Enterovirus 71 encephalomyelitis and Japanese encephalitis can be distinguished by topographic distribution of inflammation and specific intraneuronal detection of viral antigen and RNA


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Enterovirus 71 encephalomyelitis and Japanese encephalitis can be distinguished by topographic distribution of inflammation and specific intraneuronal detection of viral antigen and RNA

Aims: To investigate if two important epidemic viral encephalitis in children, Enterovirus 71 (EV71) encephalomyelitis and Japanese encephalitis (JE) whose clinical and pathological features may be nonspecific and overlapping, could be distinguished. Methods: Tissue sections from the central nervous system of infected cases were examined by light microscopy, immunohistochemistry and in situ hybridization. Results: All 13 cases of EV71 encephalomyelitis collected from Asia and France invariably showed stereotyped distribution of inflammation in the spinal cord, brainstem, hypothalamus, cerebellar dentate nucleus and, to a lesser extent, cerebral cortex and meninges. Anterior pons, corpus striatum, thalamus, temporal lobe, hippocampus and cerebellar cortex were always uninfamed. In contrast, the eight JE cases studied showed inflammation involving most neuronal areas of the central nervous system, including the areas that were uninflamed in EV71 encephalomyelitis. Lesions in both infections were nonspecific, consisting of perivascular and parenchymal infiltration by inflammatory cells, oedematous/necrolytic areas, microglial nodules and neuronophagia. Viral inclusions were absent. Conclusions: Immunohistochemistry and in situ hybridization assays were useful to identify the causative virus, localizing viral antigens and RNA, respectively, almost exclusively to neurones. The stereotyped distribution of inflammatory lesions in EV71 encephalomyelitis appears to be very useful to help distinguish it from JE.

Keywords: diagnosis, Enterovirus 71 encephalomyelitis, Japanese encephalitis virus, pathology

Introduction

Despite advances in general medicine and vaccine development, frequent outbreaks of epidemic viral encephalitides continue to be reported globally [1–5]. In the paediatric age group, apart from HIV encephalitis,
probably the most important causes of epidemic viral encephalitis in terms of incidence, morbidity and mortality are Enterovirus 71 (EV71) and Japanese encephalitis (JE) virus. EV71 (family: Picornaviridae) has a worldwide distribution, and person-to-person transmission is by the faecal–oral or oral–oral routes [6]. The JE virus (family: Flaviviridae) is found in large regions of Asia and is zoonotically transmitted by mosquitoes from birds and pigs [7]. Both are positive, single-strand RNA viruses. In some outbreaks, thousands of children may be infected in a short period of time [3–5] although symptomatic EV71 infection usually manifests as self-limited hand, foot and mouth disease, and JE infection is usually mild, presenting with fever, headache and gastrointestinal symptoms [7]. Unfortunately, both infections may be complicated by clinically similar neuroinvasive disease such as aseptic meningitis, acute flaccid paralysis (AFP) and encephalomyelitis [8–14].

In any outbreak of epidemic viral encephalitis, virus identification is crucial to enable suitable preventive measures and effective treatments to be instituted. As clinical neurological features alone are usually insufficient to establish the type of viral encephalitis, a combination of brain magnetic resonance (MR) imaging, serology and viral detection by culture and molecular methods is more useful to confirm the diagnosis [15–18]. Yet, even with molecular techniques, the viral aetiology in a significant number of viral encephalitic syndromes still remains elusive [15,19]. Even autopsy examination of the central nervous system (CNS) may be insufficient to clinch the diagnosis all the time partly because pathological features can be nonspecific. However, combined with ancillary aids like immunohistochemistry (IHC), in situ hybridization (ISH) and electron microscopy, the diagnostic yield can be improved [20–23]. This was clearly demonstrated in a recent EV71 outbreak in which EV71 encephalomyelitis was initially confused with JE, and only after careful autopsy studies were the respective causative viruses confirmed [20].

