The Epidemiology of and Risk Factors for Central Nervous System Infections in Vietnam

'Thesis submitted in accordance with the requirements of the

University of Liverpool for the degree of Doctor in Philosophy

by

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ABSTRACT

Dr Hannah Brindle: The Epidemiology of and Risk factors for Central Nervous System Infections in Vietnam

In Vietnam, central nervous system (CNS) infections due to acute encephalitis syndrome (AES) and meningitis are caused by multiple aetiologies. Despite implementation of vaccination, the most common cause in children is Japanese encephalitis virus (JEV), and in adults, *Streptococcus suis*. In at least half of the cases, the cause is unknown however, there are peaks in incidence of acute encephalitis syndrome (AES) in the summer months in northern Vietnam.

This thesis is composed of four results chapters with the following main objectives: 1) to understand the spatio-temporal distribution and risk factors of CNS infections in Vietnam; 2) to assess the feasibility and acceptability of conducting a case control study which aims to determine the risk factors for CNS infections; 3) to evaluate human movement and contact patterns in relation to risk factors for CNS infections and 4) to determine whether there are associations between the seroprevalence of JEV in pigs and incidence of AES and Japanese encephalitis (JE) in humans.

The first results chapter uses monthly national surveillance data of the incidence of AES and meningitis at the provincial level from 1998 to 2016. The incidence of AES was highest in provinces bordering Lao PDR and seasonal patterns corresponded closely with those of JE. Negative binomial Integrated Nested Laplace Approximation (INLA) multivariate models showed a positive correlation of the number of cases of AES and meningitis with temperature and absolute humidity, and AES with the Normalised Difference Vegetation Index (NDVI) at a lag of one month. This might suggest that many cases of CNS infections are due to vector-borne diseases.

In the second results chapter, many of the participants with AES had a bacterial meningitis due to the poor specificity of the case definition for AES, the predominantly adult patient population and the season of recruitment. The recruitment of matched controls who were relatives of hospital in-patients was also insufficient. Therefore, adaptations to the methods would be required for a future, larger case control study to evaluate risk factors for AES.

The third results chapter showed that it was feasible to determine human movement patterns using global positioning system (GPS) tracking devices and contact patterns using diaries over the period of a year. Urban adults travelled the most and all participants spent the most time in areas of medium to high NDVI. Contact patterns occurred most frequently between children and adults. These findings may have implications for the transmission dynamics of pathogens causing CNS infections with larger studies recommended.

The final results chapter showed that nearly two-third of pigs were seropositive to JEV at slaughter however, the specificity of the complement enzyme-linked immunosorbent assay (cELISA) was 57.4%. Multivariate analysis showed no evidence of an association between incidence of human JE and AES and seroprevalence of JEV in pigs. However, human-pig studies at the individual level are needed to determine whether this absence of risk is true.

There is evidence that many CNS infections may be due to undiagnosed JE however, this requires further investigation before any changes to public health policy are made.

INTRODUCTION

This Wellcome Trust Clinical PhD Fellowship was originally designed to be a multi-centre case-control study conducted in Nepal. Over a period of approximately two years, cases of acute encephalitis syndrome (AES) were to be recruited from at least three tertiary hospital sites; Kanti Children's Hospital in Kathmandu, BP Koirala Institute of Health Sciences (BPKIHS), Dharan and Lumbini Zonal Hospital, Butwal with community controls from locations within the hospital catchment areas. Diagnostics were to be performed by a collaborative study, conducted by the Ministry of Health of Nepal with the University of Liverpool and the World Health Organization. An additional study whereby healthy community members were to participate in a human movement and contact pattern study in the Kathmandu (urban) and Sunsari (rural) districts was also to be conducted.

However, on 25 April 2015, there was a major earthquake of magnitude 7.8 with a depth of 15km and an epicentre 81km northwest of Kathmandu (World Health Organization, 2018a). A second earthquake of magnitude 7.3 with an epicentre 76 km northeast of Kathmandu occurred on 12 May 2015 (United Nations Office for the Coordination of Humanitarian Affairs, 2015). There were 8,659 deaths, 21,924 injuries and 88,482 people displaced (World Health Organization Country Office for Nepal, 2015).

I conducted a situation report with my local supervisor and drafted an adaptation to the PhD to account for undertaking work in a humanitarian crisis. However, following discussion with the Wellcome Trust Centre for Global Health Research, Liverpool, it was decided that the research in Nepal should be closed and the work transferred to Hanoi, Vietnam, in collaboration with the Oxford University Clinical Research Unit (OUCRU). As a result of this, the programme of work was altered particularly with regard to differences in research governance between Vietnam and Nepal. Whilst the aim of the work to evaluate risk factors associated with CNS infections through a case control study was the same, the proposed geographical distribution of the recruitment was much more limited in Vietnam. It was intended to recruit cases of AES from the National Hospital for Tropical Diseases (NHTD) and the National Hospital of Pediatrics (NHP) in Hanoi. As NHP was already included in a multicentre study of AES in southeast Asia led by Institute Pasteur, the possibility of collaborative work

was explored but was ultimately not feasible. Recruitment of paediatric cases from other hospitals within the Red River Delta region of northern Vietnam were explored but not possible to set-up within the timeframe of the grant. Without the collaboration with the WHO, diagnostics were required to be funded through the clinical PhD fellowship. The geographical area in which the movement and contact pattern work was also restricted to one province in northern Vietnam.

This thesis therefore evaluates the epidemiology and risk factors of central nervous system infections (CNS) in Vietnam. Following a literature review of the epidemiology of CNS infections in south, southeast and east Asia there are four results chapters. The first results chapter is an ecological study which uses national surveillance data of notifiable diseases and syndromes to understand the spatio-temporal distribution and risk factors for CNS infections and make suggestions as to the possible cause given that many cases are of unknown aetiology. The second results chapter is a pilot case control study which aims to determine the feasibility of the recruitment of cases and controls and the feasibility and acceptability of conducting home visits to understand participants' risk factors for AES in northern Vietnam. The third results chapter evaluates the human movement and contact patterns amongst a healthy cohort of participants living in Ha Nam province to determine their risk of CNS infections. The final results chapter examines the associations between the seroprevalence of Japanese encephalitis virus (JEV) in pigs and incidence of Japanese encephalitis (JE) and AES in humans.

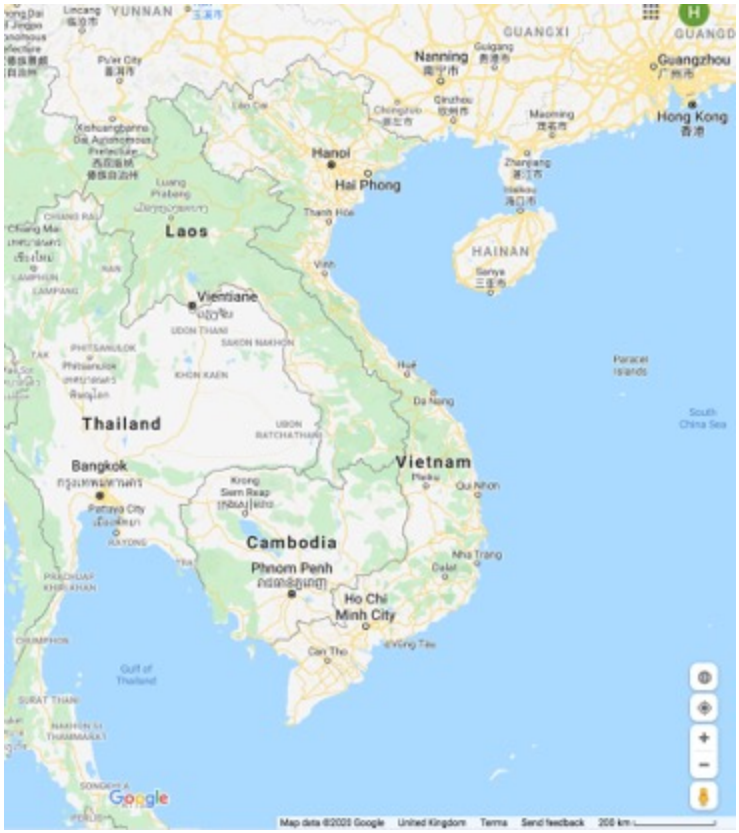
VIETNAM

Vietnam is located in southeast Asia and is bordered by China to the north, Lao Peoples' Democratic Republic (Lao PDR) and Cambodia to the west and the "East sea" to the east (figure a). It stretches from approximately 8.5°N to 23°N (Nguyen-Le, Matsumoto and Ngo-Duc, 2014).

