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Childhood encephalitis in the Greater Mekong region (the SouthEast Asia Encephalitis Project): a multicentre prospective study

Jean David Pommier, Chris Gorman*, Yoann Crabol*, Kevin Bleakley, Heng Sothy, Ky Santy, Huong Thi Thu Tran, Lam Van Nguyen, Em Bunnakea, Chaw Su Hlaing, Aye Mya Min Aye, Julien Cappelle, Magali Herrant, Patrice Piola, Bruno Rosset, Veronique Chevalier, Arnaud Tarantola, Mey Channa, Jerome Honnorat, Anne Laure Pinto, Sayaphet Rattanavong, Manivanh Vongsouvath, Mayfong Mayxay, Sommanikhone Phangmanixay, Khounthavy Phongsavath, Ommar Swe Tin, Latt Latt Kyaw, Htay Htay Tin, Kyaw Linn, Thi Mai Hung Tran, Philippe Pérot, Nguyen Thi Thu Thuy, Nguyen Hien, Phuc Huu Phan, Philippe Buchy, Philippe Dussart, Denis Laurent, Marc Eloit†, Audrey Dubot-Pérès†, Olivier Lortholary†, Xavier de Lamballerie†, Paul N Newton†, Marc Lecuit†, on behalf of the SEAe Consortium‡*

Summary

Background Encephalitis is a worldwide public health issue, with a substantially high burden among children in southeast Asia. We aimed to determine the causes of encephalitis in children admitted to hospitals across the Greater Mekong region by implementing a comprehensive state-of-the-art diagnostic procedure harmonised across all centres, and identifying clinical characteristics related to patients' conditions.

Methods In this multicentre, observational, prospective study of childhood encephalitis, four referral hospitals in Cambodia, Vietnam, Laos, and Myanmar recruited children (aged 28 days to 16 years) who presented with altered mental status lasting more than 24 h and two of the following minor criteria: fever (within the 72 h before or after presentation), one or more generalised or partial seizures (excluding febrile seizures), a new-onset focal neurological deficit, cerebrospinal fluid (CSF) white blood cell count of 5 per mL or higher, or brain imaging (CT or MRI) suggestive of lesions of encephalitis. Comprehensive diagnostic procedures were harmonised across all centres, with first-line testing was done on samples taken at inclusion and results delivered within 24 h of inclusion for main treatable causes of disease and second-line testing was done thereafter for mostly non-treatable causes. An independent expert medical panel reviewed the charts and attribution of causes of all the included children. Using multivariate analyses, we assessed risk factors associated with unfavourable outcomes (ie, severe neurological sequelae and death) at discharge using data from baseline and day 2 after inclusion. This study is registered with ClinicalTrials.gov, NCT04089436, and is now complete.

Findings Between July 28, 2014, and Dec 31, 2017, 664 children with encephalitis were enrolled. Median age was 4·3 years (1·8–8·8), 295 (44%) children were female, and 369 (56%) were male. A confirmed or probable cause of encephalitis was identified in 425 (64%) patients: 216 (33%) of 664 cases were due to Japanese encephalitis virus, 27 (4%) were due to dengue virus, 26 (4%) were due to influenza virus, 24 (4%) were due to herpes simplex virus 1, 18 (3%) were due to *Mycobacterium tuberculosis*, 17 (3%) were due to *Streptococcus pneumoniae*, 17 (3%) were due to enterovirus A71, 74 (9%) were due to other pathogens, and six (1%) were due to autoimmune encephalitis. Diagnosis was made within 24 h of admission to hospital for 83 (13%) of 664 children. 119 (18%) children had treatable conditions and 276 (42%) had conditions that could have been preventable by vaccination. At time of discharge, 153 (23%) of 664 children had severe neurological sequelae and 83 (13%) had died. In multivariate analyses, risk factors for unfavourable outcome were diagnosis of *M tuberculosis* infection upon admission (odds ratio 3·23 [95% CI 1·04–10·03]), coma on day 2 (2·90 [1·78–4·72]), supplementary oxygen requirement (1·89 [1·25–2·86]), and more than 1 week duration between symptom onset and admission to hospital (3·03 [1·68–5·48]). At 1 year after inclusion, of 432 children who were discharged alive from hospital with follow-up data, 24 (5%) had died, 129 (30%) had neurological sequelae, and 279 (65%) had completely recovered.

Interpretation In southeast Asia, most causes of childhood encephalitis are either preventable or treatable, with Japanese encephalitis virus being the most common cause. We provide crucial information that could guide public health policy to improve diagnostic, vaccination, and early therapeutic guidelines on childhood encephalitis in the Greater Mekong region.

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For the Khmer translation of the abstract see **Online** for appendix 1

For the Lao translation of the abstract see **Online** for appendix 2

For the Vietnamese translation of the abstract see **Online** for appendix 3

For the Burmese translation of the abstract see **Online** for appendix 4

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Research in context

Evidence before this study

Encephalitis is a major global health issue, associated with high mortality and frequent long-term neurological sequelae. Japanese encephalitis virus is the most frequent recorded cause of encephalitis in the Greater Mekong region, but many other local major public health threats also lead to acute encephalitis. Causes of encephalitis are unidentified in a large proportion of children and new emerging pathogens might be responsible for cases with as yet unidentified causes; hence, intensifying efforts to identify and characterise causes of encephalitis are crucial. We searched PubMed on Jan 1, 2014, for worldwide cohort clinical encephalitis studies published in English since Jan 1, 1980, using the keywords "encephalitis", "acute encephalitis syndrome", and "auto immune encephalitis". Among the studies focusing on adults or children, or both, none of them had a harmonised diagnostic procedure or spanned different countries.

Added value of this study

To our knowledge, this is the largest multicentric prospective investigation of childhood encephalitis using comprehensive and harmonised pathogen diagnosis procedures to date. Our innovative three-step laboratory diagnostic procedure aimed to diagnose the most treatable and commonly encountered pathogens rapidly, identify known pathogens, and explore other diagnoses and identify unknown pathogens. Rapid and early diagnosis for treatable encephalitis is crucial for patient outcomes and was associated with a favourable outcome in our study. The data generated by this large study also provide support to several major public health recommendations for the Greater Mekong region. Indeed, 64% of children in our cohort had an identified cause of encephalitis, which was treatable in 18% of total cases and preventable in 42%.

Implications of all the available evidence

The high rate of vaccine-preventable infections should encourage strengthening national vaccination programmes in the four study countries. Vaccination against Japanese encephalitis virus was implemented during the study period in Cambodia as a consequence of this study. We advocate for a two-step diagnostic strategy, focusing first on treatable causes and then on non-treatable known pathogens.

Introduction

Encephalitis is an acute inflammation of the CNS associated with neurological dysfunction.[1] It can be caused by direct infection of the brain parenchyma, a post-infectious process such as acute disseminated encephalomyelitis,[2] or an autoimmune response such as to the N-methyl-D-aspartate (NMDA) receptor.[3] Because of high mortality and frequent long-term neurological sequelae,[4] encephalitis is an important global health issue. Reported incidences across countries range between 3·5 and 13·8 cases per 100 000 patient-years,[5,6] with higher incidence in children (aged ≤16 years) than in adults (aged >16 years).[7]

Japanese encephalitis virus is the most frequently recorded cause of encephalitis in children in southeast Asia and is associated with a high burden of neuropsychiatric sequelae.[8] An estimated 50 000 cases of encephalitis due to Japanese encephalitis virus occur per year in the Greater Mekong region, which includes Myanmar, China, Laos, Thailand, Cambodia, and Vietnam. This estimate is probably lower than the actual burden because of imperfect surveillance and reporting.[8] Moreover, many other major public health threats (eg, dengue virus, West Nile virus, enterovirus A71 [EV-A71], *Orientia tsutsugamushi*)[9–11] can also lead to encephalitis, with high rates of hospitalisation, mortality, and long-term sequelae relative to Europe, North America, and Australia.[12]

Despite extensive microbiological investigations and use of advanced molecular biology-based assays, the cause of encephalitis is not identified for a substantial proportion of children with the condition in low-income, middle-income, and high-income countries (28–85% of cases have an unidentified cause).[13–19] New emerging pathogens, probably of zoonotic origin or related to human activities affecting the environment,[20] might be responsible for unidentified causes of the condition, suggesting that intensifying efforts to identify and characterise them is critical.[21] Southeast Asia is a biodiversity hotspot at high risk for pathogen emergence, with increasing human density, urbanisation, and frequent contact with wildlife and domesticated animals.[22] The southeast Asian population is particularly exposed to infectious agents, irrespective of their method of transmission.[23] Thus, the surveillance and investigation of acute encephalitis syndrome is of critical public health importance there. However, surveillance and diagnostic capabilities for encephalitis remain weak in most low-income and middle-income countries in southeast Asia.

