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Observational study on the clinical epidemiology of infectious acute encephalitis syndrome including Nipah virus disease, Bangladesh: BASE cohort study protocol

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ABSTRACT

Introduction Nipah virus (NiV) is a bat-transmitted paramyxovirus causing recurrent, high-mortality outbreaks in South and South-East Asia. As a WHO priority pathogen, efforts are underway to develop therapies like monoclonal antibodies and small-molecule antivirals, which require evaluation in clinical trials. However, trial design is challenging due to limited understanding of NiV's clinical characteristics. Given the rarity of NiV infections, strategies targeting improved outcomes for the broader acute encephalitis syndrome (AES) patient population, including those with NiV, are essential for advancing therapeutic research. To address these gaps, we designed the Bangladesh AES cohort study to characterise the patient population, clinical features, treatment practices, common aetiologies and outcomes in patients presenting with AES, including NiV infection, as a clinical characterisation study to inform the design of clinical trials for NiV and AES more broadly.

Methods and analysis This prospective cohort study will be conducted in Bangladesh, a NiV endemic country with annual outbreaks. In collaboration with the ongoing NiV surveillance programme in Bangladesh, we aim to enrol up to 2000 patients of all ages presenting with AES at three tertiary care hospitals within the Nipah belt. Patients who provide informed consent to participate will be monitored throughout their hospital stay until 90 days post enrolment. Data will be systematically collected through interviews and medical record reviews at several time points: on the day of enrolment, day 3, day 7, the day of critical care admission (if applicable), discharge day and 90 days post enrollment. Additionally, a portion of the cerebrospinal fluid collected under the concurrent NiV surveillance protocol will be tested for an array of viral and bacterial pathogens responsible for encephalitis at the International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b) laboratory.

Ethics and dissemination The study received ethical approval from the Oxford Tropical Research Ethics Committee, University of Oxford, UK (OxTREC Ref: 576–23) and the institutional review board of icddr,b, Bangladesh

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first large-scale prospective cohort study in Bangladesh to characterise acute encephalitis syndrome (AES) patient, including Nipah virus disease (NiVD), providing valuable insights into patient outcomes.
⇒ The study will integrate with the national surveillance system, enabling efficient identification and enrolment of AES patients from multiple tertiary hospitals.
⇒ Participants will be followed for 90 days post enrollment, allowing for comprehensive assessment of long-term neurological outcomes and recovery trajectories.
⇒ While the study includes diagnostic testing for specific pathogens, its primary aim is to characterise the AES patient population rather than determine the exact cause of each case.
⇒ Recruiting patients exclusively from tertiary hospitals may limit the generalisability of the findings to other healthcare settings or regions within Bangladesh.

(icddr,b protocol number: 24016). By characterising the AES patient population, this study will generate essential evidence on key clinical parameters, which will be pivotal in optimising the design of clinical trials for potential interventions aimed at improving outcomes in patients with AES, including those with NiV disease. Findings will be shared with participating hospitals, patients and relevant government stakeholders. Results will also be disseminated through conference presentations and peer-reviewed publications.

Clinical trial number Not applicable (this is an observational study).

INTRODUCTION

Nipah virus (NiV) is a highly lethal zoonotic paramyxovirus transmitted through contact with infected bats, pigs, humans or consumption of contaminated raw date palm sap.[1 2] It causes severe neurological and respiratory illness with a high mortality rate.[3 4] Since its discovery in 1998/1999 in Malaysia and Singapore, outbreaks have occurred in Bangladesh, India and the Philippines, with concerns about its spread to regions where *Pteropus* bats, the virus' natural reservoir, are found.[5 6] Mortality has remained consistently high across outbreaks, and there are no approved treatments.[7 8] Current management relies on supportive care, but delays to diagnosis and the lack of standardised protocols and limited access to intensive care in affected regions pose major challenges.[9] Strengthening clinical management and optimising interventions through data-driven approaches is critical to prepare for larger future epidemics.