Enterovirus 71 and JE virus are known to be neurotropic and share common nonspecific, neuropathological features that consist of perivascular cuffing and parenchymal infiltration by inflammatory cells, microglial nodule formation and neuronophagia [10,24–26]. In contrast to JE in which most CNS areas may be affected [24,27], the topographic distribution of inflammation in EV71 encephalomyelitis was reported to be stereotyped and unique [25]. The most severe inflammation was found in the neuronal areas of the spinal cord, brainstem, hypothalamus and cerebellar dentate nucleus. The cerebral cortex shows focal mild inflammation. Strikingly, anterior pons, cerebellar cortex, thalamus and corpus striatum are usually spared. These findings were based on a few cases and, if confirmed, could provide very helpful diagnostic clues.

The purpose of this study is to investigate if the stereotyped topographic distribution of inflammation described so far only in Malaysian cases could be confirmed in more cases of EV71 encephalomyelitis from different countries. We hypothesize that most, if not all, fatal cases of EV71 encephalomyelitis would have the same topographic distribution of inflammation as the clinical presentation appears to be similar, with patients mainly presenting with relatively mild nonspecific symptoms of infection followed by fatal sudden collapse within hours of hospital admission [10,11,28,29]. We also compared neuropathological findings with JE which has been confused with EV71 encephalomyelitis in regions where both infections can be found [20]. Moreover, specific IHC and ISH assays to detect EV71 and JE virus in infected CNS tissues were developed and investigated for their usefulness in diagnosis. ISH has been previously used in EV71 infection [25,30] but as far as we know, this diagnostic procedure has not been reported in JE.

Materials and methods

A total of 13 suspected or confirmed autopsy cases of EV71 encephalomyelitis from Hong Kong (n = 3), Singapore (n = 2), Taiwan (n = 3), Malaysia (n = 4) and France (n = 1), and a total of eight autopsy cases of JE from India (n = 7) and Malaysia (n = 1) were studied. Most of the EV71 and all the JE cases have not been previously published. Except for the four Malaysian EV71 cases [10,25], published cases from Singapore [31,32] and France [33] have not been carefully examined from the neuropathological perspective. All cases used in this study have been obtained ethically and approved by the various institutional ethical committees.

Formalin-fixed, paraffin-embedded tissue blocks from various areas of the CNS of both infections were available for examination, including spinal cord, medulla, pons, midbrain, hypothalamus, thalamus, corpus striatum, cerebral cortex, cerebellum and hippocampus. Both EV71 and JE cases were initially examined by light microscopy after routine H&E stains and selected tissue sections were...
further tested using specific IHC and ISH assays for the respective viruses. Cases were included in the study only when either or both assays were unequivocally positive in at least one tissue section. In addition, several cases were also confirmed by reverse transcriptase-polymerase chain reaction (PCR) and/or virus culture (Table 1).

**Enterovirus 71 detection**

Immunohistochemistry and ISH assays were used separately to detect EV71 in tissue sections as previously described [25,30]. Briefly, for IHC, deparaffinized and rehydrated tissue sections were reacted with specific primary antibodies (dilution 1:2000, rabbit polyclonal, source: Dr Shimizu) [34] to EV71 in a standard immunoperoxidase procedure after antigen retrieval in a microwave processor (20 min, 99°C, citrate buffer).

Specific digoxigenin (DIG)-labelled DNA probes were produced as previously described [25] and applied onto similar tissue sections in the ISH assay. The probe, which was designed to hybridize to the 5′ non-translated region of the virus genome, was labelled with DIG using a specific PCR that incorporated DIG-11-deoxyuridine triphosphate nucleotide in the reaction. Sections were pretreated with 0.2 M HCl and proteinase K (100 μg/ml; 20 min at 37°C) and hybridized with probes in a standard hybridization solution overnight at 42°C. Hybridization was detected using an anti-DIG antibody (Roche, Mannheim, Germany) conjugated to alkaline phosphatase and visualized using nitroblue tetrazolium/5-bromo-4-chloro-indol-3-indolyl phosphate (Roche) as substrate chromogen.