The SouthEast Asia Encephalitis Project (SEAe) aimed to strengthen and harmonise the identification of causes of encephalitis and microbiological diagnostic capacity of public referral laboratories in Cambodia, Laos, Vietnam, and Myanmar. In participating hospitals, within the first 24 h of inclusion of a child with suspected encephalitis, the project provided clinicians with state-of-the-art laboratory diagnostics for 23 treatable microorganisms, along with subsequent assays for an additional 45 micro-organisms, giving a total of 68 pathogens that could be screened for diagnosis of childhood encephalitis. To intensify efforts to diagnose unidentified causes of encephalitis, next-generation sequencing was used for selected patients with severe disease without an identified cause. The SEAe project also aimed to establish a system for monitoring the emergence of new pathogens and

outbreaks. We present here the results of these investigations.

Methods

Study design and participants

In this multicentre, observational, prospective study, children with suspected encephalitis were recruited at Kantha Bopha IV Children's Hospital (Phnom Penh, Cambodia), Vietnam National Children's Hospital (Hanoi, Vietnam), and Mahosot Hospital and National Children's Hospital (Vientiane, Laos) between July 28, 2014, and Dec 31, 2017, and at Yangon Children's Hospital (Yangon, Myanmar) between Oct 1, 2016, and Dec 31, 2017 (appendix 5 p 4). Inclusion criteria were adapted from the International Encephalitis Consortium 2013 case definition (appendix 5 p 4).[1] Briefly, children were eligible if they were aged 28 days to 16 years and presented with altered mental status (ie, confusion or inability to talk, decreased or altered level of consciousness, or personality change) lasting more than 24 h. Additionally, children needed to meet at least two of the following minor criteria: fever (≥38°C axillary) within the 72 h before or after presentation, one or more generalised or partial seizures (excluding febrile seizures), cerebrospinal fluid (CSF) white blood cell count of 5 per mL or higher, or brain imaging (CT or MRI) suggestive of lesions of encephalitis, or a new-onset focal neurological deficit. At each country's site, a maximum of one child per day, each being the first suspected case of the day, and four per week, were enrolled to maintain timely laboratory diagnoses. All children gave written informed assent and their parents or guardians gave written informed consent, and the ethics committee of each country approved the research study (appendix 5 p 5).

Procedures

Encephalitis was defined according to the International Encephalitis Consortium case definition[1] after lumbar puncture and brain imaging (via CT or MRI); additionally, chest x-ray and abdominal ultrasound were performed at treating paediatrician discretion. At inclusion, for each child, the referring paediatrician completed a comprehensive case report form, recording demographic, environmental, clinical, radiological, and biological data. Complete details of information collected are in appendix 5 (p 5). Children were then followed up on day 2, at discharge, and 1 year after inclusion. Liverpool outcome score was assessed at discharge and at 1 year (see appendix 5 pp 6–9).

The following samples were collected for pathogen assays upon inclusion only (ie, baseline), unless otherwise stated: CSF samples (up to 3·5 mL); blood samples (up to 10 mL for children aged ≥1 year, and 6 mL for those aged <1 year) at admission and discharge (or one of days 7–10 of the hospital stay); nasopharyngeal, throat, and rectal swabs; and urine (5 mL) and faecal samples (5 g). Blood cell count, serum electrolytes, liver enzymes, blood cultures, HIV serology, and CSF culture, biochemistry, and cell count were performed. Testing was done in referral laboratories (appendix 5 p 10). First-line testing, done in duplicate at the national and referral laboratories, was done from CSF and blood samples within 24 h and screened for 23 treatable and frequent pathogens with PCR or rapid diagnostic tests, or both. Full details of first-line testing are provided in appendix 5 (pp 10–15). Second-line testing was done without time constraints, for all patients regardless of first-line result, with samples tested for 45 additional pathogens either by PCR or serology, or both, from all relevant samples collected (appendix 5 p 16). All cutoff values and target oligonucleotide sequences are defined in appendix 5 (pp 17–22). CSF samples from children with no causes of encephalitis yet identified, with symptoms lasting fewer than 5 days and with diffuse or multifocal lesions on MRI or a Glasgow coma scale (GCS) score of less than 8 upon admission associated with isolated oedema or a small number of lesions visible by MRI, regardless of CSF cellularity, were identified and subjected to a pathogen discovery pipeline (appendix 5 p 14). CSF samples were sent to the Pathogen Discovery Laboratory, Institut Pasteur (Paris, France), and processed as described previously.[24]

Regardless of the aetiology identified in each child, all CSF samples were assayed for auto-antibodies for NMDA receptors via cell-based assay using HEK293 cells (ATCC-CRL-293T/17; embryonic kidney; human) expressing both the GluN1 and GluN2B subunits of the NMDA receptor, as previously described,[25] and all positive CSF samples were also confirmed by immunohistofluorescence[25] on samples of rat brain tissue. We did not test for other antibodies associated with autoimmune encephalitis because encephalitis associated with NMDA receptor antibodies is the only autoimmune cause described in children with acute symptoms of encephalitis.[26]

A medical panel (JDP, YC, OL, ML, PNN, XdL, and AD-P) reviewed all anonymised charts (appendix 5 p 5). If an aetiology was detected, it was classified as confirmed or probable for a given patient based on the definitions provided in appendix 5 (pp 23–25). Otherwise, the aetiology was classified as unidentified. Patient outcome was assessed at discharge (or before in case of death) and 1 year after enrolment by the physician in charge using the Liverpool outcome score[27] (appendix 5 pp 6–9), where a score of 1 corresponds to death, 2–4 to alive with neurological sequelae (2 being severe, 3 being moderate, and 4 being minor), and 5 to complete recovery. We defined an unfavourable outcome as a Liverpool outcome score of 1 or 2 at (or before) hospital discharge. All patients were assessed at discharge, and some were lost to follow-up before 1 year follow-up.

Statistical analysis

Because this study was observational without a prespecified method for error control, summary statistics

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See Online for appendix 5

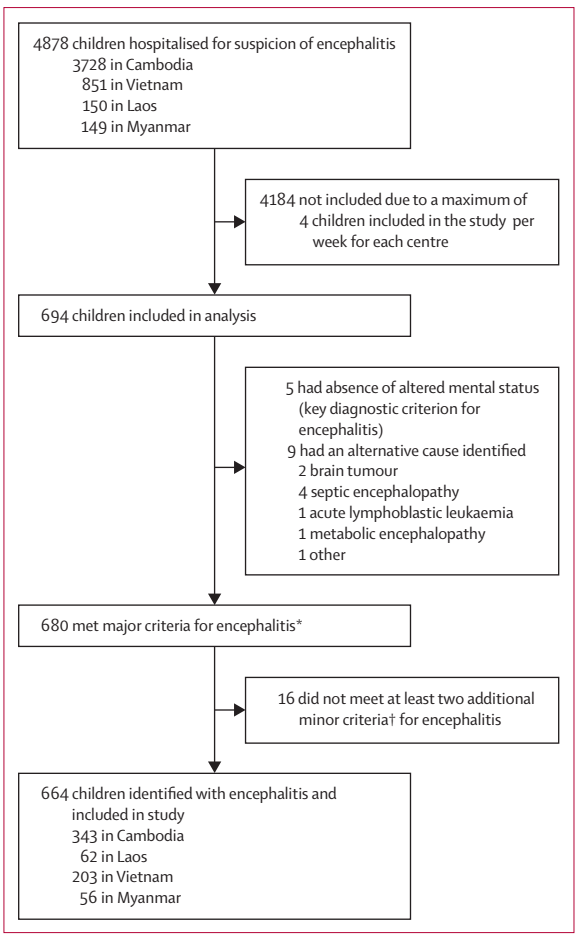

Figure 1: **Flow chart of children included in SouthEast Asia Encephalitis Project**
*Presence of altered mental status lasting >24 h. †Including fever (≥38°) within the 72 h before or after presentation; generalised or partial seizures not fully attributable to a pre-existing seizure disorder; new onset of focal neurological findings; cerebrospinal fluid white blood cell count of ≥5 per mL; and brain imaging (CT or MRI) suggestive of lesions of encephalitis.

were limited to point estimates and 95% CIs. We did not adjust the widths of these intervals for multiplicity, so inference based on them might not be reproducible. We report variables in terms of median (IQR) for continuous data and number (%) for categorical data. We calculated summary statistics quantifying the association between variables and a Japanese encephalitis virus infection (*vs* other cause identified or not identified) and an identified cause (*vs* unidentified cause). We did univariate tests for association with an unfavourable outcome (*vs* a favourable outcome) at discharge (or earlier if the patient died) using data from baseline (ie, day 1) and day 2 after inclusion using Fisher's exact tests for categorical variables and Student's *t* test for continuous variables. We further modelled the probability of an unfavourable outcome using multivariate logistic regression. We included variables in an initial multivariate logistic model if all of the following factors held: a univariate p value of less than 0·2, measured on baseline or day 2, and with fewer than 10% missing values. We imputed missing values for included variables using random forest imputation (appendix 5 p 26).[28] We then did backward selection on this model, stopping when the Akaike information criterion[29] reached its minimum. We ran a principal component analysis and multiple component analysis to investigate whether any clinical phenotypes were visible as clusters on the principal axes (appendix 5 p 26). Further details of statistical analyses, including post-hoc analyses, are in appendix 5 (pp 26–27).