NiV disease (NiVD) typically manifests as acute encephalitis syndrome (AES), with or without pulmonary disease.[10] AES carries substantial morbidity and mortality, particularly impacting individuals living in low- and middle-income countries, including those also affected by NiVD.[11] Reports from India, a Nipah endemic country, showed an all-cause encephalitis mortality ranging from 14% to 36%.[12 13] Reviews on AES outcomes revealed that nearly half of surviving children experienced neurodevelopmental sequelae, including developmental delay and motor impairment.[14] In adults, 26–62% faced significant long-term consequences, including epilepsy, memory issues, inappropriate behaviour, social skill deficits, fatigue, personality changes, cognitive problems and difficulties in daily living skills.[15]

Diagnosing AES remains a significant challenge, with up to 85% of cases globally undiagnosed, even in high-income settings with advanced diagnostic capabilities, due to the need for extensive sampling and complex analyses.[16–20] In countries like Bangladesh and India, where NiVD is endemic, AES diagnosis is further complicated. NiVD, a relatively rare cause of AES (approximately 3% of AES cases in Bangladesh), often presents with nonspecific symptoms.[21–23] Limited diagnostic access, especially in rural areas, increases diagnostic uncertainty and highlights limitations within current AES diagnostic protocols.

To gain a comprehensive understanding of the clinical epidemiology of NiVD, including neurological and pulmonary involvement, a prospective observational study is needed. Clinical characterisation of NiVD disease including the broader group of patients with AES should form the foundation for developing standardised clinical trial methodologies, including enrolment criteria, outcome measures and subgroup analyses. The clinical epidemiology of AES can also facilitate the identification of high-risk patients, optimising the allocation of diagnostic resources and enhancing overall disease management strategies. This involves improving contact tracing and implementing infection prevention and control protocols within hospitals, especially in settings with limited resources.

During the 1998–99 Malaysian outbreak, 10 out of 11 NiV patients cared for in a Singapore hospital survived,[24] suggesting that improving elements of supportive care improves patient outcomes. Currently, there are several potential NiV-specific therapeutics in different phases of development, including monoclonal antibodies and small molecule antivirals.[25] By characterising the range of causes of AES, we will generate evidence on key clinical parameters necessary to inform the design of clinical trials for potential interventions aimed at improving clinical outcomes in the broader group of patients with AES, including those with NiVD.

AIM AND OBJECTIVES

The aim of this study is to describe the patient population, clinical presentation, natural history, common infectious aetiologies, treatment practices and clinical outcomes of patients presenting with AES (including NiVD) to inform the design of future clinical treatment trials.

The primary and secondary objectives of the study with relevant outcome measures are listed in table 1.

METHODS AND ANALYSIS

Study timeline: the recruitment is ongoing. The key timelines are below:

Institutional review boInstitutional Review Board approval: February 2024.

Recruitment initiated: March 2024.

Anticipated completion of enrolment and follow-up: July 2026.

The Bangladesh acute encephalitis (BASE) study is a hospital-based prospective observational cohort study. Patients who meet the eligibility criteria will be invited to provide informed consent to participate. Enrolled patients will have demographic and clinical data collected during hospital admission and will be followed up at several specific time points up until 90 days after enrollment.

The study will be integrated with clinical care and with an ongoing surveillance programme titled 'National Nipah surveillance in Bangladesh' jointly implemented by the International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b) and the Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh with technical support from the US Centers for Disease Control and Prevention.[26] Clinical staff,

Table 1 Study objectives and relevant outcome measure

	Objectives	Outcome measures
Primary objective	To estimate the proportion of hospitalised patients with AES who develop adverse clinical outcomes	90-day mortality and neurological sequelae *
Secondary objectives	To estimate if the frequency of adverse outcomes differs by patient characteristics, clinical phenotype, disease severity, duration of illness at presentation and common aetiologies	*Outcomes:* 90-day mortality and neurological sequelae *Predictors (at enrolment):* ► Age and sex ► Comorbidities ► Presence of acute signs and symptoms ► Glasgow Coma Scale (GCS) score, or paediatric GCS ► Oxygen saturation ► Days since symptom onset ► Lab-confirmed common AES aetiologies
	To describe the treatment and clinical management of patients with AES	Proportion of patients treated with antivirals, antibiotics, antifungal and/or steroids Proportion of patients requiring oxygen supplementation (mask, high-flow oxygen, non-invasive ventilation, invasive ventilation)
	To estimate the proportions of AES that are caused by common, identifiable, infectious aetiologies†	► Proportions of AES patients with a specific lab-confirmed infection

*This will include disabilities identified by neurological examination including vision and hearing.
†See table 3.
AES, acute encephalitis syndrome.

surveillance staff and BASE study staff will collaborate to align procedures to minimise any burden to participants. Further details on the routine clinical assessment and investigations of encephalitis patients, as well as the ongoing NiVD surveillance activities, are provided in online supplemental appendix methods sections 1.1 and 1.2.