The geography varies from the low-lying Red River Delta in northern Vietnam and the Mekong Delta in southern Vietnam, to mountainous areas (up to 3,000 m elevation) in the northwest and the Central Highlands. Northern Vietnam has four seasons (Trang *et al.*, 2016) with a cool and humid winter and a warm, wet summer whereas

southern Vietnam has a tropical climate (Kelly-Hope *et al.*, 2007) with a wet and dry season (Lin *et al.*, 2000). The total area of the country is 331,235.7 km^2 (General Statistics Office of Viet Nam, 2019).

Figure a) A map of Vietnam.



The population of Vietnam is 97 million with 70% of the population aged under 35 years (The World Bank, 2020a). The capital city, Hanoi, in northern Vietnam, has a population of 8 million within the municipality and the largest city, HCMC (HCMC) in southern Vietnam, a population of 9 million within the municipality (General Statistics Office of Viet Nam, 2019).

From 111 BC to 905 AD, Vietnam was under Chinese colonial rule however, it alternated between being independent and under Chinese rule until 1427 (Adger, 1999). Vietnam was colonised by France from 1867 until the end of Franco-Vietnamese War in 1954. Following this, the country was divided into the communist North and non-communist South under the Geneva Accord (Adger, 1999; Tucker-

Jones, 2014). Ongoing conflict led to the Vietnam War between 1965 and 1975 with the south being supported by the United States. Following victory by the north, the country was reunified in 1976 however, economic growth was hindered (Asselin, 2018). During the 1980s Vietnam suffered from food shortages despite three-quarters of the Vietnamese labour force being employed in agriculture. As a result of this, the country was reliant on foreign aid until the economic reform in 1986 'Doi Moi' which increased the workforce in the modern sector and integrated Vietnam into the world markets with a rise in exports. As a result of this, the Vietnamese economy grew rapidly with a decline in the ratio of those living in poverty (Tran, 2013). In 2019 the growth of the gross domestic product (GDP) was one fastest in the world at 7% (The World Bank, 2020a). However, there are still economic discrepancies with 86% of those who remain poor, being from ethnic minorities. Despite this, 70% of those living in rural areas and 95% of those in urban areas have access to clean water and 99% use electricity for lighting (The World Bank, 2020a).

As economic growth increased and living standards improved, health outcomes also improved with a reduction in infant mortality rate from 32.6 per 1000 live births in 1993 to 16.7 per 1000 live births in 2017 and an increase in life expectancy from 70.5 years in 1990 to 76.3 years in 2016 (The World Bank, 2020a).

The healthcare system in Vietnam consists of both private and public facilities. Public hospitals are divided into central level hospitals, provincial level hospitals and district level hospitals with most people visiting hospital as their first point of care (World Health Organization, 2020g). However, hospital autonomy reforms initiated in the 1990s allow the public sector to charge user fees to mobilise finance from the private sector and individuals (World Health Organization, 2020g; The World Bank, 2012). This has led to increases in total hospital revenues with improved salaries of hospital staff and an expansion of healthcare services. However, it appears that central hospitals and those in large cities have benefited more than those in rural areas (The World Bank, 2012).

THE ROLES AND RESPONSIBILITIES OF THE WORK

The work for this thesis was designed by myself with support of my supervisors and collaborators.

The analysis of the data was undertaken by me and reviewed by my supervisors. I sought advice from Dr Marc Choisy (OUCRU) and Dr Leo Bastos (London School of Hygiene and Tropical Medicine) regarding the choice of spatio-temporal models for chapters two ("The spatio-temporal distribution of and risk factors associated with Acute Encephalitis Syndrome and meningitis in Vietnam: an ecological study") and five ("The seroprevalence of Japanese encephalitis virus in pigs in northern Vietnam"). I co-supervised Maria Malik, an intern from Princeton University who performed an initial descriptive analysis of the surveillance of AES in Vietnam. However, none of her work was included in the thesis. Dr Marc Choisy (supervisor in Vietnam) provided the interpolated data for climatic variables from the weather stations and due to the size of computation required, the NDVI for the provinces in chapter two.

Data cleaning was undertaken by myself with support of the OUCRU Clinical Trials Unit for chapter three ("A pilot case-control study to assess the risk factors for Acute Encephalitis Syndrome (AES) in patients admitted to the National Hospital for Tropical Diseases from the Red River Delta region in northern Vietnam") and staff from the National Institute of Hygiene and Epidemiology (NIHE) for chapter four ("Evaluation of the daily activities and contact patterns of healthy adults and children in rural and urban settings in Ha Nam province, Vietnam to explore potential risk factors for acute encephalitis syndrome") where required. The national surveillance data used for chapter two was already cleaned and made available as an R package prior to my use for analysis.

Laboratory work for chapter three was undertaken by staff at OUCRU and for chapter five by staff at OUCRU, the National Institute for Veterinary Research (NIVR), Hanoi, and Mahidol University, Bangkok. The results were checked by me.

Consent in Vietnamese was undertaken by study staff from the National Hospital for Tropical Diseases (NHTD), and the Preventive Medicine Center (PMC) Ha Nam for chapters three and four, respectively. Primary data collection was undertaken in Vietnamese by local staff. For chapter three, clinical data collection was performed by the staff from NHTD with support of OUCRU staff where required and at the homes of participants by field staff employed by OUCRU. For chapter four, the contact pattern diaries were completed by staff from the PMC. Data entry into the electronic data system CliRes was undertaken by OUCRU staff. The focus group discussions and discussion with staff were facilitated, transcribed and translated into English by staff from OUCRU.

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Finally, thank you to all of the study participants both in Vietnam and Nepal. Your input was much appreciated and I hope that this work helps to better understand and provide guidance for the control of CNS infections in your communities.

CHAPTER 1 THE EPIDEMIOLOGY AND AETIOLOGY OF CENTRAL NERVOUS SYSTEM INFECTIONS IN SOUTH, SOUTHEAST AND EAST ASIA

1.1 AN INTRODUCTION TO CENTRAL NERVOUS SYSTEM (CNS) INFECTIONS

The central nervous system (CNS) is composed of the brain and spinal cord. This differs from the peripheral nervous system which is composed of the nerves which branch off the spinal cord. CNS infections typically include encephalitis, meningitis and meningo-encephalitis (Sigfrid *et al.*, 2019) but can also include brain and spinal epidural abscesses, ventriculitis and subdural empyemas (Ziai and Lewin, 2008). For the purpose of this thesis, CNS infections refers to encephalitis, meningitis and meningo-encephalitis.