The negative tissue controls for both IHC and ISH included human brain tissues of normal individuals, measles and toxoplasmic encephalitides, mouse brain tissues experimentally infected with dengue virus, West Nile virus and tick-borne encephalitis virus, respectively. Further negative controls omitted either the primary antibody or probe from the IHC and ISH procedures, respectively. RNAase-treated test slides provided additional negative controls for ISH.

**Japanese encephalitis detection**

Immunohistochemistry and ISH methods were used separately to detect JE virus in tissue sections.

The IHC was based on a standard EnVision (Dako, Glostrup, Denmark) immunoperoxidase technique. Briefly, deparaffinized and rehydrated tissue sections were pretreated in a microwave processor followed by primary polyclonal antibody (dilution 1:200, 2 h, room temperature; source: Dr Takasaki, prepared by inoculating whole virus into mice and harvesting immune ascites fluid) followed by secondary antibody and substrate 3′ diaminobenzidine, according to manufacturer’s instructions. The slides were counterstained with haematoxylin and routinely mounted.

For ISH, a new DIG-labelled, JE-specific DNA probe was prepared from a plasmid template containing the full-length E gene in a standard PCR that used a specific primer set (5′-TGGGACTTTGGCTCTATTGG-3′ and 5′-AGAACACGAGCACACCTCCT-3′) and that incorporated DIG-11-deoxyuridine triphosphate nucleotide (Roche) in the reaction. The PCR conditions were: (1) 15 min, 95°C; (2) 15 s at 94°C, 30 s at 53°C. 1 min at 72°C, for 35 cycles; and (3) 5 min. 72°C. The 208-bp, DIG-labelled DNA probes were purified with High Pure PCR purification kit (Roche) and stored at −80°C before use. The ISH protocol was the same as the procedure for EV71.

**Results**

All 13 EV71 and eight JE cases were found to be IHC- and/or ISH-positive for the respective viruses and were included in the study (Table 1).

**Topographic distribution of inflammation**

The inflammation found in both EV71 and JE cases was mainly in the neuronal areas and consisted of varying degrees of perivascular cuffing by inflammatory cells, parenchymal inflammatory cell infiltration with formation of occasional microglial nodules (Figure 1e) and focal neuronophagia (Figure 1f). The inflammatory cells consisted of numerous macrophages and also lymphocytes, neutrophils and plasma cells. Rarefied, paler staining areas of oedema and/or necrosis are observed in both infections (Figures 1e and 2e). There was no evidence of viral inclusions. Vasculitis was absent in all the EV71 cases. Similarly, most of the JE cases did not show evidence of vasculitis with the exception of Case 7 (Table 1) in which one or two small cerebral cortex vessels showed features of possible vasculitis (Figure 2d).

The distribution of inflammation in the EV71 cases was confirmed to be stereotyped and generally confined to neuronal areas of the spinal cord (both anterior and posterior horns) while sparing the long spinal tracts. The entire medulla was inflamed except for the area of the pyramids.
## Table 1. Demography, country of origin and clinicopathological findings of Enterovirus 71 (EV71) encephalomyelitis and Japanese encephalitis (JE) autopsies