Study sites

The main study sites are three tertiary care public hospitals[1 2] who are engaged with the ongoing NiV surveillance programme located in the Nipah belt (figure 1):

1. Rajshahi Medical College Hospital, Rajshahi.
2. Rangpur Medical College Hospital, Rangpur.
3. Faridpur Medical College Hospital, Faridpur.

The hospitals have been chosen primarily based on their location within the Nipah belt and their record of reporting the most NiVD cases. They also ensure diverse geographical representation, have high bed capacity, manage high patient volumes and are equipped with facilities for routine laboratory tests such as cerebrospinal fluid (CSF) examination. Additionally, patients with confirmed NiVD diagnosis at any hospital in Bangladesh will be alerted to the surveillance programme, and subsequently, to BASE study staff, for potential recruitment. Study staff based at the main sites will travel to sites across the country when alerted of a patient with confirmed NiV infection. Over the past 20 years, an average of 14 cases of NiVD have been reported per year in Bangladesh.[10] Though unlikely, additional NiVD cases will be enrolled if a larger outbreak occurs.

Study population

Enrolment started in March 2023 and will continue until 2000 participants are enrolled. AES patients of any age and sex admitted to study hospitals and meeting the

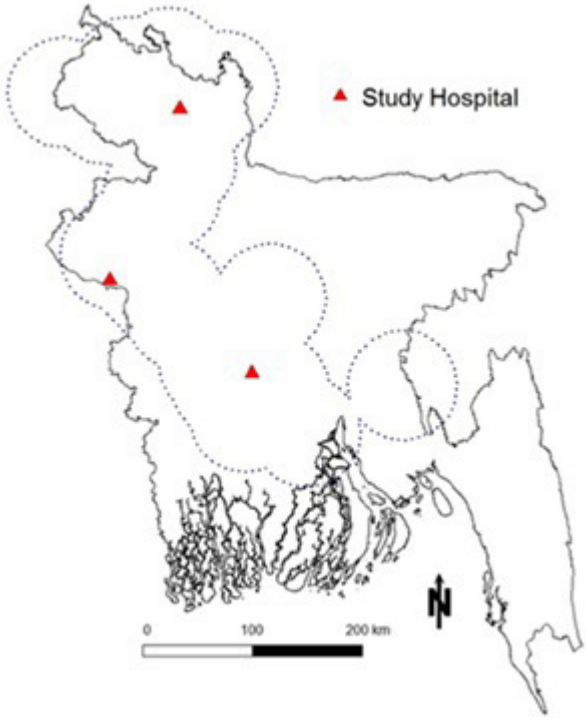

Figure 1 Location of main Bangladesh acute encephalitis syndrome study sites, the dotted line indicates the Nipah belt within Bangladesh.

inclusion criteria are eligible to enrol. In addition, all laboratory confirmed NiVD cases identified by National Nipah surveillance programme during the study period, regardless of location of clinical care, are eligible to enrol.

Inclusion criteria

We will use the enrolment criteria used by the National Nipah surveillance programme.

Patients of any age and sex admitted with *acute encephalitis* defined as:

1. Reported or measured fever (axillary temperature >38.5°C) *and*
2. Evidence of acute brain pathology (eg, altered mental status, new onset seizures or new neurological deficit either diffuse or localised to the brain).
   - The participant is enrolled in the National Nipah surveillance programme.
   - A participant or their legal representative/guardian is willing and able to provide informed consent for participation in the study.

Exclusion criteria

The participant may not enter the study if they have a clear alternative non-infectious disease diagnosis (either clinical or laboratory/imaging confirmed diagnosis) that explains the acute presentation.

Screening and eligibility assessment

For potential participants, enrolment in the Nipah surveillance programme will be an inclusion criterion for the BASE study due to reliance on the programme's NiV testing which is the primary pathogen of interest. Surveillance programme staff will inform the BASE study team of all patients who consent to enrol in the Nipah surveillance programme in the three study hospitals and of any confirmed NiVD patients located at any hospital. BASE study staff will verify the eligibility of patients against the study inclusion and exclusion criteria and initiate a discussion with the patient or their parent/guardian/representative regarding the BASE study. An anonymised screening log tracking the number of patients who meet or do not meet the enrolment criteria and who consent or do not consent to the BASE study will be maintained. Anonymised information regarding the number of patients screened and enrolled to the surveillance programme will be requested from the surveillance programme staff to inform understanding of complete AES patient numbers at each site.

Enrolment

Participants will be enrolled in the study after written informed consent has been obtained and their enrolment has been registered on the electronic case record form. An enrolment log including patient names and contact details will be completed and securely stored at the study site.

