.

## Materials and Methods

### Specimen sources and extraction of nuclear acid samples

This is a retrospective co-operative study between HPPH and China National Center for Disease Control & Prevention (CNCDCP), monitoring the CNS virus infection in children of Hunan Province. CNS virus surveillance was initiated in July 2010. The initial sample size was 93 specimens. For this study, a total of 161 CSF specimens were collected from the patients with a clinical diagnosis of viral cerebritis at CPM, HPPH during the period of October 2012 to July 2014. The specimens were preserved at a temperature of -80°C. Prior to collection, informed consent was obtained from patient relatives and the protocol approved by our Institutional Ethics Committee. All CSF samples were shipped within dry ice. After de-frosting, 200  $\mu$ L aliquots were dispensed. And viral nuclear acid was extracted according to the user's instructions of QIAamp MinElute Virus Spin Kit (Qiagen, Shanghai, China). The specimens were promptly delivered to CNCDCP for further detection and analysis.

### Materials

The positive samples of MV and JEV were donated by National Institute for Viral Disease Control & Prevention (NIVDCP), China CDC. And the specific plasmids containing the segments of target genes of EV, MuV, HPIV, HSV-1, HSV-2, ADV, EBV and HHV-6 were supplied and maintained by NIVDCP. And wild-type VZV-specific plasmids were synthesized by Beijing Tianyi Huiyuan Biotech Inc. All kits of MinElute Mini DNA Extraction, QIAquick Gel Extraction and QuantiTect Probe PCR were purchased from German Qiagen Inc.; High-Speed Plasmid Mini Kit from Taiwan Geneaid Inc.; pGEM-T Easy vector (Promega, Madison, WI, USA); fluorescent real-time quantitative polymerase chain reaction (PCR) cyclers were Step One Plus real-time fluorescent quantitative PCR.

### Primers and probes

For EV, MV, MuV, HPIV, HSV-1, HSV-2, ADV, VZV, EBV and HHV-6, the sequences of their primers and corresponding Taqman probes followed those of the references [11,13-20]. For JEV detection, the primers and corresponding Taqman probe sequences derived from the patented method and reagent kit of testing type B encephalitis virus [21] were generously provided by NIVDCP, China CDC. Both

primers and probes were synthesized by Shanghai Invitrogen Biotech Inc. PAGE was used for purifying primers. EV, MuV, HPIV, HSV-1, HSV-2, ADV, JEV, EBV, HHV-6, etc. The 5'ends of 9 viral probes were labeled with FAM while the 3'ends with TRAMA. And the 5'end of MV probe was labeled with FAM and the 3'end with BHQ1. The 5'end of VZV probe was labeled with FAM and the 3'end with MGB.

### Specimen detections

A 1:10 dilution of standard was used as a positive control and sterile de-ionized water as a negative control. And the FQ-PCR method was employed for detecting 11 types of viruses in CSF samples. The minimal sensitivity detection thresholds were established for all individual viruses. StepOne Software v2.2 software was used for analyzing the results.

### Statistical analyses

The SPSS (Statistical Product & Service Solutions) version 13.0 software was employed for data processing. The quantitative values were expressed as medians and quartiles. Two samples were tested by the method of Mann-whitney U while Kruskal-Wallis H test was employed for multiple samples. For multiple samples, Nemenyi test was used for paired comparison; quantitative data were expressed as percentage and  $\chi^2$  test was adopted. The Pearson's correlation coefficients were used for correlation analysis; a testing threshold was set at 0.05. And a difference of  $p < 0.05$  was deemed statistically significant.

## Results

From October 2012 to July 2014, a total of 161 CSF samples were collected from children with acute VE at our center. And viruses were detected in 71 cases with an overall detection rate of 44.10%. EV had the highest detection rate of 18.01% while MuV yielded the second highest of 8.07%. And there was no detection of VZV (Table 1).