<table>
<thead>
<tr>
<th>Age/sex (country of origin)</th>
<th>Clinical presentation</th>
<th>Inflammation in specific areas of the central nervous system†</th>
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<tbody>
<tr>
<td></td>
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<td>CC</td>
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<tr>
<td>EV71 cases</td>
<td></td>
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<tr>
<td>1 2 years, M (Hong Kong)</td>
<td>Acute viral encephalitis syndrome (fever ( \times 3 ) days. Mouth ulcer, shock, tachycardia, coma and death a few hours after admission)‡</td>
<td>+</td>
</tr>
<tr>
<td>2 6 months, M (Hong Kong)</td>
<td>Acute viral encephalitis syndrome (fever ( \times 3 ) days. Vomiting, lethargy, drowsiness, tachycardia and death a few hours after admission)</td>
<td>+</td>
</tr>
<tr>
<td>3 9 months, F (Hong Kong)</td>
<td>Acute viral encephalitis syndrome (fever ( \times 2 ) days. Vomiting, shock, died within 1 day of deterioration)</td>
<td>+</td>
</tr>
<tr>
<td>4 14 months, F (Singapore)</td>
<td>Acute viral encephalitis syndrome (fever ( \times 3 ) days. Vomiting, mouth ulcers, skin rashes, haemodynamic instability. Died 2 days after admission)§</td>
<td>+</td>
</tr>
<tr>
<td>5 5 years, M (Singapore)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 3 ) days, skin rash, seizures. Died on arrival to hospital)§</td>
<td>NA</td>
</tr>
<tr>
<td>6 Child (Taiwan)</td>
<td>Acute viral encephalitis syndrome</td>
<td>-</td>
</tr>
<tr>
<td>7 Child (Taiwan)</td>
<td>Acute viral encephalitis syndrome</td>
<td>-</td>
</tr>
<tr>
<td>8 Child (Taiwan)</td>
<td>Acute viral encephalitis syndrome</td>
<td>NA</td>
</tr>
<tr>
<td>9 3 years, M (Malaysia)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 3 ) days. Tachypnoea, haemodynamic instability, diffuse pulmonary oedema. Died 6 h after admission)¶</td>
<td>+</td>
</tr>
<tr>
<td>10 3 years, M (Malaysia)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 2 ) days. Tachypnoea, haemodynamic instability, diffuse pulmonary oedema. Died 2 h after admission)¶</td>
<td>+</td>
</tr>
<tr>
<td>11 15 months M (Malaysia)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 3 ) days. Rashes in hands and feet, haemodynamic instability, pulmonary oedema. Died 2.5 h after admission)¶</td>
<td>+</td>
</tr>
<tr>
<td>12 4 years, F (Malaysia)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 3 ) days. Oral ulcers, limb weakness, tachypnoea, pulmonary oedema. Died 2.5 h after admission)¶</td>
<td>+</td>
</tr>
<tr>
<td>13 17 months, M (France)</td>
<td>Acute encephalitis syndrome (fever ( \times 2 ) days. Vomiting, severe respiratory distress, altered state of consciousness. Died within 12 h of admission)**</td>
<td>+</td>
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JE cases