Data collection

Clinical and laboratory information will be documented on enrolment, day 3, day 7, on admission to critical care (if applicable) and at hospital discharge. Clinical information between admission and enrolment will also be collected. Clinical and laboratory information will be gathered from the hospital records, patient examination/discussion and surveillance programme records. Study staff will record all laboratory test values and imaging results done as part of the routine care such as complete blood count, liver function tests, renal function tests, electrolytes, CSF examination and imaging such as chest X-ray, CT scan and MRI (see table 2 below).

If CSF has been taken as a part of clinical care, the hospital laboratory will provide an aliquot to be tested for infectious pathogens by the study laboratory at the icddr,b.

If permission from the patient is given, patients who are discharged before day 7 may get a phone call from the study team to request that the clinical information from the day 3 and/or 7 should be included. Patients will be invited to attend a follow-up visit on day 90 of enrollment. These visits will take place at one of the three study hospitals or, if more convenient for the patient, at a nearby hospital where the patient is located or as a home visit if the patient is unable to travel. The visit will include a clinical assessment and a neurological assessment, including vision and hearing testing, that will take approximately 1 hour. Patients who are unable or unwilling to attend the follow-up visit in one of the hospitals or at home will be invited to have a telephone consultation to collect follow-up data.

Patients who report to be pregnant at any point during the study will be asked if the study team may contact them to know the outcome of their pregnancy.

A window period of +/-2 days will be accepted for the day 3, day 7, intensive care unit admission and discharge data collection. A ±14-day window period will apply for the 90-day visit.

Sample processing, transportation and laboratory testing

Data will be collected on the results of samples and laboratory analysis that are part of routine clinical care or the Nipah surveillance programme. If CSF specimens are collected for routine care or Nipah surveillance, an aliquot will be stored for infectious pathogen testing at icddr,b (table 3). The broad panel of pathogens tested was selected based on the most frequently reported infectious causes of AES in South Asia, as supported by previous epidemiological studies.[24] Aliquots made for this study will depend on availability of volume. Clinical and surveillance laboratory processing will take precedence in the absence of sufficient volume for all analyses. Samples will be handled and shipped by the hospital laboratory according to routine procedures. Shipments will be made to the icddr,b laboratory in Dhaka where processing and testing will follow established protocols.

Table 2 Schedule of study procedures

	Day 1	Day 3	Day 7	ICU admission	All available routine investigation	Discharge	Day 90
	Enrolment	In hospital (or by telephone if discharge before day 7)					Outpatients follow-up
Eligibility screen	×						
Informed consent	×						
Data collection: clinical features					×		
Demographic	×						
Exposure	×						
Baseline clinical information	×						
Physical examination	×	×	×	×			
Clinical observation	×	×	×	×		×	
Treatment and interventions	×	×	×	×		×	
Complications	×	×	×	×		×	
Outcome						×	×
Neurological assessment						×	×
Laboratory testing							
Testing of available routine or surveillance CSF aliquot for target pathogens	× (1)						
Data collection: available routine laboratory record							
Complete Blood Count	×				×		
Blood glucose					×		
CRP	×				×		
Liver function tests	×				×		
Renal function	×				×		
Electrolytes	×				×		
CSF	×				×		
Data collection - available routine imaging records							
Chest X-Ray	×				×		
MRI of brain	×				×		
CT brain	×				×		
Data collection: available routine or surveillance programme microbiological tests							
Nipah virus	×				×		

*If CSF sample taken for clinical care or surveillance programme.
CRP, C reactive protein; CSF, cerebrospinal fluid; ICU, intensive care unit.

In brief, an aliquot of CSF will be transferred into lysis buffer (NucliSENS easyMag, bioMerieux, Rodolphe St, Durham, North Carolina, USA) to inactivate potential high-risk pathogens while stably preserving nucleic acids, ensuring the specimens are safe for handling in a biosafety level-2 environment. Total nucleic acids from the CSF will be extracted using an InviMag Virus DNA/RNA Mini Kit (INVITEK Molecular, Berlin-Buch GmbH, Germany) on Kingfisher Flex 96 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) automated nucleic acid extraction system according to the manufacturer's instruction. The eluted nucleic acid will be of high quality and suitable for downstream molecular analyses.