We did all analyses using R (version 3.5). This study is registered with ClinicalTrials.gov, NCT04089436.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between July 28, 2014, and Dec 31, 2017, 4878 children (3728 in Cambodia, 851 in Vietnam, 150 in Laos, and 149 in Myanmar) were admitted to referral hospitals with suspicion of acute encephalitis syndrome, of whom 694 (14%) were enrolled in the study and 664 (96%) met the case definition (343 [52%] in Cambodia, 203 [31%] in Vietnam, 62 [9%] in Laos, and 56 [8%] in Myanmar) and were included in the study (figure 1). 295 (44%) of 664 children were female, 369 (56%) were male, and the median age was 4·3 years (IQR 1·8–8·8; table 1). The geographical distribution of participants is in appendix 5 (p 28). No child had a known history of diabetes or chronic renal impairment, two (<1%) of 664 children were HIV seropositive (one from Vietnam with a final identified cause of measles and one from Cambodia with a final identified cause of Epstein-Barr virus infection), and one (<1%) child was on daily steroids (from Cambodia, with a final identified cause of Japanese encephalitis virus infection). 334 (50%) of 664 children had pigs in their compounds, 472 (71%) had cats, 529 (80%) had chickens, and 291 (44%) had ducks (appendix 5 p 29).

On the day of inclusion, 637 (96%) children had fever, 374 (57%) had limb weakness, 349 (55%) had abnormal limb reflexes (increased, reduced, or absent), 281 (43%) had seizures, 156 (27%) had language disorders, 58 (9%) had one or more cranial nerve palsies, and 179 (27%) were in a coma at admission (GCS of <8). Other symptoms were: respiratory symptoms (272 [41%] of 664), including cough (229 [35%] of 659), shortness of breath (128 [20%]), and abnormal lung examination (89 [13%]); gastrointestinal symptoms (507 [76%] of 664), including vomiting (458 [70%] of 658), abdominal pain (95 [17%] of 559]), and diarrhoea (100 [15%]); and rash (34 [5%] of 664). The median interval from onset of symptoms to hospital admission was 3 days (IQR 2–5), with patients with *M tuberculosis*-associated encephalitis having the longest

	Unidentified aetiology (n=239)	Japanese encephalitis virus (n=216)	Dengue virus (n=27)	Influenza virus (n=26)	Herpes simplex virus 1 (n=24)	*Mycobacterium tuberculosis* (n=18)	Enterovirus A71 (n=17)	*Streptococccus pneumoniae* (n=17)	Other (n=80)*	All (n=664)
Demographics and sociodemographics										
Age, years	3·5 (1·1–9·0)	5·9 (3·5–9·3)	6·4 (3·1–11·1)	4·8 (2·3–9·7)	1·1 (0·8–3·1)	2·0 (0·9–4·5)	1·8 (1·1–2·5)	2·3 (0·6–4·3)	3·6 (0·9–6·5)	4·3 (1·8–8·8)
Sex										
Female	113 (47%)	79 (37%)	12 (44%)	16 (62%)	9 (38%)	11 (61%)	10 (59%)	6 (35%)	39 (49%)	295 (44%)
Male	126 (53%)	137 (63%)	15 (56%)	10 (38%)	15 (63%)	7 (39%)	7 (41%)	11 (65%)	41 (51%)	369 (56%)
Country										
Cambodia	101 (42%)	164 (76%)	12 (44%)	12 (46%)	3 (13%)	3 (17%)	17 (100%)	1 (6%)	30 (38%)	343 (52%)
Laos	31 (13%)	14 (6%)	2 (7%)	2 (8%)	0	5 (28%)	0	0	8 (10%)	62 (9%)
Vietnam	81 (34%)	33 (15%)	6 (22%)	6 (23%)	21 (88%)	9 (50%)	0	16 (94%)	31 (39%)	203 (31%)
Myanmar	26 (11%)	5 (2%)	7 (26%)	6 (23%)	0	1 (6%)	0	0	11 (14%)	56 (8%)
Living condition (roof type)										
Straw	19/237 (8%)	20/214 (9%)	2/27 (7%)	5/25 (20%)	0/24 (0%)	0/18 (0%)	0/17 (0%)	0/17 (0%)	6/80 (8%)	52/659 (8%)
Sheet metal	103/237 (43%)	97/214 (45%)	16/27 (59%)	9/25 (36%)	2/24 (8%)	6/18 (33%)	11/17 (65%)	5/17 (29%)	29/80 (36%)	278/659 (42%)
Tiled	50/237 (21%)	64/214 (30%)	5/27 (19%)	5/25 (20%)	7/24 (29%)	4/18 (22%)	4/17 (24%)	4/17 (24%)	24/80 (30%)	167/659 (25%)
Concrete	54/237 (23%)	17/214 (8%)	2/27 (7%)	6/25 (24%)	11/24 (46%)	5/18 (28%)	1/17 (6%)	6/17 (35%)	14/80 (18%)	116/659 (18%)
Other	11/237 (5%)	16/214 (7%)	2/27 (7%)	0/25 (0%)	4/24 (17%)	3/18 (17%)	1/17 (6%)	2/17 (12%)	7/80 (9%)	46/659 (7%)
Vaccination in the past month	17/234 (7%)	5/215 (2%)	3/25 (12%)	0/26 (0%)	2/24 (8%)	0/17 (0%)	1/17 (6%)	6/17 (35%)	8/79 (10%)	42/654 (6%)
Interval between symptom onset and hospital admission, days	3 (2–5)	3 (2–4)	4 (3–5)	3 (1–4)	5 (4–6)	12 (7–18)	2 (2–3)	3 (2–6)	4 (2–5)	3 (2–5)
Clinical features at inclusion										
Fever	222/239 (93%)	214/216 (99%)	26/27 (96%)	26/26 (100%)	23/24 (96%)	17/18 (94%)	17/17 (100%)	17/17 (100%)	75/80 (94%)	637/664 (96%)
Vomiting	159/236 (67%)	146/215 (68%)	19/27 (70%)	17/25 (68%)	17/24 (71%)	15/17 (88%)	16/17 (94%)	15/17 (88%)	54/80 (68%)	458/658 (70%)
Cough	81/238 (34%)	73/214 (34%)	9/27 (33%)	12/26 (46%)	8/24 (33%)	4/17 (24%)	8/17 (47%)	4/17 (24%)	30/79 (38%)	229/659 (35%)
Shortness of breath	44/229 (19%)	52/213 (24%)	4/27 (15%)	3/26 (12%)	3/23 (13%)	2/17 (12%)	3/17 (18%)	1/16 (6%)	16/77 (21%)	128/645 (20%)
Abnormal lung examination	34/239 (14%)	24/216 (11%)	3/27 (11%)	4/26 (15%)	4/24 (17%)	2/18 (11%)	1/17 (6%)	3/17 (18%)	14/80 (18%)	89/664 (13%)
Diarrhoea	34/237 (14%)	28/213 (13%)	1/27 (4%)	6/26 (23%)	6/24 (25%)	2/17 (12%)	3/17 (18%)	2/17 (12%)	18/80 (23%)	100/658 (15%)
Abdominal pain	24/197 (12%)	47/187 (25%)	5/22 (23%)	4/24 (17%)	0/21 (0%)	1/14 (7%)	3/9 (33%)	0/17 (0%)	11/68 (16%)	95/559 (17%)
Rash	13/230 (6%)	8/212 (4%)	3/26 (12%)	1/26 (4%)	1/24 (4%)	1/17 (6%)	3/17 (18%)	0/17 (0%)	4/78 (5%)	34/647 (5%)
Coma (Glasgow Coma Scale score of <8)	65/238 (27%)	65/213 (31%)	7/27 (26%)	6/26 (23%)	6/24 (25%)	6/18 (33%)	1/17 (6%)	3/17 (18%)	20/80 (25%)	179/660 (27%)
Neck stiffness	96/237 (41%)	112/213 (53%)	13/26 (50%)	6/26 (23%)	13/24 (54%)	11/18 (61%)	3/17 (18%)	13/17 (76%)	42/79 (53%)	309/657 (47%)
Limb weakness	123/234 (53%)	148/215 (69%)	14/27 (52%)	12/26 (46%)	12/24 (50%)	8/17 (47%)	17/17 (100%)	3/17 (18%)	36/80 (45%)	374/657 (57%)
Abnormal limb reflex	126/232 (54%)	116/201 (58%)	17/27 (63%)	19/26 (73%)	18/24 (75%)	8/18 (44%)	5/17 (29%)	6/17 (35%)	34/75 (45%)	349/637 (55%)
Seizures	97/234 (42%)	105/214 (49%)	5/27 (19%)	8/26 (31%)	15/24 (63%)	4/18 (22%)	7/17 (41%)	7/17 (41%)	33/79 (42%)	281/656 (43%)
Language disorder	48/194 (25%)	77/200 (39%)	4/22 (18%)	4/23 (17%)	3/21 (14%)	1/12 (8%)	2/12 (17%)	1/13 (8%)	16/71 (23%)	156/568 (27%)
Cranial nerve palsy	26/216 (12%)	9/204 (4%)	4/26 (15%)	1/25 (4%)	5/24 (21%)	5/16 (31%)	0/17 (0%)	3/17 (18%)	5/74 (7%)	58/619 (9%)