<table>
<thead>
<tr>
<th>Age/sex (country of origin)</th>
<th>Clinical presentation</th>
<th>Inflammation in specific areas of the central nervous system†</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CC</td>
</tr>
<tr>
<td>1 8 years, M (India)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 3 ) days)</td>
<td>+</td>
</tr>
<tr>
<td>2 4 years, M (India)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 8 ) days)</td>
<td>+</td>
</tr>
<tr>
<td>3 2 years, M (India)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 8 ) days)</td>
<td>+</td>
</tr>
<tr>
<td>4 6 years, F (India)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 7 ) days)</td>
<td>+</td>
</tr>
<tr>
<td>5 8 years, F (India)</td>
<td>Acute viral encephalitis syndrome (illness ( \times 5 ) days)</td>
<td>+</td>
</tr>
<tr>
<td>6 24 years, M (India)</td>
<td>Acute viral encephalitis syndrome</td>
<td>+</td>
</tr>
<tr>
<td>7 8 years, F (India)</td>
<td>Acute viral encephalitis syndrome</td>
<td>+</td>
</tr>
<tr>
<td>8 9 years, F (Malaysia)</td>
<td>Acute viral encephalitis syndrome</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Sex: F female; M, male.
†Central nervous system areas: CC, cerebral cortex; CE, cerebellar cortex; DN, dentate nucleus; CS, corpus striatum; HY, hypothalamus; HI, hippocampal area; TH, thalamus; MB, midbrain; PO, pons; ME, medulla; SP, spinal cord.
‡Positive Enterovirus 71 culture from stool and brain tissue (Dr W. F. Ng, pers. comm.).
§Case 4, positive Enterovirus 71 culture from stool, brain tissue, intestines and oral cavity. Case 5, positive culture from brain tissue, trachea and small intestine [11,12].
¶Case 9, positive Enterovirus 71 culture in brain; Case 10 positive culture in brain and stool; Case 11 positive culture in brain, stool and throat swab; and Case 12 positive culture in brain and throat swab [10,25].
**Positive Enterovirus 71 culture from bronchial aspirate and nasal swab [13].
††Japanese encephalitis virus isolated from brain (Dr M. J. Cardosa, pers. comm.).
IHC, immunohistochemistry; ISH, in situ hybridization; NA, not available.
Figure 1. Pathological findings in Enterovirus 71 encephalomyelitis. (a) Perivascular and parenchymal infiltration of inflammatory cells in the fourth ventricle floor of thepons with no inflammation in the adjacent neuronal areas of the anterior pons (arrow). (b) Nonspecific inflammation in the cerebellar dentate nucleus (arrow) and adjacent areas. (c) Parenchymal inflammation in the midbrain with mild perivascular cuffing. (d) Meningitis (arrow) over consistently uninflamed cerebellar cortex parenchyma. (e,f) Oedematous, necrolytic areas in medulla associated with neuronophagia (arrow). (g,h) Viral antigens and RNA (arrows) in motor neurones of the spinal cord and neuropil. H&E stains (a-f), immunoperoxidase with haematoxylin counterstain (g) and in situ hybridization with haematoxylin counterstain (h). Magnification: ×10 objective (a–e); ×40 objective (f–h).
Figure 2. Pathological findings in Japanese encephalitis. (a) Focal inflammation in pontine nuclei of the anterior pons (arrow). (b) cerebellum molecular layer (arrow) and (c) thalamus (arrow). (d) The rare vasculitis in cerebral cortex (arrow). (e) Typical oedematous, necrolytic areas (arrows) in the cerebral cortex. (f) Intraneuronal viral antigens in cerebellar Purkinje cells (arrows) with minimal surrounding inflammation and in spinal cord motor neurone and neuropil (g, arrow). (h) Neuronal viral RNA (arrow) in thalamus. H&E stains (a–e), immunoperoxidase with haematoxylin counterstain (f,g) and in situ hybridization with haematoxylin counterstain (h). Magnification: × 10 objective (a–c,e); × 40 objective (d,f–h).
In the pons, the tegmentum and floor of fourth ventricle were inflamed but the anterior pons (basis pontis) was spared (Figure 1a). The whole midbrain was inflamed except for the long tracts in the cerebral peduncles (Figure 1c). The hypothalamus and dentate nucleus area of the cerebellum (Figure 1b) also showed the same intensity of inflammation. Invariably, the inflammation observed in the cerebral cortex including the motor cortex, insular cortex and cingulate gyrus was much more focal and milder. Mild meningitis was observed in most areas of the CNS (Figure 1d). No inflammation was detected in the corpus striatum, thalamus, mamillary body, hippocampus, temporal lobe and cerebellar cortex (Figure 1d).

In contrast, the inflammation in the JE cases was distributed more widely, mainly involving neuronal areas in the spinal cord, brainstem, cerebral and cerebellar cortex, dentate nucleus and molecular layer of the cerebellum (Figure 2b), hypothalamus, thalamus, hippocampus and corpus striatum. In particular, the anterior pons (basis pontis) together with other pontine areas was observed to be inflamed (Figure 2a). The degree of inflammation in the cerebral cortex (Figure 2c) generally did not seem to vary much compared to other areas of the CNS. Like EV71 encephalomyelitis, mild meningitis was also observed in most areas of the CNS. Figure 3 summarizes the distribution of inflammation in the CNS in EV71 and JE infections, respectively.