Real-time reverse transcription PCR (rRT-PCR) assays will be conducted using commercially recommended kits specific for both DNA and RNA targets (table 3). The

Table 3 List of target pathogens tested by real time RT-PCR

Pathogen	Primer/probe	Oligo sequences
Viral pathogens		
Nipah virus[29]	NVBN593F	GGTCTCTGCAGTTATCACCATCGA
	NVBN705R	ACCTTAGCCCATCTTCTAGTTTCA
	NVBN654P	FAM-CAGCTCCCGACACTGCCGAGGAT-BHQ1
Herpes simplex virus type 1[30]	VZV-Taqman3	CCGATTCTGGATTTTCGTTGTT
	VZV-Taqman4	AAAGTCGATTTCCCCCCAAA
	VZV-g28-Fam	6-Fam-AGCCCCTGGCCTAGACGCGTGA-Tamra
HSV type 2 (HSV2)[30]	HSV1-Taqman3	GATGCCGGTTTCGGAATTC
	HSV1-Taqman4	CCCATGGAGTAACGCCATATCT
	HSV1-GC-Tet	Tet-ACCCGCATGGAGTTCCGCCTC-Tamra
Cytomegalovirus (CMV)[30]	CMV-Pol-F	CATGCGCGAGTGTCAAGAC
	CMV-Pol-R1	ACTTTGAGCGCCATCTGTTCCT
	CMV-Pol-R2	ACTTTGAGTGCCATCTGTTCCT
	CMV-Pol-Vic	Vic-TGCGCCGTATGCTGCTCGACATA-Tamra
Epstein-Barr virus (EBV)[30]	EBV-EBER-F	AAACCTCAGGACCTACGCTGC
	EBV-EBER-R	AGACACCGTCCTCACCAC
	EBV-EBER-Fam	6-Fam-TAGAGGTTTTGCTAGGGAGGAGACGT GTG-BHQ
Varicella Zoster virus (VZV)[30]	VZV-Taqman3	CCGATTCTGGATTTTCGTTGTT
	VZV-Taqman4	AAAGTCGATTTCCCCCCAAA
	VZV-g28-Fam	6-Fam-AGCCCCTGGCCTAGACGCGTGA-Tamra
Dengue virus (DENV)[31]	DEN-UTR-F	GCATATTGACGCTGGGARAGAC
	DEN-UTR-R1	TTCTGTGCCTGGAATGATGCTG
	DEN-UTR-R2	YTCTGTGCCTGGATWGATGTTG
	DEN-UTR-P	6FAM-CAGAGATCCTGCTGTC-MGB(NFQ)
Influenza virus[32]	FluA-M-F	GAC CRA TCC TGT CAC CTC TGA C
	FluA-M-R	AGG GCA TTY TGG ACA AAK CGT CTA
	FluA-M-P	6 FAM-TGC AGT CCT CGC TCA CTG GGC ACG-BHQ1
	FluB-F	TCC TCA AYT CAC TCT TCG AGC G
	FluB-R	CGG TGC TCT TGA CCA AAT TGG
	FluB-P	6 FAM-CCA ATT CGA GCA GCT GAA ACT GCG GTG-BHQ1
Bacterial pathogens		
Streptococcus pneumoniae[33]	lytA-F	5′-ACGCAATCTAGCAGATGAAGCA-3′
	lytA-R	5′-TCGTGCGTTTTAATTCCAGCT-3′
	lytA-P	5′-FAM-GCCGAAAACGCTTGATACAGGGAG-3′-BHQ1
Pan Rickettsia (PanR8)[34]	PanR8_F	AGC TTG CTT TTG GAT CAT TTG G
	PanR8_R	TTC CTT GCC TTT TCA TAC ATC TAG T
	PanR8_P	FAM-CCT GCT TCT ATT TGT CTT GCA GTA ACA CGC CA-BHQ1
Orientia tsutsugamushi (scrub typhus)[35]	OriJ_F	AAC TGA TTT TAT TCA AAC TAA TGC TGC T
	OriJ_R	TAT GCC TGA GTA AGA TAC RTG AAT RGA ATT
	OriJ_P	FAM-TGG GTA GCT TTG GTG GAC CGA TGT TTA ATC T-BHQ1
Mycobacterium tuberculosis[27]		

A, adenine; C, cytosine; G, guanine; RT-PCR, reverse transcription PCR; T, thymine.

mastermix will be prepared following the manufacturer's guidelines, and the template will be added accordingly. The rRT-PCR assays will be performed on a BioRad CFX-Opus PCR system (BioRad, Hercules, California, USA) using the recommended thermal profile provided by the assay developer. Positive and negative controls will be included in each rRT-PCR experiment to ensure assay accuracy. Amplification curves will be visually checked,

and tests with poor-quality curves will either be repeated or considered negative. A cycle threshold value of <35 will be classified as positive, while values ≥35 will be regarded as negative.