Viral classification	Number of positivity	Detection rate (%)	Composition ratio (%)
EV	29	18.01	34.94
MuV	13	8.07	15.66
MY	12	7.45	14.46
EBV	8	4.97	9.64
HPIV	8	4.97	9.64
ADV	6	3.73	7.23
HHV-6	4	2.48	4.82
JEV	1	0.62	1.2
HSV-1	1	0.62	1.2
HSV-2	1	0.62	1.2
VZV	0	0	0

**Note:** Multiple viruses might be detected in one single specimen.

**Table 1:** Detection status of CSF samples in hospitalized AVE children n=161.

### Gender and age distributions of hospitalized AVE children

Among hospitalized AVE children, viruses in CSF samples were detected in 38/93 males and 33/68 females. The male-to-female ratio was 1.37:1. No statistical difference existed in viral detection rate between two genders ( $\chi^2=0.937$ ,  $p=0.333$ ). Among 161 hospitalized AVE children, the minimal age was 3 months, the maximal age 13 years and the average age  $52.51 \pm 38.35$  months. Overall the age group of 3-6 years was the most common and the second common age group was 1-3 years. However, viral detection rate showed no statistically significant difference among all age groups ( $\chi^2=3.076$ ,  $p=0.380$ ) (Table 2).

Age group	Age & gender distributions of AVE			Age & gender distributions of viral-positive children		
	Total	Male	Female	Total	Male	Female
< 1 yr	19	11	8	8	4	4
1-<3 yr	47	27	20	17	10	7
3-<6 yr	61	35	26	32	17	15
6-14 yr	34	20	14	14	7	7
Total	161	93	68	71	38	33

**Table 2:** Age and gender distributions of viral -positive hospitalized AVE children.

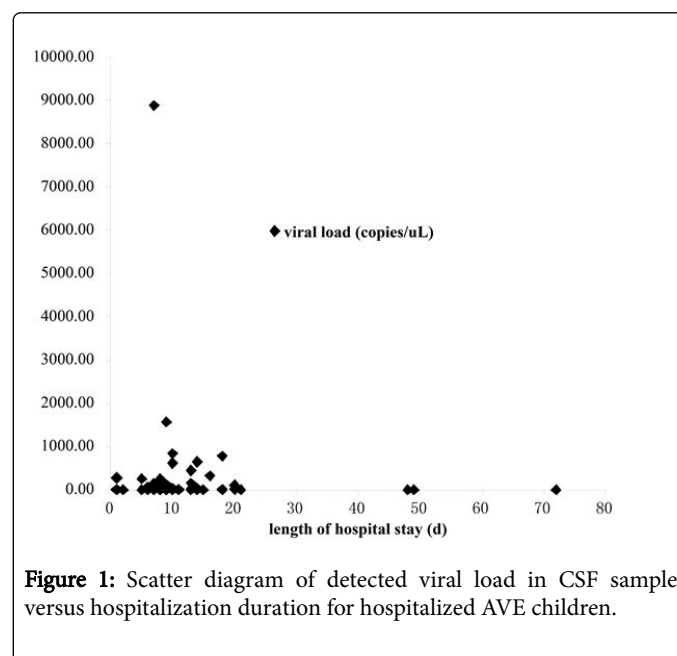
### Clinical characteristics and auxiliary examinations of viral-positive CSF samples in hospitalized AVE children

**1. Clinical symptoms and signs:** Their common symptoms and signs included fever (91.93%), nausea & vomiting (73.29%), dizziness & headache (51.55%), convulsion (38.51%), mental disturbance (18.63%) and skin rash (17.39%). As seen from Table 3, the most common clinical manifestations of AVE included fever, vomiting and headache. However, clinical symptoms and signs showed no differences between children with viral-positive versus negative CSF samples.