THE CASE DEFINITIONS OF CNS INFECTIONS

THE CASE DEFINITION OF ENCEPHALITIS

Encephalitis is defined as inflammation of the brain parenchyma (tissue) leading to dysfunction of the neurological system (Johnson, 1996; Venkatesan *et al.*, 2013; Sejvar *et al.*, 2007). Confirmed diagnosis would be based on evidence of oedema, inflammation and the death of neuronal cells (neurophagia) however, performing pre-mortem biopsies is challenging and not often undertaken (Sejvar *et al.*, 2007; Venkatesan *et al.*, 2013). Diagnosis may therefore be based on a clinical definition alone however, where resources are available neuroimaging, laboratory diagnosis and electroencephalography (EEG) may also be used (Venkatesan *et al.*, 2013; Granerod *et al.*, 2010a). However, no standard case definitions exist for encephalitis (Granerod and Crowcroft, 2007) and often vary between research studies (Hills *et al.*, 2009; Jmor *et al.*, 2008). Additionally, studies may use the World Health Organization (WHO) International Classification of Disease (ICD) codes (Jmor *et al.*, 2008).

In 2006, the World Health Organization (WHO) provided a clinical definition for an ‘acute encephalitis syndrome’ as part of the surveillance standards for the pathogen Japanese encephalitis (JE) (Solomon *et al.*, 2008; Hills *et al.*, 2009). AES is defined as ‘a person of any age, at any time of year, with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) AND/OR new onset of seizures (excluding simple febrile seizures). Other early clinical findings can include an increase in irritability, somnolence or

abnormal behaviour greater than that seen with usual febrile illness' (World Health Organization, 2006). However, the specificity of detecting cases with Japanese encephalitis virus (JEV) was only found to be 39% (95%CI 30-48%) in those admitted to the Centre for Tropical Disease, HCMC, Vietnam (Solomon *et al.*, 2008). Examples of the use of the WHO AES case definition as an inclusion criteria includes studies in Nepal (Rayamajhi *et al.*, 2011) and India (Goel *et al.*, 2017).

The case definition for AES developed by the Brighton Collaboration Encephalitis Working Group has been one of the most widely used (Venkatesan *et al.*, 2013). However, this uses criteria based on varying levels of diagnostic certainty to aid the standardisation of encephalitis as an adverse event following immunisation. Level 1 of diagnostic certainty includes the confirmation of inflammation by histopathology; and levels 2 and 3, the presence of an encephalopathy e.g. a reduction in the level of consciousness, lethargy or a change in personality lasting more than 24 hours with additional specific symptoms and signs of encephalitis in combination with a number of the following: fever, raised white cell count in the cerebrospinal fluid (CSF pleocytosis), EEG findings consistent with encephalitis or neuroimaging consistent with encephalitis. Neuroimaging should show evidence of parenchymal inflammation on computerised tomography (CT) or magnetic resonance imaging (MRI) (Sejvar *et al.*, 2007).

In March 2012, the International Encephalitis Consortium in the United States stated that the sensitivity and specificity of the case definition produced by the Brighton Collaboration Encephalitis Working Group was unknown and they had concerns about the overlap between the definitions of encephalitis and encephalopathy which refer to altered mental status but without inflammation of the brain tissue. The consortium therefore developed diagnostic criteria to capture both encephalitis and encephalopathy with major criteria including a change in consciousness and minor criteria including the presence of fever, seizures, new focal neurology, a CSF pleocytosis, abnormalities of the brain parenchyma on neuroimaging or a EEG consistent with encephalitis. Confirmation of encephalitis required one of 1) brain inflammation on pathology, 2) evidence of acute infection e.g. by microbiology, pathology or serology or 3) evidence of an autoimmune condition known to be strongly associated with encephalitis as provided by laboratory diagnosis (Venkatesan *et al.*, 2013). An overlap of the WHO case definition for AES has also been seen with

encephalopathy, meningitis and other neurological manifestations including a myelitis (inflammation of the spinal cord), abscesses, febrile convulsions and non-infectious causes (Solomon *et al.*, 2008).

A study of the causes of encephalitis in the United Kingdom (UK) from 2005 to 2006 amongst 203 patients found that the most common clinical symptoms and signs were fever (72%, 95%CI 66-78); headache (60%, 95%CI 53-67%), a change in personality or behaviour (64%, 95%CI 57-71%), lethargy (55%, 95%CI 48-62%) and seizures (52%, 95%CI 45-59%). 80% (95%CI 74-86%) had a CSF pleocytosis, 60% (95%CI 53-68%) changes on MRI and 83% (95%CI 75-89%) an EEG suggestive of encephalitis. The case definition included “any person of any age admitted to hospital with encephalopathy (altered consciousness that persisted for longer than 24 hours, including lethargy, irritability or a change in personality and behaviour) and with two or more of the following: fever or history of fever (≥38°C) during the presenting illness; seizures and/or focal neurological findings (with evidence of brain parenchyma involvement); CSF pleocytosis (more than four white blood cells per µL); electroencephalographic (EEG) findings indicative of encephalitis; and abnormal results of neuroimaging (CT or MRI) suggestive of encephalitis” (Granerod *et al.*, 2010a).

THE CASE DEFINITION OF MENINGITIS

Where encephalitis is due to inflammation of the brain parenchyma, meningitis is caused by inflammation of the meninges which surround the brain and spinal cord and the subarachnoid space which contains the CSF. However, inflammation of the brain parenchyma and cortex (a layer which surrounds the brain) can also occur. When the meninges become inflamed this can lead to symptoms and signs of fever, headache and neck stiffness and a CSF pleocytosis. Although meningitis can be caused by a variety of agents (bacterial, viral, fungal etc), the WHO provide a case classification for bacterial meningitis only due the severity of the impact of epidemics and the need for surveillance (World Health Organization, 2020a).

The WHO case classification for bacterial meningitis is as follows

Suspected Any person with sudden onset of fever (> 38.5 °C rectal or 38.0 °C axillary) and one of the following signs: neck stiffness, altered consciousness or other meningeal sign.

Probable A suspected case with CSF examination showing at least one of the following: - turbid appearance; - leukocytosis (> 100 cells/mm3); - leukocytosis (10-100 cells/ mm3) AND either an elevated protein (> 100 mg/dl) or decreased glucose (< 40 mg/dl).

Confirmed A case that is laboratory-confirmed by growing (i.e. culturing) or identifying (i.e. by Gram stain or antigen detection methods) a bacterial pathogen (Hib, pneumococcus or meningococcus) in the CSF or from the blood in a child with a clinical syndrome consistent with bacterial meningitis.

An aseptic meningitis is defined as a CSF pleocytosis without a positive Gram stain or culture (Shukla *et al.*, 2017).

THE CASE DEFINITION OF MENINGOENCEPHALITIS

Commonly, both inflammation of the meninges and brain parenchyma occur thereby eliciting symptoms and signs of encephalitis with meningitis. In these cases, the term ‘meningoencephalitis’ is used (Tunkel *et al.*, 2008).