For the rapid diagnosis of *Mycobacterium tuberculosis*, we will use the GeneXpert MTB/RIF Ultra test (Xpert Ultra), an advanced, cartridge-based nucleic acid amplification test.[27] The Xpert Ultra, recommended by the WHO, is specifically designed to detect both *Mycobacterium tuberculosis* and rifampicin resistance. Using advanced melting curve analysis, this next-generation assay targets a key region of the rpoB gene, including its critical 81-base pair 'core region', as well as sequences in the multicopy IS1081 and IS6110 insertion elements. This enhanced sensitivity and specificity make the Xpert Ultra an optimal tool for prompt and reliable tuberculosis diagnosis and drug resistance testing.

In cases where multiple pathogens are identified in a sample, an expert panel consisting of microbiologists, epidemiologists and clinicians will review the patient's clinical, laboratory and exposure records to determine the most likely causative pathogen. Test results will be regularly reported to the respective hospitals to aid in patient management.

Sample size determination

This is a descriptive observational study aimed at characterising AES, including NiVD. Our aim is to collect detailed clinical data, given our limited understanding of the clinical characteristics of AES. The primary outcome of interest (to inform clinical trial design) is the proportion of AES patients with an adverse clinical outcome (mortality or neurological sequelae). To ensure the study captures a sufficient number of outcome events to provide informative and representative data, we estimated a sample size based on the two primary outcomes of interest (mortality and neurological sequelae) to guide required enrolment.

Data from ongoing national Nipah surveillance in Bangladesh during 2022–2023 showed an in-hospital mortality rate of around 15% (756/4952) of enrolled AES participants (National Nipah surveillance team, personal communication). Studies from neighbouring India also reported an all-cause 14%–36% mortality rate among hospitalised encephalitis patients irrespective of aetiologies.[12 13]

Assuming an expected mortality outcome of 15%, the estimated required sample size is 1532 for the margin of error or absolute precision of ±2% in estimating the case fatality ratio with 95% confidence and considering the potential loss/attrition of 20%. With this sample size, the anticipated 95% CI is 13% to 17%. This sample size is calculated using the formula for estimating a proportion as previously described.[26]

The reported prevalence of long-term neurological sequelae varied between 32–53% for children and 26–62% for adults depending on the duration of follow-up.[11 14] Therefore, for the long-term neurological sequelae outcome, we assume a conservative prevalence of 20% for long-term neurological sequelae outcome of enrolled participants during our 90-day follow-up. With 20% prevalence, the estimated required sample size is 1922 for the margin of error or absolute precision of ±2% in estimating the prevalence of long-term neurological sequelae with 95% confidence and considering the potential loss/attrition of 20%. With this sample size, the anticipated 95% CI is 18% to 22%.

To capture the required number of both outcome events, we plan to enroll 2000 AES patients in our study. We plan to conduct the study in three purposively selected tertiary care hospitals that have ongoing NiVD surveillance and are situated within the Nipah belt. The enrollment of patients will vary across sites due to differences in facility size and catchment area.

Statistical analysis

We will perform descriptive analysis of patient characteristics, acute signs and symptoms, laboratory parameters, disease severity, common aetiologies, clinical management, including medications and clinical outcomes of patients. Continuous data will be presented as a mean with SD where data are normally distributed and as a median with the 25th and 75th centiles for non-parametric data along with a 95% CI for both the mean and the median. Categorical data will be summarised as frequencies and percentages.

We will also compare frequency of adverse outcomes (mortality and long-term neurological complications categorised by severity: mild, moderate or severe) by patient characteristics, exposures (date palm sap consumption, contact with infected humans, bats or pigs), acute signs and symptoms, disease severity, duration of illness at presentation and common aetiologies. For univariate comparisons, Welch's t-test for two groups or Analysis of Variance (ANOVA) for more than two groups will be used when dealing with normally distributed continuous data. Non-parametric continuous data will be compared using the Mann-Whitney U test for two groups or the Kruskal-Wallis test for three or more groups. In cases of categorical data, differences will be assessed using the χ^2 test or Fisher's exact test where applicable.

To measure the association between patient characteristics, acute signs and symptoms, disease severity, duration of illness at presentation and common aetiologies, and adverse outcomes defined as 90-day mortality and neurological sequelae, we will calculate hazard ratios (HRs) using multivariable Cox regression after adjusting for potential confounders. Table 4 describes key patient parameters, analysis framework and rationale based on clinical trial needs.