	Total Abnormality / total (%)	Viral etiology Abnormality /total (%)	Non-viral etiology Abnormality /total (%)	P value*	
				$\chi^2$	p
Fever	148/161 (91.9)	65/71 (91.5)	83/90 (92.2)	0.02	0.876
Headache	83/161 (51.6)	42/71 (59.2)	41/90 (45.6)	2.94	0.086
Vomiting	118/161 (73.3)	55/71 (77.5)	63/90 (70)	1.13	0.288
Convulsion	62/161 (38.5)	22/71 (31)	40/90 (44.4)	3.04	0.081
Mental disturbance	30/161 (18.6)	12/71 (16.9)	18/90 (20)	0.25	0.616
Respiratory problem	43/161 (26.7)	21/71 (29.6)	22/90 (24.4)	3	0.079
Diarrhea	12/161 (7.5)	7/71 (9.9)	5/90 (5.6)	1.12	0.301
Rash	28/161 (17.4)	16/71 (22.5)	12/90 (13.3)	2.34	0.126
Menigeal signs	39/161 (24.2)	18/71 (10.5)	21/90 (23.3)	0.09	0.767

Paresis	7/161 (4.3)	5/71 (7)	2/90 (2.2)	2.36	0.102
Death during hospitalization	0/161	0/71	0/90	0	0
CT (head)	16/74 (21.6)	3/23 (13)	13/51 (25.5)	0.81	0.369
MRI (head)	36/126 (28.6)	14/50 (28)	22/76 (18.9)	0.01	0.908
EEG	99/154 (64.3)	49/68 (72.1)	50/86 (58.1)	3.21	0.073
CSF pressure	88/161 (54.7)	34/71 (47.9)	54/90 (60)	2.35	0.125
CSF leucocyte count	59/161 (36.6)	30/71 (42.3)	29/90 (32.2)	1.72	0.19
CSF protein	15/161 (9.3)	7/71 (9.9)	8/90 (8.9)	0.06	0.811
CSF glucose	0/161	0/71	0/90	0	0
*Blood leucocyte	28/161 (17.4)	15/71(21.1)	13/90(14.4)	2.32	0.127
*With sequel after a 6-month follow-up	5/107 (4.7)	4/51(7.8)	1/56(1.8)	2.34	0.118
*Viral vs. non-viral etiologies					
*Blood leucocyte $<3.5 \times 10^9/L$ or $>10.0 \times 10^9/L$ was abnormal					
*Sequelae included aphasia, motor disturbance and cognitive dysfunction					

**Table 3:** Clinical manifestations, prognosis, imaging, EEG and biochemical parameters of hospitalized AVE children.



**2. Auxiliary examinations:** The CSF samples were examined for all children. And there were elevations in CSF pressure ( $>100$  mm H<sub>2</sub>O, 54.66%), leucocyte count ( $>10 \times 10^6/L$ , 36.65%) and protein ( $>400$  mg/L, 9.32%). A total of 154 hospitalized children had abnormalities (64.29%) on EEG. Among 144 cases of neuroimaging, 33.33% showed abnormalities. And there were 74 abnormal cases (21.62%) on cranial

computed tomography (CT) and 126 abnormal cases (28.57%) on magnetic resonance imaging (MRI). As seen from Table 3, no statistically significant differences existed in neuroimaging, EEG, CSF or blood routine among CSF sample viral-positive and negative hospitalized AVE children.

### Viral loads of CSF samples from hospitalized AVE children

In CSF samples of hospitalized AVE children, the maximal viral load was 8888.77 copies/ $\mu$ L, the minimal viral load 1.05 copies/ $\mu$ L and the average viral load 4.11(47.71-1.79) copies/ $\mu$ L. The variables of detected viral load and hospitalization duration were used for plotting a graph of scatter diagram (Figure 1).

According to the Pearson's correlation analysis, there was no obvious correlation ( $r=-0.51$ ,  $p=0.649$ ).

### Epidemiological analysis

EV is more prevalent in spring and summer, and HHV-6 occurs frequently in autumn, and the differences had statistical significance (Table 4).

Viral classification	Spring (n=46)	Summer (n=52)	Autumn (n=28)	Winter (n=35)	$\chi^2$	P value
EV	12	14	0	3	13.091	0.004
MuV	5	3	2	3	0.896	0.826
MY	3	6	0	1	6.487	0.09
EBY	3	1	1	3	2.46	0.483
ADV	2	0	1	3	5.692	0.128
HPEV	0	2	2	4	7.386	0.061
HHV-6	1	0	3	0		0.013
JEV	0	0	1	0		0.174
HSV-1	0	0	1	0		0.174
HSV-2	0	0	0	1		0.391
VZV	0	0	0	0		

**Table 4:** Seasonal distributions of AVE positivity in CSF samples of hospitalized children.

### Characteristic of cases with viral-positive CSF samples

**MuV:** In this cohort, 13 cases were tested positive for MuV. One 5-month-old child received no mumps vaccination; among 4 children aged 18-24 months, immunization was never ( $n=1$ ) and non-specified ( $n=3$ ); all children aged >24 months received parotitis vaccine. Among 13 positive samples, only 1 case had enlarged parotid gland.