(Table 1 continues on next page)

	Unidentified aetiology (n=239)	Japanese encephalitis virus (n=216)	Dengue virus (n=27)	Influenza virus (n=26)	Herpes simplex virus 1 (n=24)	*Mycobacterium tuberculosis* (n=18)	Enterovirus A71 (n=17)	*Streptococccus pneumoniae* (n=17)	Other (n=80)*	All (n=664)
(Continued from previous page)										
CSF tests at baseline										
Pleiocytosis (>5 cells per µL)	91/237 (38%)	180/216 (83%)	13/27 (48%)	4/25 (16%)	13/24 (54%)	17/18 (94%)	16/17 (94%)	13/17 (76%)	45/80 (56%)	392/661 (59%)
Protein >0·5 g/L	57/237 (24%)	99/214 (46%)	15/27 (56%)	7/26 (27%)	9/24 (38%)	15/18 (83%)	3/17 (18%)	13/17 (76%)	40/79 (51%)	258/659 (39%)
Glucose <2 mmol/L	21/236 (9%)	6/211 (3%)	1/27 (4%)	2/25 (8%)	1/24 (4%)	13/18 (72%)	1/17 (6%)	11/17 (65%)	17/79 (22%)	73/654 (11%)
Neutrophils (%)	22 (0–49)	45 (32–64)	10 (0–53)	0 (0–4)	18 (6–28)	17 (7–53)	38 (35–53)	64 (41–82)	39 (5–66)	38 (8–59)
Lymphocytes (%)	40 (0–60)	49 (34–65)	35 (0–53)	0 (0 – 0)	57 (38–72)	50 (25–76)	61 (47–65)	12 (10–21)	31 (1–58)	42 (20–63)
Blood test at baseline										
Anaemia (<11g/dL)	112/238 (47%)	102/216 (47%)	10/26 (38%)	9/26 (35%)	12/24 (50%)	8/18 (44%)	11/17 (65%)	10/17 (59%)	45/79 (57%)	319/661 (48%)
Thrombopenia (<150 G/L)	14/236 (6%)	2/215 (1%)	5/26 (19%)	0/26 (0%)	1/24 (4%)	1/18 (6%)	0/17 (0%)	0/16 (0%)	12/80 (15%)	35/658 (5%)
Leukocytosis (>10 G/L)	118/239 (49%)	139/215 (65%)	10/26 (38%)	9/26 (35%)	7/24 (29%)	9/18 (50%)	11/17 (65%)	8/17 (47%)	45/79 (57%)	356/661 (54%)
High C-reactive protein concentration (>10 mg/L)	80/198 (40%)	131/188 (70%)	9/25 (36%)	10/23 (44%)	3/23 (13%)	6/12 (50%)	4/17 (24%)	14/17 (82%)	35/66 (53%)	292/569 (51%)
Abnormal brain imaging	172/205 (84%)	185/202 (92%)	21/26 (81%)	18/24 (75%)	20/24 (83%)	13/17 (76%)	14/17 (82%)	8/15 (53%)	54/73 (74%)	505/603 (84%)
Follow-up										
Length of hospital stay, days	11 (8–17)	13 (10–18)	9 (7–14)	12 (7–17)	20 (15–23)	17 (11–34)	9 (9–11)	20 (14–27)	12 (8–17)	12 (9–18)
Liverpool outcome score at discharge										
1 (death)	29 (12%)	30 (14%)	4 (15%)	3 (12%)	1 (4%)	3 (17%)	1 (6%)	1 (6%)	11 (14%)	83 (13%)
2 (severe neurological sequelae)	56 (23%)	53 (25%)	5 (19%)	6 (23%)	7 (29%)	8 (44%)	0	5 (29%)	13 (16%)	153 (23%)
3 (moderate neurological sequelae)	55 (23%)	46 (21%)	2 (7%)	4 (15%)	10 (42%)	2 (11%)	0	6 (35%)	20 (25%)	145 (22%)
4 (minor neurological sequelae)	15 (6%)	18 (8%)	5 (19%)	3 (12%)	3 (13%)	2 (11%)	0	2 (12%)	8 (10%)	56 (8%)
5 (total recovery)	84 (35%)	69 (32%)	11 (41%)	10 (38%)	3 (13%)	3 (17%)	16 (94%)	3 (18%)	28 (35%)	227 (34%)
Liverpool outcome score 1 year after enrolment										
1 (death)	43/188 (23%)	33/174 (19%)	4/19 (21%)	4/21 (19%)	1/15 (7%)	6/14 (43%)	1/15 (7%)	2/9 (22%)	13/60 (22%)	107/515 (21%)
2 (severe neurological sequelae)	11/188 (6%)	7/174 (4%)	2/19 (11%)	0/21 (0%)	4/15 (27%)	4/14 (29%)	0/15 (0%)	2/9 (22%)	3/60 (5%)	33/515 (6%)
3 (moderate neurological sequelae)	32/188 (17%)	16/174 (9%)	0/19 (0%)	3/21 (14%)	1/15 (7%)	0/14 (0%)	0/15 (0%)	1/9 (11%)	6/60 (10%)	59/515 (11%)
4 (minor neurological sequelae)	16/188 (9%)	10/174 (6%)	0/19 (0%)	2/21 (10%)	3/15 (20%)	2/14 (14%)	0/15 (0%)	0/9 (0%)	4/60 (7%)	37/515 (7%)
5 (total recovery)	86/188 (46%)	108/174 (62%)	13/19 (68%)	12/21 (57%)	6/15 (40%)	2/14 (14%)	14/15 (93%)	4/9 (44%)	34/60 (57%)	279/515 (54%)

Data are n (%), n/N (%), or median (IQR). Counts for each cause include confirmed and probable causes. Due to rounding, some percentages may add up to more than 100%. CSF=cerebrospinal fluid. *Includes: human herpes virus 6 (n=13), Epstein-Barr virus (n=9), *Enterovirus* spp (n=5), hepatitis A virus (n=4), measles virus (n=3), rabies virus (n=2), flavivirus (n=2), mumps virus (n=2), rubella virus (n=1), varicella zoster virus (n=1), adenovirus (n=1), rhinovirus C (n=1), *Orientia tsutsugamushi* (n=9), *Haemophilus influenzae* (n=4), *Leptospirosis* spp (n=3), *Neisseria meningitidis* (n=2), *Streptococcus suis* (n=1), *Streptococcus agalactiae* (n=1), *Staphylococcus aureus* (n=1) *Rickettsia* spp (n=1), *Listeria monocytogenes* (n=1), *Treponema pallidum* (n=1), multiple pathogens detected (n=3), *Plasmodium falciparum* (n=3), and auto-immune encephalitis (n=6).

Table 1: **Demographic and clinical characteristics of the study population, according to respective causes od encephalitis (n=664)**