**Viral detection by IHC and ISH**

Immunohistochemistry and ISH assays to detect EV71 antigens and RNA showed similar positive results in that they were almost exclusively localized to neuronal bodies and processes in the inflamed areas (Figures 1g.h and 2f-h). Positive neurones were either isolated or in very small groups (Figure 1h). Nonetheless, in some cases, single positive neurones in a disproportionately large area of inflammation were found only after careful and systemic search. In general, the IHC and ISH assays to detect JE virus also show virus to be localized to single or small groups of neuronal bodies and processes. Hence, the pattern of staining appears similar to EV71. However, in a significant number of JE cases (five out of eight cases), where IHC was strongly positive, the ISH was negative (Table 1). Very rarely, in both EV71- and JE-infected tissues, phagocytes surrounding infected neurones may contain some viral antigens and/or RNA in their cytoplasm. There was no evidence of IHC and ISH positivity in astrocytes, oligodendrocytes, ependymal cells, blood vessels (endothelium or smooth muscle) and choroid plexus. All the negative tissue and procedural controls showed negative results as expected. The anti-JE probe showed no cross reactivity with dengue, West Nile virus and tick-borne encephalitis virus.

**Discussion**

Based on a relatively large and rare series of fatal EV71 infections drawn from several Asian countries and France, this study compares the pathological features of EV71 encephalomyelitis and JE, respectively, to uncover useful diagnostic clues to distinguish these two important paediatric epidemic viral encephalitides. We confirmed that the topographic distribution of inflammation in EV71 encephalomyelitis is stereotyped [25], affecting most severely the neuronal areas in the spinal cord, medulla, pons (tegmentum and floor of fourth ventricle only), midbrain, hypothalamus and cerebellar dentate nucleus. Milder inflammation may be detected in the cerebral grey matter and meninges. No inflammation was ever found in the neuronal areas of the anterior pons, thalamus, corpus striatum, hippocampus and cerebellar cortex. We believe this stereotyped distribution of inflammation to be very useful to help distinguish EV71 encephalomyelitis from JE, and indeed from most other viral encephalitis. MR scans of the CNS in EV71 encephalomyelitis have to a large extent confirmed the stereotyped distribution of inflammation showing corresponding topographically restricted, hyperintense lesions in the brain stem, cerebellar dentate nucleus and other areas [19,35]. We think the lack of cerebral lesions in MR scans is most likely related to the very focal inflammation found in these areas which were probably too mild to be detectable. Nevertheless, the overall strong correlation suggests that brain MR scans are useful adjuncts for antemortem diagnosis.

In the JE cases we studied and those reported in the literature [9,24,36], inflammation was detected in most neuronal areas of the CNS, including spinal cord, brainstem, hypothalamus, cerebral cortex, cerebellum, thalamus, corpus striatum and hippocampus. Hence, if suitable and adequate tissue blocks are obtained, we believe it should be possible to distinguish JE from EV71 encephalomyelitis on the basis of the distribution of inflammation alone. Important blocks to sample should include the pons (including anterior pons and floor of fourth ventricle), cerebellar dentate nucleus (to include cerebellar cortex).
Figure 3. Comparison of the topographic distribution of inflammation in Enterovirus 71 encephalomyelitis and Japanese encephalitis. The shaded (grey) areas show the known or potential neuronal areas where inflammation may be found. The relative intensity of inflammation is described elsewhere in the text.
hypothalamus (including part of thalamus) and also spinal cord, medulla, midbrain and cerebral cortex. Like EV71, the microscopic features in JE were generally non-specific, consisting of parenchymal and meningeal inflammation with perivascular cuffing, microglial nodules, neuronophagia and absence of viral inclusions. The discrete oedematous/necrolytic areas described in JE may also be found in EV71 and hence may not be diagnostically useful. However, it appears to be a more extensive and prominent feature in some JE cases [36].