**MV:** Among 12 hospitalized cases of MV (+) CSF specimen, none had a previous history of measles. One child aged 5 months received no measles vaccine; among 28-18-month-old children, one was non-immunized while another received the first dose of measles vaccine; the ages of remaining 9 cases (75%) were all >24 months, and vaccination was repeated ( $n=2$ ), non-repeated ( $n=5$ ) and non-specific ( $n=2$ ). None of them had an onset of morbilliform rash at 1 week prior

to admission and after admission (maculopapule), Koplik's spot, conjunctival hyperemia and typical measles changes. The major symptoms were fever (100%), mental disturbance (91.67%), vomiting (58.33%) and etc. On cranial MRI examination 5/12 showed imaging changes; EEG was normal ( $n=4$ ) and abnormal ( $n=8$ ). For 12 children, lumbar puncture was performed at Day 1-14 post-onset. And 9 of them were punctured at Day 1-4 post-onset. The results of CSF were normal ( $n=12$ ), mild leukocytosis ( $n=4$ ) and slightly elevated protein ( $n=1$ ).

**ADV:** Six cases were tested positive for ADV and rural children predominated. The major symptoms were fever ( $n=6$ ), nausea & vomiting ( $n=5$ ), headache ( $n=3$ ), convulsion ( $n=2$ ), mental disturbance ( $n=1$ ) and etc. Only 2 cases had associated with cough and respiratory infection signs.

**JEV:** One JEV-positive hospitalized child was 9 years old. Primary and secondary inoculations of type B encephalitis vaccine were completed. There were the symptoms of fever, headache and nausea & vomiting, etc. The meningeal irritation sign was negative while the right-sided Gorden's sign positive. Within the same onset day, CSF examination revealed a slight higher CSF pressure. And the remainder was normal. No abnormality was found on cranial CT. And EEG suggested paroxysmal multiple short-course  $\theta$  waves of hyper-active voltage. After active interventions for 9 days, the child improved and was discharged without any neuropsychiatric sequel.

### Discussion

From October 2012 to July 2014, a total of 161 CSF samples were collected from hospitalized AVE children. The technique of FQ-PCR was used for detecting 71 viral-positive cases, including superimposed infections. The overall detection rate was 44.10%. And EV had the highest detection rate of 18.01% (29/161), MuV the second highest detection rate of 8.07% (13/161) and VZV was not detected. Based upon a pilot study of July 2010 to October 2011, our project team revealed that 93 hospitalized AVE children had an overall viral detection rate of CSF at 38.71%; EV had the highest detection rate of 24.73%, ADV 5.38% and there was no detection of JEV, VZV or HSV-1 [22]. As compared with the data of our pilot study, the overall detection rate increased somewhat. And, though as the most common species, EV had a slightly lower detection rate; the detection rates of MuV, MV and HPeV increased obviously; ADV and HSV-2 had the slightly lower detection rates; the detection rates of EBV, HHV-6 and VZV showed no obvious changes. Furthermore, JEV ( $n=1$ ) and HSV-1 ( $n=1$ ) missing previously were detected in the present study. The variations of this cohort and our pilot study were probably due to different study periods and case numbers. And it had also reflected the changing patterns of viral prevalence and spectrum during difference time periods at the locality.