	Final aetiology confirmation			Biological testing used in cause identification					Sample used in cause identification			
	Total (n=664)	Confirmed (n=330)	Probable (n=95)	RT-PCR (n=173)	Serology (n=241)	Culture (n=15)	Rapid diagnostic test (n=16)	NGS (n=5)	CSF (n=301)	Inclusion blood sample (n=51)*	Discharge blood sample (n=21)†	Non-sterile site (n=46)‡
Infectious causes												
Japanese encephalitis virus	216 (33%)	207 (63%)	9 (9%)	1 (1%)	214 (89%)	..	..	1 (20%)	187 (62%)	9 (18%)	20 (95%)	..
Dengue virus	27 (4%)	21 (6%)	6 (6%)	18 (10%)	14 (6%)	..	12 (75%)	..	10 (3%)	17 (33%)	..	..
Influenza virus	26 (4%)	0	26 (27%)	26 (15%)	..	..	..	..	0	..	..	26 (57%)
Herpes simplex virus 1	24 (4%)	24 (7%)	0	24 (14%)	..	..	..	..	24 (8%)	..	..	..
Mycobacterium tuberculosis	18 (3%)	12 (4%)	6 (6%)	10 (6%)	..	2 (13%)	..	..	12 (4%)	..	..	1 (2%)
Enterovirus-A71§	17 (3%)	0	17 (18%)	17 (10%)	..	..	..	..	0	..	..	17 (37%)
Streptococcus pneumoniae	17 (3%)	17 (5%)	0	15 (9%)	..	8 (53%)	..	..	17 (6%)	..	..	..
Human herpes virus 6	13 (2%)	0	13 (14%)	13 (8%)	..	..	..	..	7 (2%)	6 (12%)	..	..
Orientia tsutsugamushi	9 (1%)	9 (3%)	0	7 (4%)	8 (3%)	..	..	..	3 (1%)	6 (12%)	..	..
Epstein-Barr virus	9 (1%)	0	9 (9%)	9 (5%)	..	..	..	..	8 (3%)	1 (2%)	..	..
Enterovirus§	5 (1%)	5 (2%)	0	5 (3%)	..	..	..	..	5 (2%)	..	..	..
Haemophilus influenzae	4 (1%)	4 (1%)	0	3 (2%)	..	2 (13%)	..	..	4 (1%)	..	..	..
Hepatitis A virus	4 (1%)	2 (<1%)	2 (2%)	2 (1%)	4 (2%)	..	..	2 (40%)	2 (1%)	1 (2%)	1 (5%)	..
Coinfection	3 (<1%)	2 (<1%)	1 (1%)	2 (1%)	1 (<1%)	0	..		2 (1%)	1 (2%)	..	..
Plasmodium falciparum	3 (<1%)	2 (<1%)	1 (1%)	2 (1%)	..	..	3 (19%)		2 (1%)	1 (2%)	..	..
Measles virus	3 (<1%)	3 (<1%)	0	2 (1%)	..	..	..	1 (20%)	1 (<1%)	2 (4%)	..	..
Leptospira spp	3 (<1%)	3 (<1%)	0	3 (2%)	..	..	..	..	0	3 (6%)	..	..
Rabies virus	2 (<1%)	1 (<1%)	1 (1%)	1 (1%)	..	..	..	..	0	..	..	1 (2%)
Flavivirus¶	2 (<1%)	1 (<1%)	1 (1%)	2 (1%)	..	1 (7%)	..	..	1 (<1%)	1 (2%)	..	..
Neisseria meningitidis	2 (<1%)	2 (<1%)	0	2 (1%)	..	..	..	..	2 (1%)	..	..	..
Mumps virus	2 (<1%)	2 (<1%)	0	2 (1%)	..	..	..	..	2 (1%)	..	..	..
Streptococcus suis	1 (<1%)	1 (<1%)	0	1 (<1%)	..	..	..	..	1 (<1%)	..	..	..
Streptococcus agalactiae	1 (<1%)	1 (<1%)	0	1 (<1%)	..	..	..	..	1 (<1%)	..	..	..
Staphylococcus aureus	1 (<1%)	1 (<1%)	0	0	..	1 (7%)	..	..	1 (<1%)	..	..	..
Rickettsia spp	1 (<1%)	1 (<1%)	0	1 (<1%)	0	..	..	..	1 (<1%)	..	..	..
Rubella virus	1 (<1%)	1 (<1%)	0	1 (<1%)	..	..	..	..	0	1 (2%)	..	..
Varicella zoster virus	1 (<1%)	0	1 (1%)	1 (<1%)	..	..	..	..	0	..	..	1 (2%)
Adenovirus	1 (<1%)	0	1 (1%)	1 (<1%)	..	..	..	..	0	1 (2%)	..	..
Listeria monocytogenes	1 (<1%)	1 (<1%)	0	1 (<1%)	..	1 (7%)	..	..	1 (<1%)	..	..	..
Treponema pallidum	1 (<1%)	0	1 (1%)	0	..	..	1 (6%)	..	0	1 (2%)	..	..
Rhinovirus	1 (<1%)	1 (<1%)	0	0	..	..	..	1 (20%)	1 (<1%)	..	..	..
Immune mediated causes												
NMDA receptor antibody	6 (1%)	6 (2%)	0	..	..	..	..	..	6 (2%)	..	..	..
Unidentified cause‖	239 (36%)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

For biological testing done on each sample to determine the cause of encephalitis, sometimes more than one test led to the diagnosis, so numbers might not add up to total shown in the left column. For cells with 0 in them, this indicates that the test was done with a negative result, whereas the blank cells indicted with .. indicate that the test was not done or does not exist for the pathogen in question. CSF=Cerebrospinal fluid. NA=not applicable. NGS=next-generation sequencing. NMDA=N-methyl-D-aspartate. *Blood obtained on the day of inclusion. †Blood obtained 7–10 days after inclusion or at discharge. ‡Usually nasopharyngeal, throat, or rectal swabs, or both. §Enterovirus A71 excluded from enteroviruses. ¶Pan flavivirus RT-PCR positive, undefined species. ‖Six identified causes were not laboratory confirmed but were only based on clinical data: one child had rabies and five had tuberculosis (appendix 5 p 15). Furthermore, 47 children had multiple identified pathogens (appendix 5 pp 31–32), of which two were confirmed coinfections and one was probable.

Table 2: **Burden and diagnostic strategy to identify causes of encephalitis**

median interval (12 days [IQR 7–18]; table 1). CSF pleocytosis was present in 392 (59%) of 661 children, most commonly in children with Japanese encephalitis (180 [83%] of 216 with data), but was rare among children with influenza-associated encephalitis (four [16%] of 25 with data; table 1). The combination of pleocytosis, low CSF glucose concentration, and high CSF protein concentration was present in 38 (6%) of 664 children, mainly in those identified to have bacterial or mycobacterial encephalitis (26 [68%] of 38) but also

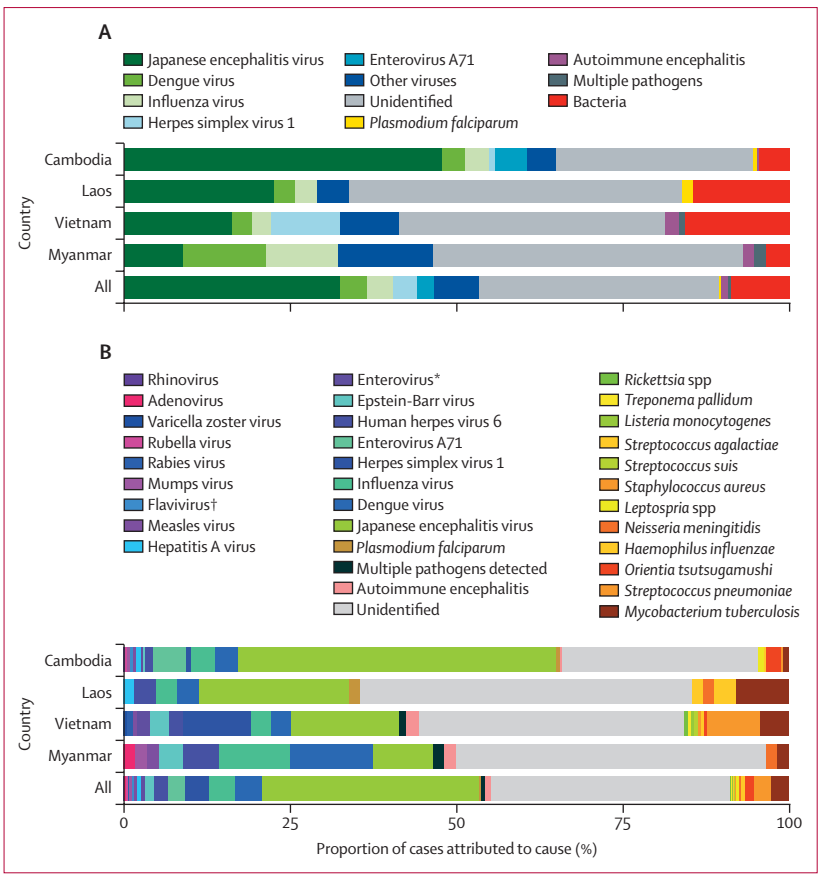

Figure 2: **Major causes (A) and all causes (B) of childhood encephalitis according to the participating country (n=664)**

Major causes refer to most frequent cause categories. *Does not include enterovirus A71. †Pan flavivirus RT-PCR positive undefined species.

in children with a viral or unidentified aetiology (12 [32%] of 38). Brain imaging (MRI or CT scan) was suggestive of lesions of encephalitis in 505 (84%) of 603 children.