THE GLOBAL BURDEN AND AETIOLOGY OF CNS INFECTIONS

In 2016, the Global Burden of Diseases, Injuries and Risk Factors Study (GBD) conducted a systematic analysis of estimate the global, regional and national burden of neurological disorders. In addition to CNS infections this analysis included malignancy, stroke, trauma and dementia and other manifestations of neurological disorders from 1990 to 2016 (G. B. D. Neurology Collaborators, 2019). Case definitions for the neurological disorders were based on ICD criteria as provided in the 2015 systematic analysis (G. B. D. Neurological Disorders Collaborator Group, 2017) with modelling used to estimate prevalence, incidence and mortality (G. B. D. Neurology Collaborators, 2019). However, the authors noted that due to the various

iterations of the ICD classifications, comparison of the data sources required extensive effort and all sources of measurement bias could not be accounted for. In 2016, encephalitis accounted for 653.4 million cases (95% confidence interval (CI) 595.7-716.5 million), 103,000 deaths (95% CI 84,000-138,000), 670.4 million Disability-Adjusted Life Years (DALYS) (95%CI 546.9-857.4 million). Although the total number of cases of meningitis in 2016 was lower at 2.821 million (95% CI 2.464-3.310 million) the number of deaths was higher with a total of 318,000 (95%CI 265,000-409,000) and the number of DALYS much higher at 2.19 billion (95%CI 1.82-2.83 billion) total (G. B. D. Neurology Collaborators, 2019). However, from 1990 to 2016, there was an increase in the incidence of meningitis globally from 2.5 million (95% uncertainty interval 2.19-2.91) to 2.82 million (2.46-3.31) but with a 21% reduction in deaths. The meningitis belt which includes countries in sub-Saharan Africa which are peri-Sahelian e.g. border the Sahara from Ethiopia to Senegal saw the highest age-standardised incidence. However, some countries outside of this region also experienced some of the highest mortality rates including Afghanistan, Pakistan, India and China (G. B. D. Neurology Collaborators, 2019).

ENCEPHALITIS

The infectious aetiologies of encephalitis include viruses, bacteria, rickettsia, parasites and fungi. Non-infectious aetiologies include immune-mediated or toxic causes (Granerod *et al.*, 2010a). A systematic review of forty-one studies from 1950 to 2002 found that in twenty-six studies the proportion of cases of AES of unknown aetiology was 50% or greater (Granerod *et al.*, 2010b) and worldwide it is estimated that up to 85% of cases may be due to unknown causes (Granerod *et al.*, 2010a). However, both the incidence of AES and the proportion of cases in which no cause is found is dependent on the number of pathogens tested for and the methods used in addition to the case definition (Granerod *et al.*, 2010b). Similarly, the breadth of aetiologies detected is dependent on the type of testing undertaken with studies conducted in high-resourced settings more likely to detect more pathogens. This potentially leads to reporting biases between studies.

The risk of encephalitis varies by age, geographic region and seasonality (Granerod and Crowcroft, 2007). For example, in the United Kingdom, encephalitis is most commonly caused by herpes simplex virus (HSV) (Granerod *et al.*, 2010a; Ambrose

et al., 2011). HSV is also a common cause of encephalitis in Asian countries however, Japanese encephalitis virus (JEV) which is unique to the continent accounts for the majority of cases in many countries, particularly in children (Tan le *et al.*, 2014; Rayamajhi *et al.*, 2011; Tarantola *et al.*, 2014). There is also strong evidence for a seasonal pattern of JEV, peaking in incidence in the warmer months or during the monsoon (Kumar Pant *et al.*, 2017; Robertson *et al.*, 2013). Table 1 outlines a selection of the pathogens more frequently known to cause encephalitis.

Table 1: Pathogens causing encephalitis by category (Granerod *et al.*, 2010a; Ludlow *et al.*, 2016b; Morfopoulou *et al.*, 2016; Moriguchi *et al.*, 2020; Arabi *et al.*, 2015; Glaser *et al.*, 2003; Tunkel *et al.*, 2008; Dittrich *et al.*, 2015; Granerod and Crowcroft, 2007; Lewthwaite *et al.*, 2010; Marinho *et al.*, 2019).

Category	**Family/classification**	**Pathogens**
Virus	*Herpesviridae*	Herpes simplex virus 1 and 2 (HSV 1 and HSV 2) Varicella zoster (VZV) Cytomegalovirus (CMV) Epstein-Barr virus (EBV) Human herpesvirus-6/7 (HHV-6/7) B virus Epstein-Barr virus (EBV)
Virus	*Flaviviridae*	Japanese encephalitis virus (JEV) Dengue virus (DENV) West Nile virus (WNV) St. Louis encephalitis virus (SLEV) Tick-borne encephalitis virus (TBEV) Zika virus Murray Valley encephalitis virus (MVEV) Powassan virus Yellow Fever virus (YFV)
Virus	*Picornaviridae*	Enteroviruses e.g. 71, 75 and 68 Coxsackie viruses Human parechoviruses Polioviruses
Virus	*Paramyxoviridae*	Measles virus Mumps virus Henipaviruses e.g. Nipah virus and Hendra virus

Category	Family/classification	Pathogens
Virus	*Togoviridae*	Chikungunya virus (CHIKV) Eastern equine encephalitis virus (EEEV) Venezuelan equine encephalitis virus (VEEV) Western equine encephalitis (WEEV) Rubella virus
Virus	*Bunyaviridae*	La Crosse virus (LACV) Rift Valley fever virus (RVFV) Toscana virus (TOSV)
Virus	*Orthomyxoviridae*	Influenza A and B viruses
Virus	*Rhabdoviridae*	Rabies virus (RABV) European Bat Lyssavirus
Virus	*Phenuiviridae*	Rift Valley Fever
Virus	*Arenaviridae*	Lymphocytic choriomeningitis virus (LCMV)
Virus	*Retroviridae*	Human Immunodeficiency Virus (HIV)
Virus	*Polyomaviridae*	Human polyomavirus 2/JC virus
Virus	*Orthocoronaviridae*	Human coronavirus OC43 (HCoV-43) Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) Middle-East respiratory syndrome coronavirus (MERS-CoV)
Virus	*Adenoviridae*	Adenovirus
Virus	*Reoviridae*	Banna virus
Bacteria	Gram positive cocci	*Streptococcus pneumoniae* *Enterococcus faecium* Group A *streptococci*
Bacteria	Gram positive bacilli	*Listeria monocytogenes* *Tropheryma whipplei*
Bacteria	Gram negative cocci	*Neisseria meningitidis*

Category	Family/classification	Pathogens
Bacteria	Gram negative bacilli	*Brucella* species *Pseudomonas* species
Bacteria	Gram negative cocco-bacilli	*Haemophilus influenzae*
Bacteria	*Mycobacteria*	*Mycobacterium tuberculosis* (MTB)
Bacteria	Intracellular	*Chlamydia* species *Bartonella henslae* *Rickettsia* species *Coxiella burnetii* *Bartonella quintana* *Ehrlichia chaffeensis* *Anaplasma phagocytophilum*
Bacteria	Mollicutes	*Mycoplasma pneumoniae*
Bacteria	Spirochetes	*Treponema pallidum* *Leptospira* species *Borrelia burgdorferi*
Bacteria	Rickettsiaceae	*Orientia tsutsugamushi* *Rickettsia* species
Parasite	Obligate intracellular	*Toxoplasma gondii*
Parasite	Protozoa	*Plasmodium falciparum*
Parasite	Kinetoplastid	*Trypanosoma brucei gambiense* *Trypanosoma brucei rhodesiense*
	Nematode	*Gnanthostoma* species *Baylisascaris procyonis*
	Cestode	*Taenia solium*
Fungi		*Coccidioides immitis* *Histoplasma capsulatum* *Cryptococcus neoformans*