We do not anticipate major differences between study sites given their similar infrastructure and roles as tertiary referral centres for AES within their respective regions. However, any observable variation in patient characteristics, clinical management or outcomes between hospitals will be described where relevant.

Table 4 Key patient parameters, analysis framework and rationale based on clinical trial needs

Parameter	Metrics	Analytic approach	Justification based on clinical trial needs
Patient population	► Age and gender ► Comorbidities	► To estimate if the frequency of adverse outcomes differs by patient characteristics	► A clinical trial may need to stratify randomisation or adjust analyses by key predictors of outcomes to minimise or adjust for chance imbalances between treatment arms in the frequency of major predictors of outcome. ► Subgroup analysis may be needed to identify if there is a differential treatment effect by patient characteristics.
Clinical presentation	► Oxygen saturation ► GCS ► Patterns of signs, symptoms/complications (respiratory vs CNS)	► To estimate if the frequency of adverse outcomes differs by disease severity or disease phenotype (eg, CNS only vs CNS+respiratory) at presentation	► A clinical trial may need to stratify randomisation or adjust analyses by key predictors of outcomes to minimise or adjust for chance imbalances between treatment arms in the frequency of major predictors of outcome. ► Subgroup analysis may be needed to identify if there is a differential treatment effect by disease phenotype.
Duration of illness at presentation	► Days since symptom onset	► To estimate if the frequency of adverse outcomes differs by duration of illness at presentation ► To estimate proportion of the target population who present early or late to inform decision about plausible treatments	► Stratified randomisation or adjusted analyses may be needed if duration of illness is a major predictor of outcome. ► There may be a therapeutic window for certain interventions which may preclude a proportion of the target population. ► Subgroup analysis may be needed to identify if there is a differential treatment effect by time since disease onset.
Treatment	► Use of antibiotic, antiviral, antifungal and/or steroid ► Oxygen supplementation (mask, high-flow oxygen, non-invasive ventilation, invasive ventilation)	► To estimate the proportion of patients treated with antivirals, antibiotics, antifungal and/or steroids ► To estimate the proportion of patients who required oxygen supplementation (mask, high-flow oxygen, non-invasive ventilation, invasive ventilation)	► A clinical trial may wish to define the baseline or minimum standard of care or may wish to evaluate an intervention that alters an aspect of standard of care.
Complications and clinical outcome	► Organ dysfunction ► Deaths (in-hospital and post discharge up to 90 days) ► Neurological sequelae (up to 90 days)	► To estimate the proportion of patients that develop vital organ dysfunctions ► To estimate the frequency and timing of 90-day mortality ► To estimate the frequency and duration of 90-day neurological sequelae	► A clinical trial will need to define primary and secondary end points that are readily and reliably measurable and are sufficiently common. ► Trial power calculations will require estimates of the frequency and timing of the primary outcome and a judgement of a meaningful and realistic treatment effect size.
Aetiologies	► Lab-confirmed aetiologies	► To estimate the proportion of patients with specific aetiologies and if clinical presentation and outcome varies by aetiologies	► Subgroup analyses may be needed to identify and quantify if there is a differential treatment effect by disease aetiology.

CNS, central nervous system; GCS, Glasgow Coma Scale.

Missing data handling

We will minimise missing data through regular data quality checks during recruitment and data collection, in line with best practices in clinical research. A missing at random (MAR) assumption will be made for missing data; however, this will be investigated to determine whether reasons for missing data can be obtained and to determine if such an assumption is realistic. Patterns of

missing data across sites and patient characteristics will be reported. In this study, we will address the issue of missing data using multiple imputation, which is particularly adapted to data that are missing completely at random or MAR. This method involves creating multiple complete datasets by imputing missing values, analysing each dataset separately and then pooling the results to account for the uncertainty caused by the missing data. This approach aims to preserve statistical power and reduce bias in the analyses. Additionally, sensitivity analyses will be conducted to evaluate the robustness of our results under different assumptions about the missing data. This strategy should ensure that the conclusions reached are reliable and reflective of the true effects, despite the presence of missing data.

ETHICS AND DISSEMINATION

Ethics

The study received ethical approval from the Oxford Tropical Research Ethics Committee (OxTREC Ref: 576–23) and the Institutional Review Board (Research Review and Ethics Review Committee) of icddr,b (icddr,b protocol number: 24016). Any future amendments to the study protocol will also be submitted for review and approval to these committees.