As compared with the relevant figures reported in the domestic and foreign literatures, the overall detection rate of our cohort was higher than those of New York City, USA (14.4%) 23, Guangzhou, China (33.6%) 24 etc. However, the figures of Spain (44.2%) 25, and Zhengzhou, China (61.98%) 6 were somehow underreported. The above variations were probably correlated with time, region, detection methodologies, target populations and disease classification, etc. The prevalence of a large majority of acute viral infectious diseases in CNS has shown a marked seasonal distribution, particularly summer [23,24]. In accordance with the present study, children aged under 10 years were the most common among all age groups [26]. In our cohort,

the age group of 3-6 years had the largest case load. It was somewhat similar. With regards to genders, male predominated [26,27]. In our cohort, the ratio of male-to-female was 1.37:1 and there was some correspondence. However, the present study has failed to detect statistical differences in viral detection rate among different age groups or genders. And our findings were similar to Shandong, China etc. [28].

For acute CNS infectious diseases caused by different viral pathogens, the clinical manifestations and auxiliary examinations are somewhat similar. As revealed by a study of California, 170 AVE patients developed fever (75%), convulsion (38%) and MRI changes (60%) [29]. Another retrospective study of Bo et al. [30] was conducted for analyzing the clinical data of 124 VE patients in Beijing. CSF abnormality was detected in 105 cases. In CSF, pressure increased (37.1%); leukocytosis (75.8%) and elevated protein content (56.5%). Clinical manifestations and auxiliary examinations of our cohort are similar to those of domestic and foreign reports. Furthermore, no difference existed in CSF specimens between viral positive and negative cases. According to a Swedish report, 29 cases of acute HSV encephalitis were assayed by quantitative FQ-PCR. After analyzing the relationship between disease course and viral load, they found that the higher level of viral load in CSF, the graver disease severity and the longer disease course [10]. However, our study revealed no relevance between viral load and hospitalization duration. The contradiction was probably related with viral species and study subjects. Further studies are warranted. A Dutch team employed the technique of FQ-PCR for detecting the CSF samples of 232 children of acute viral meningitis and the detection rate of EV was 24.1%.8 According to a Spanish report, the CSF specimens were collected from 566 encephalitis children and FQ-PCR was performed. And 161 cases were tested positive for EV (28.4%) [31] It suggested that EV is the most common pathogen of VE and the present study had detected the largest number of EV with a detection rate of 18.01%. There was some correspondence.

As reported by domestic surveys, acute CNS infections caused by EV have demonstrated a distinct seasonal pattern. The occurrences were clustered in summer and autumn in Zhengzhou and Guangzhou, 6, 24 April-September in Jiangsu Province [27]. In the present study, the highest detection rates of spring and summer were similar as the above. All 13 children positive for MuV were feverish and there was only 1 case of enlarged parotid gland. During an outbreak of parotitis, a Spanish team detected MuV nuclear acid in CSF of 158 meningoencephalitis patients without the signs of epidemic parotitis and 6 cases were Muv (+) [32]. It may be seen that, despite a lack of typical manifestations of swollen and painful parotid gland and epidemic parotitis, the possibility of CNS MuV infection cannot be ruled out. Also it has highlighted the importance of vaccinating the susceptible populations. However, among 13 MuV (+) cases, 8 had received vaccination of parotitis. Thus immunized children could be re-infected by MuV. It was probably due to the fact that, with the elongation of immunization time, the resulting antibody level declined gradually. And the necessity of re-vaccination is highlighted.

In the present study, the detection rate of MV was 7.45%. In this cohort, the detection rate of MV increased markedly over our pilot study. Based upon the data of dynamic epidemic briefing of categories A, B & C infectious diseases in China from Division of Public Health Surveillance & Information Service, Chinese Center for Disease Control and Prevention, there was a nationwide trend of rising incidence of measles during the period of February-June 2013 [33-37]. And it was endemic in Hunan Province [38-42]. During the period of

the present study, a markedly higher detection rate of MV was probably correlated with a local outbreak of measles. An outbreak of measles is often related with non-immunization, invalid vaccine and inadequate immunization. According to literature reports, due to a lower rate of immunization coverage than elsewhere, MV became pandemic in some European Orthodox and Ultra-Orthodox Jewish communities and Israel [43-45]. In May 2010, there was an outbreak of 71 measles cases in a non-immunized community of Essen City, Germany. And 68 children were affected [46]. Different from the above, among 12 MV (+) children, 10 had received an immunization of measles vaccine. After vaccination, the potency of resulting neutralizing antibody might decrease gradually with elapsing time. And 7 children of our cohort had no record of secondary immunization. Thus a higher detection rate of MV might be associated with inadequate immunity. In our study, among 12 MV (+) cases, none had an onset of typical measles-like rash before and after admission. Non-typical rash-measles encephalitis was once reported in India [47]. It was worth noticing that MV infection in CNS might also manifest some non-typical measles-like changes. It was probably related with insufficient immunity in children. And further confirmatory studies are warranted.