Category	Family/classification	Pathogens
Amoeba		*Acanthamoeba* species *Naegleria fowleri*
Prions		Sporadic and new variant Creutzfeldt-Jakob (CJD)
Immune-mediated		Acute disseminated encephalomyelitis Anti-N-methyl D-aspartate receptor (anti-NMDAR) Anti-voltage-gated potassium channel (VGKC) Paraneoplastic

MENINGITIS

Meningitis can also be caused by a number of pathogens, with causes of community-acquired meningitis differing in incidence according to the age of those infected and the geographical region (van de Beek *et al.*, 2016). Globally, the most common causes of bacterial meningitis are *S. pneumoniae*, *N. meningitidis* and *Haemophilus influenzae* type b (Hib) (McIntyre *et al.*, 2012). However, incidence of these pathogens has reduced due to the introduction of vaccines, particularly Hib (van de Beek *et al.*, 2016). The incidence of these pathogens also differs by age with *S. pneumoniae* more common amongst those aged less than five years and the elderly, *N. meningitidis* amongst older children and young adults and Hib in those less than 12 months (van de Beek *et al.*, 2016). However, in southeast Asian countries where pigs are farmed, the *Streptococcus suis* is an important cause in adults (van Samkar *et al.*, 2015; Wertheim *et al.*, 2009b). Table 2 outlines a selection of the pathogens more frequently known to cause meningitis.

Table 2: Pathogens causing meningitis by category (McGill *et al.*, 2016; Molyneux *et al.*, 2009; Jarvis *et al.*, 2010; Federspiel, Skovmand and Skarphedinsson, 2020).

Category	**Family/classification**	**Pathogens**
Virus	Picornaviridae	Enteroviruses
Virus	*Herpesviridae*	HSV 1 and 2 VZV EBV CMV
Virus	Paramyxoviridae	Measles virus Mumps virus
Bacteria	Gram positive cocci	*S. pneumoniae* *Streptococcus pyogenes* *Streptococcus agalactiae* *Streptococcus oralis* *S. suis* *S. pasteurianus*
Bacteria	Gram positive bacilli	*L. monocytogenes*
Bacteria	Gram negative cocci	*N. meningitidis*
Bacteria	Gram negative cocco-bacilli	*H. influenza* type b
Bacteria	Gram negative bacilli	*Fusobacterium* species *Escherichia coli* *Pseudomonas* species *Klebsiella* species *Salmonella enterica* *Salmonella typhimurium*
Bacteria	Mycobacteria	*M. tuberculosis*
Bacteria	Mollicutes	*M. pneumoniae*
Bacteria	Spirochetes	*T. pallidum* *Leptospira* species
Fungi		*C. neoformans*
Parasite	Nematode	*Angiostrongylus cantonensis*

1.2 THE EPIDEMIOLOGY AND AETIOLOGY OF CNS INFECTIONS IN SOUTHEAST ASIA

Table 3, develop by myself, shows examples of studies evaluating the aetiology of CNS infections in southeast and south Asia. In summary, the most common cause of CNS infections amongst adults in Vietnam is *S. suis* and in children, JEV (Tan le *et al.*, 2014; Le *et al.*, 2010; Ho Dang Trung *et al.*, 2012; Taylor *et al.*, 2012).

The aetiology was unknown in 44.9-72.7% of cases. In addition to infectious causes and not shown in table 3, autoimmune and toxic causes of AES have been identified in Vietnam. Of ninety-nine patients admitted with encephalitis to the Hospital for Tropical Diseases in HCMC, Vietnam, 9.1% (n=9) had antibodies to NMDA receptors (Nguyen Thi Hoang *et al.*, 2017). In Bac Giang province in northern Vietnam, a retrospective analysis of data from 2004-2009 showed an association between the proportion of land covered by lychee plantations and the incidence rate ratio of acute encephalitis in children (Paireau *et al.*, 2012b). A later case control study conducted in northern India found that consumption of lychees was associated with an encephalopathy in children (odds ratio (OR) 9.6, 95%CI 3.6-24) (Shrivastava *et al.*, 2017).

JEV was the most common cause identified in a study of paediatric patients in Cambodia (Horwood *et al.*, 2017). In other paediatric studies in Cambodia and in China enterovirus was identified as the most common cause (Turner *et al.*, 2017; Ai *et al.*, 2017). The remaining studies did not differentiate the aetiology between adults and children however, in Lao PDR the median age of those with JEV was 13 years with an interquartile range of 8-20 years compared to the Cryptococcus species, the second most common pathogen with a median age of 33 years and an interquartile range of 27-41 years (Rattanavong *et al.*, 2020). In Nepal, the median age of those with enterovirus, the most common pathogen was 39 years with an interquartile range of 33-53 years and JEV, 42 years with an interquartile range of 27-67 years (Giri *et al.*, 2013). In the study in Lucknow, the age distribution was only given for those with JEV and DENV with 59.2% of those with JEV and 57.7% of those with DENV being less than 15 years and 57.7% (Jain *et al.*, 2017).

Many studies categorised a CNS infection as either probable for example, based on clinical judgement with or without abnormalities in the CSF or WHO criteria; or confirmed based on a laboratory diagnosis of a pathogen (Tan le *et al.*, 2014; Le *et al.*, 2010; Taylor *et al.*, 2012; Ho Dang Trung *et al.*, 2012; Turner *et al.*, 2017; Horwood *et al.*, 2017).

Table 3 Publications giving the aetiology of CNS infections in south, southeast and east Asia.

Country	Authors	Site (s)	Year(s)	Adults or children	Number of cases	*S. suis* (%)	*S. pneumoniae* (%)	*M. tuberculosis* (%)	*H. influenzae* type b (%)	*N. meningitidis* (%)	JEV (%)	HSV (%)	DENV (%)	Enterovirus (%)	*O. tsutsugamushi* (%)	Other or dual infections (%)*	Unknown (%)
Vietnam	(Tan le *et al.*, 2014)	HCMC	1996-2008	Adults	291	NA	NA	NA	NA	NA	12.4	6.5	6.5	2.7	NA	22.7	68.0
Vietnam	(Le *et al.*, 2010)	HCMC	2004	Children	194	0	3.1	NA	3.1	0	25.8	0.5	4.6	9.3	NA	8.7	44.9
Vietnam	(Taylor *et al.*, 2012)	Hanoi	2007-2008	Adults	352	13.6	2.0	2.6	0	0.6	NA	3.4	NA	0.6	NA	4.5	72.7
Vietnam	(Ho Dang Trung *et al.*, 2012)	Multiple	2007-2010	Adults	617	23.8	5.7	5.5	0	0.7	1.8	3.6	3.7	3.2	NA	4.2	47.8
Vietnam	(Ho Dang Trung *et al.*, 2012)	Multiple	2007-2010	Children	624	0	5.9	1.8	6.3	1.0	22.8	2.2	2.2	5.8	NA	3.0	49.0
Cambodia	(Horwood *et al.*, 2017)	Phnom Penh and Siem Reap	2010-2013	Children	1160	0	1.6	0	0.7	0.2	24.5	0.9	4.6	3.5	4.7	3.3	55.8
Cambodia	(Turner *et al.*, 2017)	Siem Reap	2014-2015	Children	284	0	2.5	0	0.4	0.7	6.0	0.4	NA	7.4	NA	2.1	79.2
Lao PDR	(Rattanavong *et al.*, 2020)	Vientiane	2003-2011	Both	1065	?	2.1	1.9	?	?	8.8	?	2.5	?	2.9	?**	?
China	(Ai *et al.*, 2017)	Multiple	2009-2012	Children	546	NA	NA	NA	NA	NA	0.34	10.8	2.9	15.4	NA	18.0***	47.4
Nepal	(Giri *et al.*, 2013)	Kathmandu	2009-2011	Adults	87	0	3.5		0	5.8	8.1	2.3	0	10.3	NA	5.8	63.2
India	(Rathore *et al.*, 2014)	Odisha	2011-2012	Both	526	NA	NA	NA	NA	NA	1.3	4.0	0.2	0	NA	16.2	78.3
India	(Jain *et al.*, 2017)	Lucknow	2014-2016	Both	4092	NA	0.94	NA	0.97	0	8.3	0.8	7.8	0.4	31.8	1.5	47.5