Immunohistochemistry and/or ISH were generally useful for confirming JE virus and EV71 infections. Our findings confirmed that these viruses are neuronotropic, and the intense ISH staining strongly suggests viral replication in the neuronal cytoplasm as expected for RNA viruses. Astrocytes, oligodendrocytes, ependyma, blood vessels and choroid plexus were not found to be IHC- or ISH-positive. However, one previous study reported JE viral antigens localized to ependyma and subependymal astrocytes [24]. Our results in the same case/tissue block did not invariably show positive results for both IHC and ISH assays (Table 1). Sampling errors arising from non-identical adjacent sections that were used separately for these assays could account for some of these discrepancies. There may also be problems arising from different protocols and duration of fixation, and possible delays after autopsy that could impact on the results. As expected, these variations in conditions appear to affect ISH more critically presumably because the quality of viral RNA is more likely to suffer from long-term fixation [37]. Thus, wherever possible, we suggest that both assays should be attempted in inflamed tissues to increase the chances of a positive result.

In one unique JE case in our series, probable focal vasculitis was noted but this feature was not seen in the EV71 cases. We did not find any evidence of virus localized to endothelium in this or other cases. Focal vasculitis and endothelial localization of JE viral antigens have been reported previously [9]. In another study, endothelial viral antigen but not vasculitis was reported [24]. Although true viral encephalitis-associated vasculitis has been reported in other viral encephalitides, for example, acute Nipah and varicella zoster encephalitides [22,38,39], this feature in JE may be a very rare phenomenon [36] and certainly needs to be confirmed. The distribution of inflammation in JE appears to be similar to Tick-borne encephalitis and West Nile encephalitis [40,41], suggesting that the different types of flaviviral encephalitides may not be easily distinguished on this basis. IHC, ISH and other molecular methods would be probably more useful for this purpose.

Interestingly, poliovirus, another well-known enterovirus, apart from being an important cause of AFP, also causes a very rare bulbospinal form of poliomyelitis that has a topographic distribution of inflammation that appears to be very similar to EV71 [42]. Clinical observations and animal experiments have long suggested that poliovirus crosses into the CNS via either a haematogenous/blood–brain barrier or a retrograde peripheral nerve route [43]. More recent data from transgenic mice expressing the poliovirus receptor have confirmed the latter [44]. Similarly, human and animal studies have supported a role for retrograde peripheral motor nerve EV71 transmission into the CNS [25,30]. It is thought that once virus has gained entry, further spread within the CNS possibly occurred along motor or other neural pathways, such as pathways that connect medullary inferior olivary and cerebellar dentate nuclei. As far as we know, there are no data to support viral entry via the blood–brain barrier although viraemia has been demonstrated in the EV71 murine model [30,45]. Despite not sharing the same virus entry receptors [46–48], one possible explanation for the overlap of neurological syndromes in EV71 and poliovirus may be that both viruses share a common peripheral motor nerve pathway for CNS invasion. If this hypothesis is correct, it follows that AFP associated with these two enteroviruses represents a more limited form of neuroinvasive disease involving only the cord’s motor neurones without spreading beyond.

Demonstration of virus in spinal cord motor neurones (anterior horn cells) in our JE autopsies is entirely consistent with clinical AFP reported in some non-fatal JE cases [12]. We speculate that the extensive distribution of inflammation in JE is more consistent with viral CNS entry via the haematogenous/blood–brain barrier route rather than the peripheral nerve route, but this needs to be further investigated [49]. The sudden collapse and associated neurogenic pulmonary oedema in fatal EV71 infection has not been reported in JE. This may be related to the differences in EV71 and JE virus entry into the CNS. Rapid retrograde motor cranial nerve EV71 transmission and damage to the medullary respiratory and cardiovascular centres could occur to cause sudden collapse [10,25,50].

We conclude that the stereotyped distribution of inflammatory lesions appears to be very useful to distinguish EV71 encephalomyelitis from JE. In addition to
Aiding diagnosis, neuronal localization of respective viral antigens and RNA suggests that viral replication and cytolysis is an important mechanism of CNS injury in both viral infections.

Acknowledgements

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