30 pathogens were identified (table 2, figure 2). For the 664 cases of encephalitis, a cause was identified in 425 (64%) children, with 330 (50%) confirmed and 95 (14%) probable cases. 355 (53%) children had viral encephalitis, 59 (9%) had bacterial or mycobacterial encephalitis, five (1%) had other infectious causes, and six (1%) had autoimmune encephalitis. Japanese encephalitis virus was the most commonly identified cause, being found in 216 (33%) children, followed by dengue virus (27 [4%]), influenza virus (26 [4%]), herpes simplex virus 1 (HSV-1; 24 [4%]), *Mycobacterium tuberculosis* (18 [3%]), EV-A71 (17 [3%]), and *Streptococcus pneumoniae* (17 [3%]). PCR testing identified the cause of encephalitis in 173 (26%) children: 96 (14%) from CSF samples, 32 (5%) from blood samples, and 45 (7%) from a non-sterile site (ie, nasopharyngeal and throat or rectal swab; table 2). Notably, we diagnosed nine (53%) of 17 children with *S pneumoniae* only by PCR, with negative aerobic CSF culture. Serological testing identified the cause of encephalitis in 241 (36%) children: 193 (29%) from CSF samples, 27 (4%) from blood taken at inclusion, and 21 (3%) from blood taken at discharge (only 448 [67%] of 664 children had blood samples that were available at discharge). First-line testing within 24 h of study inclusion identified the cause of encephalitis in 83 (13%) children (appendix 5 p 30), with 55 (8%) of these being treatable causes. Data for 41 children for whom multiple pathogens were detected are in appendix 5 (pp 31–32). After full diagnostic work-up, 119 (18%) of 664 children had treatable causes of encephalitis and 276 (42%) had conditions that could have been prevented by vaccination.

CSF samples from 31 children who had an unidentified aetiology were selected and subjected to next-generation sequencing-based diagnoses. Two (6%) of 31 children had sequences of hepatitis A virus in their CSF, one (3%) had a sequence of measles virus, one (3%) had a sequence of Japanese encephalitis virus, and one (3%) a sequence of rhinovirus C. Other presumed non-pathogenic viruses were identified, including circovirus-like DCCV-7 in six (19%) of 31 children screened. Hepatitis A virus sequences in CSF samples that were identified by next-generation sequencing were confirmed by RT-PCR and serology in CSF and blood samples. The two children with measles and Japanese encephalitis virus whose CSF samples were sequenced by next-generation sequencing were negative by RT-PCR for these viruses. In post-hoc analyses, all 664 children were screened for hepatitis A virus by PCR in the admission serum and CSF. In case of positive PCR, serological testing was done; two further children had IgM antibodies for hepatitis A virus and no other identified aetiologies. Two (50%) of four children with hepatitis A virus-associated encephalitis had normal liver functions. All children were screened for autoimmune encephalitis antibodies and six (1%) of 664 children had NMDA receptor antibodies, confirmed by immunohisto-chemistry (appendix 5 p 33). No pathogen was identified in these six patients with NMDA receptor antibodies. Compared with older children, children younger than 1 year more commonly had unidentified aetiology (59 [49%] of 120 children aged <1 year *vs* 180 [33%] of 544 children aged ≥1 year) and HSV-1-associated encephalitis (12 [10%] of 120 *vs* 12 [2%] of 544), whereas Japanese encephalitis was less common among younger children (seven [6%] of 120 *vs* 209 [38%] of 544; appendix 5 pp 34–35). By contrast, most cases of EV-A71-associated encephalitis were among children aged 4 years and younger (16 [94%] of 17 cases). A larger proportion of cases of encephalitis in Cambodia were due to Japanese encephalitis virus than in Vietnam (164 [48%] of 343 *vs* 33 [16%] of 203), whereas 21 (88%) of 24 cases of HSV-1-associated encephalitis were in Vietnam, and three (12%) were in Cambodia (figure 2). All 17 cases of EV-A71-associated encephalitis were identified in Cambodia.

Elevated serum C-reactive protein concentration was more common in blood samples taken at inclusion from children with an identified aetiology than in those with an unidentified aetiology (212 [65%] of 327 *vs* 80 [41%] of 197), as was CSF pleocytosis (301 [71%] of 425 *vs* 91 [38%] of 239); all odds ratios [ORs] and 95% CIs are in appendix 5 (p 36). The frequencies of nasopharyngeal viruses and bacteria identified by PCR did not differ between children with identified or unidentified aetiologies, except perhaps for respiratory syncytial virus (RSV; 2% [nine of 425] *vs* 5% [12 of 239]; OR 2·43 [95% CI 0·93–6·65]) and the combined set of human coronaviruses (229E plus NL63 plus HKU1 plus OC43; 3% [14 of 425] *vs* 7% [16 of 239]; OR 2·10 [0·94–4·74]; appendix 5 p 37). Notably, molecular detection in CSF was negative for the aforementioned respiratory pathogens. EV-A71 RNA was detected in the rectal swab samples of 19 (3%) of 664 children and was the only attributed cause of encephalitis in 17 (89%) of these 19 children. 18 (58%) of 31 children with a positive enterovirus RT-PCR other than EV-A71 in throat or nasopharyngeal swabs and 80 (62%) of 130 with a positive enterovirus RT-PCR rectal swab had another identified cause of encephalitis. *Mycoplasma pneumoniae* was identified by PCR in nasopharyngeal or throat swabs in children with an alternative cause of encephalitis (n=6) or an unidentified (n=4) cause of encephalitis, but was not detected by PCR in these children's CSF samples.

Japanese encephalitis was more likely to be diagnosed in the rainy season (159 [74%] of 216 Japanese-encephalitis virus-associated cases), whereas cases not associated with Japanese encephalitis virus were only marginally more frequent in the rainy season than at other times of the year (255 [57%] of 448 cases not associated with Japanese encephalitis virus; appendix 5 p 38). Notably, 20 (74%) of 27 dengue virus-associated encephalitis cases were also observed during the rainy season. Children with Japanese encephalitis more often had pigs (135 [63%] of 213 *vs* 199 [45%] of 444) and chickens (192 [90%] of 213 *vs* 337 [76%] of 445) in their compound, and were less likely to have been vaccinated against Japanese encephalitis virus (24 [12%] of 199 *vs* 127 [33%] of 387) than were children with other causes of encephalitis (appendix 5 p 39). Children with Japanese encephalitis presented more frequently with a subset of neurological symptoms including language disorders (77 [39%] of 200 *vs* 79 [21%] of 368), generalised seizures (101 [47%] of 214 *vs* 144 [33%] of 442), decrease or absence of limb reflexes (88 [44%] of 201 *vs* 134 [31%] of 436), and presence of limb weakness (148 [69%] of 215 *vs* 226 [51%] of 442) than did children with other causes of encephalitis (appendix 5 p 39). No overall difference in clinical outcome between children with or without Japanese encephalitis virus-associated encephalitis was observed (appendix 5 p 39). Notably, *Orientia tsutsugamuchi* was associated with a favourable outcome in all patients (n=9) who had a Liverpool outcome score of more than 2 (appendix 5 p 33).

No clear clinical features associated with specific causes or clusters of cases of encephalitis with unidentified causes were noted, despite extensive investigations using demographic, clinical, and biological data at inclusion (appendix 5 p 40).

83 (13%) of 664 children died during their hospital stay, with a median time to death of 4 days (IQR 1–13). At discharge, 354 (61%) of 581 survivors had neurological sequelae and 227 (39%) had recovered completely (table 1). The median duration of hospital stay among survivors was 12 days (IQR 9–19 days). At discharge, of 354 children with neurological sequelae (Liverpool outcome score 2–4), 153 (43%) had severe sequelae, 145 (41%) had moderate sequelae, and 56 (16%) had minor sequelae. The most common neurological sequelae at discharge were behavioural disorders (312 [88%] of 354), communication disorders (229 [65%]), feeding disorders (216 [61%]), difficulty standing (188 [53%]), difficulty walking (175 [49%]), and limb weakness (159 [45%]; appendix 5 p 41). No neurological sequelae were found to be specifically related to Japanese encephalitis virus (data not shown).

We assessed determinants for unfavourable outcome (Liverpool outcome score 1–2) at discharge (or earlier if patients died) using data from baseline and day 2 after inclusion using univariate logistic regression and multivariate logistic regression with backward selection (11 variables selected; table 3). In multivariate analyses, an unfavourable outcome was associated with a GCS score of 8 or lower at day 2 (OR 2·90 [95% CI 1·78–4·72]), abnormal limb reflexes (2·83 [1·86–4·31]), and increase in GCS score of at least 1 point within 24 h was protective (0·82 [0·74–0·91]). Unfavourable outcome was also associated with requiring oxygen, limb weakness, shortness of breath, absence of pleocytosis, and a long interval between symptom onset and hospital admission (table 3). *M tuberculosis* was the only aetiology associated with an unfavourable outcome (table 3; appendix 5 pp 42–43). There were no significant differences in the proportion of unfavourable outcomes between the different inclusion centres (data not shown). Post-hoc analyses, including a complete-case analysis and keeping the Liverpool outcome score as an ordinal score from 1–5, are reported in appendix 5 (pp 44–45).