? = it is unknown whether the pathogen was tested for
NA = the pathogen was not tested for

* This can also include probable diagnoses
** 6.8% of the total were due to *Cryptococcus species*
*** 1.8% of the total were due to TBEV

As demonstrated in tables 1 and 2, the aetiology of CNS infections globally is very broad. However, given that this thesis aims to explore the epidemiology and risk factors of CNS infections in Vietnam, only those infections which are common in Vietnam and its neighbouring countries will be discussed in detail including JEV, DENV, *O. tsutsugamushi*, HSV, enterovirus 71 and *S. suis*. For each of these pathogens an overview is given of the epidemiology and transmission; clinical symptoms, signs, and prognosis; diagnostics; treatments and control and surveillance on a global level with a focus on Asia. A separate section on the epidemiology of the pathogen in Vietnam is also given.

Other pathogens which are known to be important but less common causes of CNS infections in Vietnam and its neighbouring countries or are not found in Vietnam but are important globally are discussed in less detail including VZV, measles virus, mumps virus, rubella virus, influenza virus, CMV, EBV, CHIKV, Nipah virus, rabies virus, *S. pneumoniae*, *N. meninigitidis*, *H. influenzae*, *M. tuberculosis*, *Leptospira* species, *R. typhi*, WNV, Zika virus and TBEV. For each of these pathogens an overview of their epidemiology, neurological manifestations and control is given with a focus on Vietnam.

In addition to infectious, autoimmune and toxic causes will be mentioned briefly. This list is based on a list of pathogens causing encephalitis in the countries of the Mekong Region (Tarantola *et al.*, 2014) and the wider literature.

1.3 COMMON PATHOGENS CAUSING CNS INFECTIONS IN VIETNAM AND ITS NEIGHBOURING COUNTRIES

JAPANESE ENCEPHALITIS VIRUS (JEV)

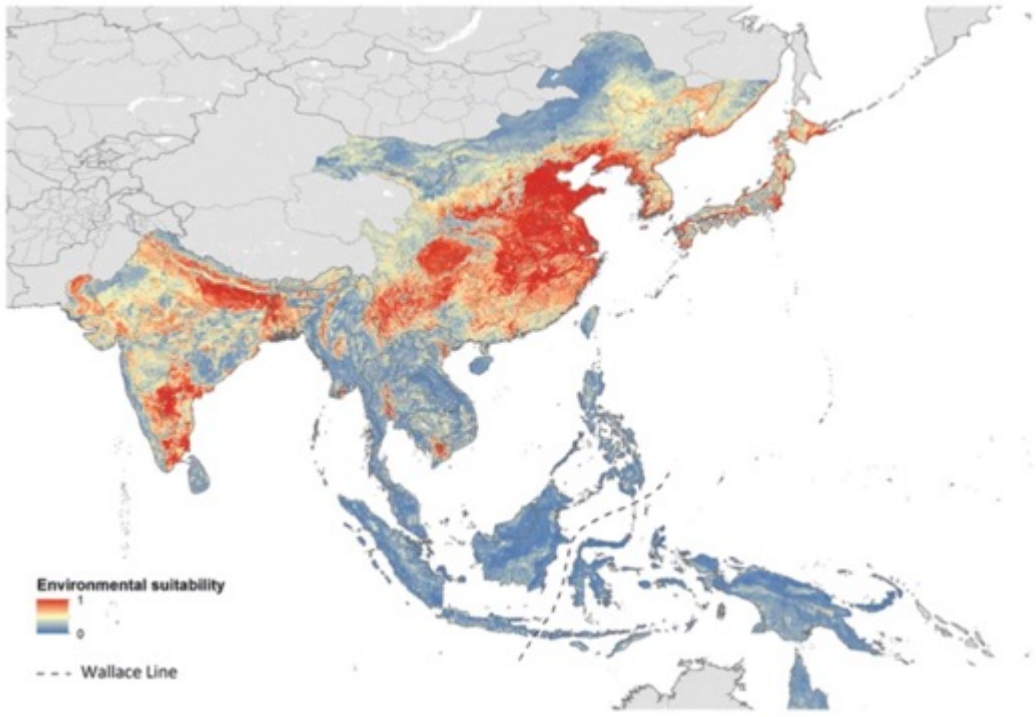
TRANSMISSION AND EPIDEMIOLOGY

JEV, is a single-stranded positive-sense ribonucleic acid (RNA) virus with five genotypes, based on the nucleotide sequence of the viral envelope (E) gene (Li *et al.*, 2011). JEV is transmitted in an enzootic cycle between *Culex* mosquitoes, animals such as wild birds and pigs and humans (Solomon *et al.*, 2000b; Scherer *et al.*, 1959a; Buescher *et al.*, 1959; Scherer *et al.*, 1959b). The replication of the virus in both the vertebrate and non-vertebrate hosts makes this an arbovirus (Rosen, 1986). JEV is predominantly transmitted by *Culex tritaeniorhynchus* Giles, 1901 mosquitoes of the order Dipetra and family Culicidae however, it can also be transmitted by other species of *Culex* including Culex *gelidus,* Culex *fuscocephala* and Culex *annulirostris* (van den Hurk, Ritchie and Mackenzie, 2009; Longbottom *et al.*, 2017; Reuben *et al.*, 1994). JEV has an extrinsic incubation period (EIP) (the time taken for the virus to be transmitted by a mosquito after a blood meal) (Ye *et al.*, 2016) of 7-14 days in the mosquito (van den Hurk, Ritchie and Mackenzie, 2009).

Cx. tritaeniorhynchus is found across east, south and southeast Asia, northern Australia, the Middle East, Africa and Greece (Longbottom *et al.*, 2017; Reisen, Aslamkhan and Basio, 1976; Sallam *et al.*, 2013; Lytra and Emmanouel, 2014; Samy *et al.*, 2018). The mosquito larvae mainly breed in irrigated rice fields but also pools of water which may be temporary or semi-permanent including swamps and marshes (Keiser *et al.*, 2005; Longbottom *et al.*, 2017; Self *et al.*, 1973). However, the vector can also be found in urban areas (Murthy *et al.*, 2002). Climatic factors including rainfall and temperature, and altitude also influence the abundance of the larval breeding sites and habitats for adult mosquitoes (Keiser *et al.*, 2005). A reduction in rainfall can result in fewer breeding sites as the eggs will not survive dessication. If rainfall is too heavy, breeding sites can be flooded (Reisen, Aslamkhan and Basio, 1976; Longbottom *et al.*, 2017). However, this flooding is not necessarily detrimental as populations of *Cx. tritaeniorhynchus* have been shown to remain high even during these periods (Gould *et al.*, 1974). Higher temperatures are associated with longevity

of the mosquitoes (Niaz and Reisen, 1981). However, temperatures which are too high can negatively impact both the activity and survival of the mosquitoes (Liu *et al.*, 2018). Higher altitudes may also result in fewer mosquitoes, due to cooler temperatures and absence of suitable breeding grounds. In Yunnan, China, there was an absence of *Cx. tritaeniorhynchus* above 3000m (Sun *et al.*, 2009). However, more recently *Cx. tritaeniorhynchus* infected with JEV were found in Tibet at elevations of 3100m *(Zhang et al., 2017a)*. *Cx. tritaeniorhynchus* is an exophagic (biting outdoors) mosquito with biting times after sunset and the middle of the night (Longbottom *et al.*, 2017; Rohani, Zhong and King, 2010; Reisen and Aslamkhan, 1978). Figure 1.1 shows the predicted environmental suitability for *Cx. tritaeniorhynchus* within areas where there is a risk of transmission of JEV based on models including covariates such as land surface temperature, tasselled cap wetness, elevation and vegetation cover (Longbottom *et al.*, 2017). This model should be interpreted with some caution as the authors acknowledge that countries such as Malaysia and Indonesia show low suitability for *Culex* yet are areas of known JEV transmission. This effect is also seen in areas of Vietnam, particularly the northwest.