Participant safety

The BASE study will be conducted in strict adherence to ethical standards, ensuring the confidentiality of all participants in accordance with applicable good clinical practice guidelines, national regulations and the Declaration of Helsinki. Informed consent will be obtained from all participants or their legal representatives prior to enrolment. The consent process will clearly explain the study's purpose, procedures, potential risks and benefits and the right to withdraw at any time without affecting future care. The consent form will be provided in Bengali, and participants will have ample opportunity to ask questions before and after deciding on participation. For those unable to read or write, the consent process will include an impartial witness to confirm comprehension and voluntary participation.

To protect participant confidentiality, data will be anonymised or pseudonymised, with each patient assigned a unique patient identification number, which will be consistently used throughout the study. This number will be assigned and managed securely by the centre coordinator, and personal data will only be released in accordance with local regulations and ethics committee approvals.

Dissemination

We plan to share the study findings with participating hospitals, patients, relevant government stakeholders and funders. Academic dissemination will include peer-reviewed publications, conference presentations and open-access data sharing in line with study partners' data governance framework.

In addition, a policy brief summarising key findings and their implications for clinical management and outbreak preparedness will be developed and shared with the Ministry of Health and Family Welfare, Government of Bangladesh and the IEDCR, which is one of our study partners and the national authority responsible for encephalitis and NiVD management guideline development. We anticipate that findings from this study will directly inform updates to supportive care protocols, AES case management practices and preparedness strategies.

To support broader regional knowledge-sharing, study findings will also be presented at international forums, including the WHO Coordinated Research and Collaboration initiative for the Paramyxoviridae family, led by the Indian Council of Medical Research (ICMR). This initiative fosters collaboration across countries affected by high-consequence paramyxoviruses, including Nipah, and provides a key platform to align BASE study outputs with regional preparedness and research priorities.

Patient and public involvement

Patients and the public were not involved in the design phase of this study. However, the results will be shared with study participants and the wider community through accessible summaries and public engagement activities.

DISCUSSION

The BASE study is designed to address critical gaps in the clinical understanding of AES, including NiVD and to provide essential data that will inform future clinical trials aimed at evaluating potential treatments for NiVD. While the study is designed to integrate with existing clinical and surveillance infrastructure, we anticipate operational challenges during implementation. These include ensuring consistent training across study sites, maintaining sample integrity during transport and avoiding dependence on overstretched hospital systems for essential logistics. However, several strategies are in place to mitigate these risks. The national Nipah surveillance programme, active in Bangladesh since 2007, is well established and includes robust systems for sample transport, testing and data reporting, which the BASE study is leveraging (see online supplemental appendix methods, sections 1.2). Research staff involved in this study are employed by icddr,b and have been additionally recruited and trained specifically for this protocol, rather than being drawn from the hospital clinical workforce. This helps ensure standardised data collection and reduces disruption to routine clinical care. Supplies for sample collection and transport, including lumbar puncture for CSF sampling, are provided directly through the study and do not rely on hospital inventories. All microbiological and virological testing will be conducted at icddr,b's central laboratories, which routinely handle surveillance samples and are equipped to support the study's diagnostic needs. These

proactive measures aim to reduce logistical bottlenecks and promote high-quality data collection across all participating sites.

Several limitations may also influence the study's objectives. Missing data and unmeasured confounders, common in observational studies, could introduce bias and affect outcome estimates. However, the study incorporates rigorous data monitoring and multiple imputation techniques to mitigate these issues, ensuring the data's robustness for future trial design. Additionally, variability in diagnostic and care practices across hospitals may impact data consistency. Although such differences are presumed to be minimal, as all hospitals are public tertiary care centres with broadly similar infrastructure and clinical capacity, any minor variations that are observed will be described and interpreted accordingly. Furthermore, as the study is conducted in tertiary-care hospitals, individuals with milder illness or limited access to inpatient care, particularly in rural settings, may be underrepresented. While this may affect the generalisability of the findings to all infectious encephalitis patients, the primary aim of the study is to characterise hospitalised cases in order to inform clinical trial design in settings where inpatient care is provided.

The BASE study offers a valuable opportunity to overcome the limitations of conducting NiVD-specific trials by focusing on AES as a broader syndrome. Expanding this syndromic approach beyond Bangladesh to other regions with a high burden of infectious encephalitis could form the basis of a global network for harmonised data collection, similar to the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC)-WHO model for COVID-19.[28] Such a network would generate standardised, actionable data, optimising clinical trial designs and improving patient outcomes for AES, including those affected by NiVD.

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