432 (74%) of the 581 children discharged alive from hospital were followed-up at 1 year after inclusion. Of these children, 24 (6%) had died due to encephalitis, 129 (30%) had neurological sequelae, and 279 (65%) had completely recovered. Among the 129 children with neurological sequelae at 1 year, the most common were behavioural disorders (100 [78%]), limb weakness (63 [49%]), and communication disorders (59 [46%]; appendix 5 p 46).

	Unfavourable outcome (n=236)*	Favourable outcome (n=428)†	Univariate odds ratio for unfavourable outcome (95% CI)	p value	Multivariate odds ratio for unfavourable outcome (95% CI)	p value
More than 1 week between symptom onset and hospital admission	43/235 (18%)	35/393 (9%)	2·50 (1·55 to 4·05)	0·0002	3·03 (1·68 to 5·48)	0·0002
Rainy season‡	158/236 (67%)	256/428 (60%)	1·36 (0·98 to 1·90)	0·079	..	..
Clinical features						
Cough	94/234 (40%)	135/425 (32%)	1·44 (1·03 to 2·01)	0·033	..	..
Abnormal lung examination	53/236 (22%)	36/428 (8%)	3·14 (1·99 to 5·01)	0·0002	..	..
Shortness of breath	65/227 (29%)	63/418 (15%)	2·26 (1·52 to 3·35)	<0·0001	1·86 (1·15 to 3·00)	0·011
Costal indrawing	27/233 (12%)	17/428 (4%)	3·15 (1·69 to 6·04)	0·0003	..	..
Generalised seizures	101/233 (43%)	144/423 (34%)	1·48 (1·07 to 2·06)	0·023	..	..
Limb weakness	161/234 (69%)	213/423 (50%)	2·17 (1·55 to 3·05)	<0·0001	1·69 (1·12 to 2·57)	0·013
Dysautonomia	12/231 (5%)	10/424 (2%)	2·26 (0·95 to 5·49)	0·069	..	..
Abnormal limb reflex	163/227 (72%)	186/410 (45%)	3·06 (2·17 to 4·36)	<0·0001	2·83 (1·86 to 4·31)	<0·0001
Abnormal sensory function	45/215 (21%)	37/410 (9%)	2·66 (1·66 to 4·29)	<0·0001	..	..
Cranial nerve palsy presence	27/214 (13%)	31/405 (8%)	1·74 (1·00 to 3·01)	0·058	..	..
Coma (GCS ≤8) at day 2	120/232 (52%)	61/427 (14%)	6·40 (4·42 to 9·35)	<0·0001	2·90 (1·78 to 4·72)	<0·0001
Intubation	34/236 (14%)	18/428 (4%)	3·81 (2·12 to 7·07)	<0·0001	..	..
Supplemental oxygen	118/232 (51%)	100/427 (23%)	3·38 (2·40 to 4·76)	<0·0001	1·89 (1·25 to 2·86)	0·0023
Biological and radiological features						
Pleiocytosis (>5 cells per µL)	124/236 (53%)	268/428 (63%)	0·66 (0·48 to 0·91)	0·013	0·57 (0·38 to 0·86)	0·0067
CSF glucose concentration <2·2mmol/L	32/235 (14%)	41/419 (10%)	1·45 (0·88 to 2·38)	0·15	..	..
CSF protein concentration >1g/L	37/236 (16%)	45/423 (11%)	1·56 (0·97 to 2·49)	0·065	1·82 (1·02 to 3·22)	0·041
Abnormal CNS imaging	198/217 (91%)	307/386 (80%)	2·66 (1·59 to 4·66)	0·0001	..	..
Difference between GCS on day 2 and at baseline	0 (–2 to 1)	0 (0 to 2)	0·75 (0·69 to 0·81)	<0·0001	0·82 (0·74 to 0·91)	0·0002
Pathogens						
RSV in nasopharyngeal swab	13/235 (6%)	8/428 (2%)	3·05 (1·25 to 7·90)	0·018	2·49 (0·88 to 7·05)	0·086
Parainfluenza 4 in nasopharyngeal swabs	11/235 (5%)	8/428 (2%)	2·56 (1·01 to 6·79)	0·050	..	..
Mycobacterium tuberculosis-associated encephalitis	11/236 (5%)	7/428 (2%)	2·91 (1·12 to 8·14)	0·026	3·23 (1·04 to 10·03)	0·043
Bacterial encephalitis	8/236 (3%)	33/428 (8%)	0·43 (0·18 to 0·90)	0·028	..	..

Outcomes are either n/N (%) or median (IQR). All variables were entered in the multivariable logistic regression model simultaneously; we then did backward selection on this model, stopping when the Akaike information criterion reached its minimum, and we report only p values and odds ratios with their 95% CIs for variables retained in the final model. CSF=cerebrospinal fluid. GCS=Glasgow coma scale. RSV=respiratory syncytial virus. *Death or a Liverpool outcome score of 1 (death) or 2 (severe neurological sequalae) at discharge. †Liverpool outcome score >2. ‡Rainy season is the period between May 1 and Oct 31.

Table 3: **Determinants of an unfavourable outcome at discharge (or earlier if patient died) using data from baseline (ie, day 1) and day 2 after inclusion**

Discussion

To our knowledge, this is the largest multicentre prospective study of childhood encephalitis to use comprehensive and harmonised pathogen screening procedures. At the four referral paediatric hospitals, Japanese encephalitis virus was the most frequently identified cause of encephalitis, comprising approximately a third of cases, followed by 29 other identified pathogens (30% of cases together) and autoimmune-associated encephalitis (1%). However, the cause of encephalitis was unidentified in 36% of children, which, although a relatively high proportion of this cohort, is one of the lowest reported rates in the international literature,[13–18,30] probably in part because of the high incidence of Japanese encephalitis and the exhaustive and standardised diagnostic work-up done for each patient.

This study is original because of its comprehensive and harmonised three-step laboratory diagnostic procedure designed to rapidly diagnose the most treatable and frequent pathogens first, then other known pathogens, and, finally, other causes, including unknown pathogens. We identified at least one pathogen of the 23 tested within 24 h for 83 (13%) of 664 children with encephalitis, and 55 (66%) of these 83 children had treatable causes of encephalitis. Rapid and early diagnosis of treatable encephalitis is critical for patient outcomes.[31,32] Diagnostic procedures are usually at the discretion of treating physicians,[13,17,33] and biological samples are tested for only a small number of pathogens.[14,18] Through broad pathogen screening, we hoped to identify clinical phenotypes characteristic of particular pathogens to allow future narrowing of aetiological testing for patients; however, no clear clinical phenotypes were uncovered using principal component analysis and multiple component analysis in children with and without identified causes of encephalitis. Notably, we diagnosed 53% children with *S pneumoniae* only by PCR, because

their CSF aerobic cultures were negative, probably due to the prescription of antibiotics before lumbar puncture. We also detected 17 unsuspected cases of EV-A71-associated encephalitis in Cambodia in the context of a hand, foot, and mouth disease outbreak between December, 2016, and March, 2017. These findings reinforce the need for comprehensive rather than targeted screening procedures in children with encephalitis.[34]

Japanese encephalitis virus was the most frequently identified pathogen (33% of cases), particularly in Cambodia, with a higher proportion of cases being reported during the rainy season than at other times of the year.[35] National vaccination policies vary between countries: immunisation against Japanese encephalitis virus began in Vietnam in 1997[36] and in Laos in 2013.[37] In Cambodia, a vaccination programme against Japanese encephalitis virus was implemented in March, 2016, as a consequence of this study, with a decrease in the proportion of children positive for Japanese encephalitis virus (from 61% to 35%) and an increase in children vaccinated against the virus (from 12% to 22%) being reported between March, 2016, and December, 2017, after study implementation.[38] Myanmar launched its policy at the end of the study inclusion period, in mid-November 2017.[39] Although HSV-1 testing was done in all children, HSV-1-associated encephalitis was diagnosed in only 4% of participants. A similar rate was previously reported in children in southern Vietnam.[16] Varicella zoster virus (VZV)-associated encephalitis was rare, occurring in only one (<1%) child, consistent with previous findings in paediatric encephalitis cohorts[15,16,33] and contrasting with reports for adults among whom the proportion of encephalitis cases due to VZV has been estimated to be 2–11%.[13,17,18] Encephalitis due to dengue virus has been described previously.[40] Serological cross-reactivity between flaviviruses is known to occur between dengue virus and Japanese encephalitis virus.[41] Therefore, analysing and interpreting Japanese encephalitis virus and dengue virus serological data jointly, especially in endemic regions, is crucial.

We identified nine children with *O tsutsugamushi*-associated encephalitis, and all had a favourable outcome after treatment. Because infection with *O tsutsugamushi* can be treated with doxycycline,[42] these data support our strategy of testing for this pathogen as part of first-line screening.