Figure 1.1 The predicted environmental suitability for *Cx. tritaeniorhynchus* within areas where there is a risk of transmission of JEV. High suitability is shown in red and low in blue (Longbottom *et al.*, 2017).



Infected mosquitoes feed on and transmit the virus to a number of amplifying and dead-end hosts. Wading birds such as herons and egrets of the family Ardeidae and wild and domestic swine are the most commonly implicated amplifying hosts (Scherer *et al.*, 1959b; Buescher *et al.*, 1959; Scherer and Buescher, 1959; Soman *et al.*, 1977). Amongst swine, clinical disease is only seen is pregnant sows where fetal abortion and stillbirth may occur (Salmon, 1984; Lindahl *et al.*, 2012a). Antibodies against JEV have been found in other animals such as ducks (Kalaiyarasu *et al.*, 2016; Pant, 2006; Ayu Mirah Adi *et al.*, 2016), chickens (Ayu Mirah Adi *et al.*, 2016), cats (Kumar *et al.*, 2018), dogs (Ohno *et al.*, 2009) and cattle (Kumar *et al.*, 2018) amongst other animals (van den Hurk, Ritchie and Mackenzie, 2009). However, it has been shown that cattle do not produce a viraemia sufficient to act as amplifying hosts and are therefore 'dead-end' (Ilkal *et al.*, 1988) and although other mammals are not thought to be implicated in the transmission cycle of JEV (van den Hurk, Ritchie and Mackenzie, 2009) the role of domestic birds is unknown (Lord, Gurley and Pulliam, 2015). Humans, infected when bitten by a mosquito carrying the virus, are a dead-end host (Solomon *et al.*, 2000a).

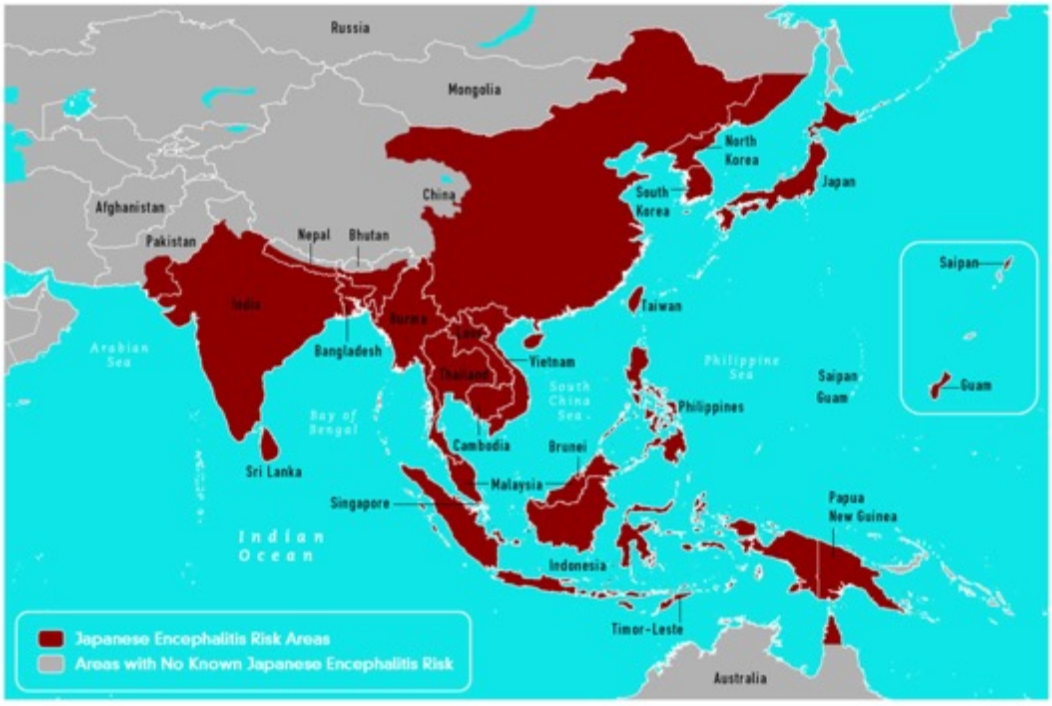
A case control study conducted in Bali, Indonesia identified living close to rice fields as a risk factors for JE in humans (Odds ratio (OR) 2.93, 95% confidence interval (CI) 1.57-5.45) (Liu *et al.*, 2010). The percentage of irrigated land was also positively associated with incidence of JE at the district level in a study in Nepal (Impoinvil *et al.*, 2011). However, an ecological study conducted in northern Vietnam found a negative association between the proportion of land covered by rice and the incidence rate ratio of acute encephalitis *(*Paireau *et al.*, 2012b*)* and a further study in Nepal found that the amount of irrigated land was not associated with a risk of JE possibly due to the increased number of cases in the Kathmandu Valley and mountainous regions (Robertson *et al.*, 2013).

In Uttar Pradesh, northern India, an association was found between an absence of indoor residual spraying (IRS) of insecticides within the past year and JE (OR 0.31, 95%CI 0.19-0.50) (Kakkar *et al.*, 2017) and in Assam, northern India, the use of insecticide-treated mosquito nets (ITMNs) was associated with a risk ratio of JE in humans of 0.28 (95% CI 0.16-0.49) (Dutta *et al.*, 2011). However, this association was not seen by Liu et al, 2010 in Bali. Similarly, the risk of JE from living close to pigs remains a matter of debate. Liu et al., 2010 found that the rearing of pigs by the

family or neighbours was associated with an increased risk (OR 1.96, 95%CI 1.07-3.56) and in Nepal, the rearing of pigs at home was also associated with JE (OR 6.12, 95%CI 2.14-17.94) (Rayamajhi *et al.*, 2007). The relocation of domestic pigs to more than 5 km from human habitation has therefore been proposed, whilst it was acknowledged that the benefit remained unknown (Solomon, 2006). However, on the Torres Strait islands, Australia it was found that the removal of pigs did not eliminate the presence of mosquitoes infected with JEV (van-den-Hurk *et al.*, 2008).

The first notification of a case of JE occurred in 1871 in Japan followed by an outbreak in 1924 (World Health Organization, *2019f;* Solomon, *2006)* and the virus is currently endemic in twenty-four countries in Asia-Pacific (Campbell *et al.,* 2011; Quan *et al.,* 2020; World Health Organization, 2019f). Figure 2 shows the distribution of areas with and without a risk of JEV (Centers for Disease Control and Prevention, 2019).