According to a team of New York City, USA, the CSF specimens of 2357 cases of VE, meningitis and meningoencephalitis, the technique of FQ-PCR detected 2 ADV(+) cases with a detection of mere 0.08% [23]. In Guangzhou area, the combined methods of FQ-PCR and enzyme-linked immunosorbent assay (ELISA) were employed for detecting the CSF and serum specimens of 198 VE children. And the positivity rate of ADV was 2.5% [24]. The detection rate of ADV was 3.73% in the present study. And it was high than that of the above studies. All local study populations were children. However, the US cohort of 2357 patients included adults. Some variations existed in age composition of study subjects. Two detection methods were combined by researchers in Guangzhou, China and arrived at the conflicting results. In the present study, all 6 children had an onset of fever, headache, vomiting and nervous manifestations. Only 2 cases were accompanied by cough. It seemed obvious that ADV could launch a direct assault on CNS without any respiratory involvement.

In Yichang City during 2006-2012, antibody testing of serum specimens of acute encephalitis and meningitis were performed with ELISA [48]. Except for 2006, the detection rate of JEV had a range of 1.10%-5.94%. And there was a gradual trend of declining yearly. However, JEV nuclear acid was detected in only one CSF sample of our study. As a non-infectious institution, our hospital has a policy of don't admitting and treating children of endemic type B encephalitis. Thus some variations existed detection rates. In this cohort, the positive cases had received vaccine of type B encephalitis. According to a report of Wuhan, China, 9 out of 31 cases of type B encephalitis were once immunized [49]. Thus, even after receiving type B encephalitis vaccine, there is still a possibility of developing the CNS infection of JEV. The underlying cause awaits further studies. In this cohort, this case was not diagnosed clinically as epidemic type B encephalitis. Thus the importance of pathogenic examination for a definite disease diagnosis can never be over-emphasized.

According to a Canadian report, the major pathogen of VE was VZV whose detection rate reached 17.7% [50]. And in Spain, the cases of VE, meningitis and meningoencephalitis were assayed by PCR. And the detection rates of VZV were 19.1%, 27.0% respectively. 25 No VZV was detected within the CSF samples of this study and our pilot study. It was probably due to the fact that our hospital was a non-specialty

hospital of infectious diseases and no patients of varicella had been ever admitted. However, in studies of Peru [51], the technique of PCR was used for detecting VE (n=97) and meningitis (n=53) in CSF samples. And there was no detection of VZV. The results corresponded to those of our study. We could not rule out the possibility of vaccine popularization lowering significantly the incidence of disease associated with this virus. Further studies may offer a confirmation.

With some limitations, the present study had only detected 11 common viruses. And some other non-monitored viruses could also cause the onsets of acute infectious diseases in CNS. Furthermore, all cases were collected from a single medical center. Therefore it failed to offer a complete epidemiological status of local prevalence in Changsha region.

## Conclusions

During the period of our study, 11 common viruses were detected in CSF among hospitalized AVE children in Changsha area. As compared with our pilot study, the detection rate increased slightly. MV and MuV were detected more frequently and EV was the most common viral strain.

A distinct pattern of seasonal distribution was observed for the prevalence of most cases of acute viral infectious diseases in CNS. Summer was particularly dramatic.

The clinical manifestations and auxiliary examinations are somewhat similar for acute infectious diseases of CNS due to different viral pathogens. And virally positive and negative cases showed no differences in gender, age, season, urban-rural variation, clinical characteristics or auxiliary examination results.

The positive cases of MuV, MV, ADV and JEV had unique characteristics of epidemiology, vaccination and clinical manifestations.

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