All respiratory and enteric pathogens known to be associated with encephalitis[43] were tested for among our cohort of children. Influenza virus was identified in nasopharyngeal or throat swabs of 36 (5%) of 664 children, while 26 (4%) children had influenza-associated encephalitis as a final diagnosis. The predominance of influenza-associated encephalitis varies greatly between studies. Several studies have reported cases of acute encephalitis during influenza outbreaks,[44] and in acute encephalitis studies, influenza has been a frequently identified aetiology.[14,17] Such cases are often interpreted as an encephalopathy associated with influenza because of the absence of CNS inflammation.[45] Children with EV-A71-associated encephalitis have a low frequency of CSF detection (approximately 24%);[34,46] therefore, rectal, nasopharyngeal, and throat swabs should be screened for EV-A71 RNA. Enterovirus RNA other than that of EV-A71 in non-sterile sites is likely to reflect carriage, because we found that most patients with a positive enterovirus RT-PCR in throat, nasopheryngeal, or rectal swabs and negative in CSF had another identified cause of encephalitis. RSV (A and B) and human coronaviruses (229E, NL63, HKU1, and OC43) were more frequently detected in children with unidentified causes of encephalitis than in children with identified causes. These viruses have already been described as being possibly associated with encephalitis,[47,48] and our results also support their possible role in encephalitis even though they were not detected in CSF samples. Furthermore, reports of SARS-CoV-2-associated encephalitis[49,50] reinforce the hypothesis that neuroinvasion by human coronaviruses can occur. Although parechovirus has been reported as the leading cause of paediatric encephalitis in Australia,[33] it was not detected in any patient in our cohort. The causal role of *M pneumoniae* detected in non-sterile site swabs should be interpreted with caution, because it was detected in both patients with other confirmed diagnoses and patients with unidentified causes, and was not detected by PCR in these children's CSF samples.

Next-generation sequencing identified two cases of hepatitis A virus-associated encephalitis, confirmed by RT-PCR and serology of CSF and blood samples. After further screening of the entire cohort, two other children were identified as being hepatitis A virus positive in CSF samples. Hepatitis A is rarely associated with encephalitis[51] but has been reported as associated with encephalitis in children with liver abnormalities.[52] The two children with hepatitis A virus detected by next-generation sequencing in our cohort had normal serum liver enzymes. Next-generation sequencing did not identify any novel pathogens, but did identify known pathogens in five (16%) of 31 children for whom no known cause was previously identified. This is slightly less than what others have reported[53] and probably reflects our thorough diagnostic procedures, ensuing high aetiology detection rate. Notably, some pathogens were identified in CSF samples of children without pleocytosis.

Autoantibody-associated encephalitis was rare in our cohort (six [1%] of 664 children) compared with other paediatric (6–10%)[15,33] and adult (7–17%) cohorts that were screened for NMDA receptor antibodies.[18,54] Viral encephalitis (HSV-1 and Japanese encephalitis virus[55]) is a known trigger of NMDA receptor antibody-mediated encephalitis[56,57] but this association was not identified in our study, most likely because inclusion was mostly done during the early phase of encephalitis symptom onset and autoimmune encephalitis symptoms are more

progressive and less frequently include fever than in viral encephalitis.

The case-fatality rate at discharge was 13%, which is similar to that in other studies in southeast Asia, which range between 7% and 27%,[11,15] and in studies from the USA, Canada, the UK, France, and Australia that have focused mainly on adults.[13,18,21] Here, the frequency of an unfavourable outcome at discharge was similar across all main causes of encephalitis, except for *M tuberculosis*, as previously observed,[13] a result probably related to delayed prescription of antituberculous drugs because they were only prescribed to 90% of children with neurotuberculosis at the referral centre (data not shown). Symptoms lasting more than 7 days before admission to hospital were also associated with an unfavourable outcome, emphasising the importance of early medical care. Finally, HSV-1 was associated with a low fatality rate (4%) at discharge but a high rate of neurological sequelae, as previously described.[4] Early treatment of *M tuberculosis* and HSV-1 is crucial for preventing unfavourable outcomes. Furthermore, long-term neurological sequelae are common (in at least 30% of children 1 year after inclusion), and rehabilitation (intense physiotherapy, neuropsychology, and speech therapy) capacity needs to increase to improve motor skills and cognitive ability and prevent long-term behavioural disorders.

Our data support several major public health recommendations. The high proportion of vaccine-preventable infections identified as the cause of encephalitis (eg, due to Japanese encephalitis virus, *Haemophilus influenzae,* influenza virus, and *M tuberculosis*) should encourage strengthening national vaccination programmes in southeast Asia and especially vaccination against Japanese encephalitis virus. We had hoped to identify clinical features that would inform future triage-specific diagnostic testing, but our evidence did not support this. On the basis of our results, we advocate a two-step diagnostic strategy, focusing first on treatable causes, specifically HSV-1, VZV, *M tuberculosis*, and common bacteria, and, if endemic, malaria. Subsequently, patients should be screened for Japanese encephalitis virus, dengue virus, EV-A71, respiratory viruses (including influenza virus), and autoimmune encephalitis autoantibodies. Regarding empirical treatment, our data suggest that for a child with encephalitis in these countries, third-generation cephalosporins plus aciclovir should be systematically administered, and could have been administered to 55 (49%) of 113 children in our study with treatable causes of encephalitis. Doxycycline should be considered if a rickettsial-associated encephalitis is suspected. Antituberculosis treatment should be systematically discussed by physicians, because neurotuberculosis is frequent and severe, and early treatment guided by local resistance patterns is associated with improved prognosis.[32] This two-step diagnostic strategy could improve early diagnosis and treatment for treatable pathogens, improve outcomes, and reduce fatality rates and long-term disability. Better understanding of long-term sequelae could also improve their prevention and treatment, as well as provide better information to families.

This study had several limitations. We included only one reference hospital per country. However, this was done with the aims of maintaining a high standard of diagnosis and strengthening laboratory diagnostic capacities. This limitation is likely to have led to an underestimation of encephalitis aetiologies occurring elsewhere in the region. Furthermore, to offer a rapid first-line testing procedure in 24 h, done in duplicate at two sites, we were obliged to limit the number of inclusions to one patient per day per country (maximum four per week) due to the workload involved. The first hospitalised patient of the day meeting criteria was included to limit selection bias; however, it might have influenced the results of the study. Because slightly more than half of the included patients were recruited in Cambodia, the generalisability of our findings is somewhat restricted. We assessed potential determinants of unfavourable outcomes, but we cannot exclude residual confounding due to unmeasured factors that contribute to encephalitis severity. Implementation of routine comprehensive testing procedures beyond the current research protocol would require substantial financial and technical resources, which are unfortunately not immediately implementable in the four study countries. Further investment to enable this is needed, in conjunction with enhanced vaccination coverage to prevent key infections, and efforts to improve the accessibility of diagnostics for pathogens treatable with specific therapies, such as HSV-1 and rickettsial pathogens.

In southeast Asia, childhood encephalitis is a major public health concern, leading to high mortality and morbidity with a high rate of neurological sequelae. For children with encephalitis, harmonised and comprehensive diagnostic procedures, independent of clinical and biological features, and tailored to target pathogens known to be prevalent in a given geographical region, could lead to increased rates of laboratory confirmed diagnoses, even in low resource areas.

Contributors

ML and PNN coordinated the study. ML, PNN, OL, XdL, AD-P, AT, PB, and YC conceived the study. JDP, CG, YC, OL, XdL, AD-P, PNN, and ML did the literature research. JH and ALP did the autoimmunity analyses. ME and PPé did the pathogen discovery studies. KB, JDP, PPi, and YC did the statistical analysis. JC, VC, and BR managed the data and did geographical analyses. CG, MC, PD, PB, AD-P, PNN, and XdL conceived and did laboratory analysis. JDP, CG, KB, and ML wrote the manuscript, with editorial assistance from YC, ME, AD-P, OL, XdL, and PNN, and all coauthors edited and agreed on the final version of the manuscript. EB, HS, KS, and DL were in charge of enrolment in Cambodia. CSH, AMMA, and KL were in charge of enrolment in Myanmar. LLK, OST, KP, SP, and HHT were in charge of laboratory analysis in Myanmar. SR, MV, and MM were in charge of enrolment in Laos. TMHT, HTTT, LVN, NTTT, NH, and PHP were in charge of enrolment, analysis, and study design in Vietnam. All authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication. JDP, KB, and ML accessed and verified the underlying study data.

Declaration of interests
PB is currently an employee of GSK, this employment has no relation with the study presented here that was initiated at the time he was head of the virology unit at Pasteur Institute in Cambodia. All other authors declare no competing interests.

Data sharing
Individual participant data will not be made publicly available because data contain protected health information. However, deidentified participant data will be shared upon reasonable request on a collaborative basis if this is approved by the committee of the SEAe project. Whether data will be provided will be based on the scientific merit of the proposals supplied. Requests for access to data should be sent to the corresponding author.

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