Figure 1.2 Distribution of Japanese encephalitis virus (Centers for Disease Control and Prevention, 2019).



In 2015, it was estimated that there were 100,308 (95%CI 61,720-157,522) cases of JE globally with 25,125 (95%CI 14,550-46,031) deaths (Quan *et al.*, 2020). This is higher than the estimate in 2011 (approximately 67,900 cases annually) (Campbell *et al.*, 2011) however, the authors attribute it to accounting for under-reporting, differences in the quality of surveillance data and changes in the population and vaccination programmes over time (Quan *et al.*, 2020). Incidence of JE has reduced over time in countries such as Japan, the Republic of Korea and Taiwan (China) due to the implementation of vaccination programmes. Many other countries rolled vaccination out more slowly and incidence rates between countries is variable (Campbell *et al.*, 2011) with annual incidence varying from 0.003 per 100,000 in Japan and the Republic of Korea to 3.7 per 100,000 in Malaysia, Cambodia and Indonesia (Campbell *et al.*, 2011; Turtle and Solomon, 2018). There are reports of incidences of greater than 10 per 100,000 per year during outbreaks (World Health Organization, 2019f).

Two epidemiological patterns exist; in tropical regions such as southern Thailand and Vietnam and Indonesia, the virus is endemic with cases of JE occurring throughout the year but with a peak after the start of the wet season. In more temperate regions such as northern Thailand and Vietnam, Nepal and China epidemics occur during the summer months (Solomon *et al.*, 2000a; Vaughn and Hoke, 1992). Major outbreaks are seen every 2-15 years in temperate regions and can be difficult to predict *(World Health Organization, 2019f; Lindquist, 2018)*. The mechanism for persistence of JEV during cooler winter months when mosquitoes are unable to survive remains unknown (Turtle and Solomon, 2018). Transmission of the virus between pigs via respiratory secretions has been shown (Ricklin *et al.*, 2016). Additionally, it has been hypothesised that the virus could be reintroduced to the area by migratory birds or persist in other animals such as lizards or snakes or even in mosquitoes (van den Hurk, Ritchie and Mackenzie, 2009).

JE is seen mostly commonly in children as adults tend to acquire natural immunity. However, JE may also be seen in adults, especially those who have no pre-existing immunity for example, countries which have experienced epidemics more recently including Nepal, India and Sri Lanka (Solomon *et al.*, 2000a).

CLINICAL SYMPTOMS AND SIGNS AND PROGNOSIS

Less than 1% of those infected with JEV develop symptoms (Campbell, 2011) which may range from a mild flu-like illness with headache and fever to encephalitis with reduced consciousness (Solomon, 2000a), seizures (Kumar *et al.*, 2006), an acute flaccid paralysis (Solomon *et al.*, 1998), a focal neurological deficit such as a hemiparesis (Rayamajhi *et al.*, 2007) or extrapyramidal features such as tremor, rigidity, hypokinesia and masking of the face (Misra and Kalita, 2002). Mortality has been reported in up to 44% of cases and as many as 91% can suffer long term neurological sequelae (Turtle and Solomon, 2018). A study in Nepal found that 68% of children hospitalised with JE had a neurological impairment at follow-up, most commonly difficulties with language and lower limb function (Griffiths *et al.*, 2013). A report in 2002, showed JE to cause 709,000 DALYs on an annual basis (Mathers, Ezzati and Lopez, 2007).

DIAGNOSTICS

The WHO recommend that confirmation of infection with JEV is based on the presence of an IgM antibody against the virus in either CSF, the preferred method to reduce the risk of a false positive result due to prior vaccination or infection, or blood (World Health Organization, 2019f). A second sample should be tested at approximately the tenth day of the onset of illness as it can take this time to develop IgM (World Health Organization, 2007b). A study in Lao PDR found that the use of dried CSF spots on pre-cut filter paper might be useful where laboratory access is limited (Bharucha *et al.*, 2016). Serological cross-reactivity with other flavivirus antibodies can present challenges (Dubot-Peres *et al.*, 2015) and although polymerase chain reaction (PCR) and cell culture are more specific, the virus is often not present in either blood or CSF when symptoms manifest. Additionally, these tests are expensive and require greater technical expertise and facilities to perform (Moore *et al.*, 2012).

TREATMENTS

There are currently no licensed treatments for JE and management is supportive (World Health Organization, 2019f). This includes careful fluid balance (Tiroumourougane *et al.*, 2003), the management of seizures using anti-epileptics (Misra and Kalita, 2001) and prevention of malnutrition, bed sores and contractures

through nursing care and physiotherapy (Solomon and Vaughn, 2002). There have been a number of trials of therapeutics, none of which have shown a detected benefit on the clinical outcome of patients with JE (Turtle and Solomon, 2018). Treatments which have been trialled include dexamethasone (Hoke *et al.*, 1992), interferon-alpha (Solomon *et al.*, 2003), ribavarin (Kumar *et al.*, 2009), intravenous immunoglobulin (Rayamajhi *et al.*, 2015) and minocycline (Kumar Singh *et al.*, 2016). There are a number of other potential treatments which have proved to be successful in animal models or in vitro which are awaiting trials in humans (Turtle and Solomon, 2018).

CONTROL AND SURVEILLANCE

Control of JE exists primarily through human vaccination programmes which have been established in a number of countries. Vaccines against JEV have been in use since the 1950s with mouse brain-derived inactivated vaccines used for over fifty years (Hegde and Gore, 2017). However, a suspension of the recommendation for the use of this vaccine was implemented in 2005 for safety reasons following rare cases of ADEM despite no causal link (Ferguson *et al.*, 2007). The live-attenuated SA-14-14-2 vaccine was licensed in China in 1988 and is recommended for children aged 1-2 years with a booster dose the following year (Hegde and Gore, 2017). In Nepal, vaccine efficacy after a single dose was shown to be 99.26% (Bista *et al.*, 2001), 98.5% one year later (Ohrr *et al.*, 2005) and 96.2% five years later (Tandan *et al.*, 2007). Lower seroprotection rates were seen in India (Indian Academy of Pediatrics *et al.*, 2013). Most inactivated vaccines are not sold internationally with the exception of the inactivated adjuvanted SA-14-14-2 vaccine which is now is licensed in Europe, North America and other countries as IXIARO (Turtle and Solomon, 2018). The first recombinant vaccine to be licensed is 'Imojev' which uses the premembrane and envelope genes of the SA-14-14-2 vaccine with a yellow fever 17D vaccine. Vaccination was first introduced in 1954 in Japan (Campbell *et al.*, 2011), however, in Lao PDR it was only introduced in 2015 (WHO, 2017), and Myanmar in 2017 (Gudmastad, 2017). Despite this approximately 81% of cases still occur in countries where there is a well-established or developing vaccination programme including China and India (Campbell *et al.*, 2011). Suggested reasons for this include a lack of vaccine coverage, waning immunity where transmission is intermittent, poor vaccine effectiveness or cold chain issues (Turtle and Solomon, 2018).

In addition to vaccination, the intermittent irrigation of rice paddy fields (alternate dry-west irrigation (ADWI) (Howell, Shrestha and Dodd, 2015)) to reduce the mosquito burden (Keiser et al., 2005), use of larvivorous fish (Keiser *et al.*, 2005), insecticide treated bed-nets (Dutta *et al.*, 2011) and vaccination of pigs (Sasaki *et al.*, 1982) and possibly relocating pigs (Solomon, 2006) may provide some benefit to the control of JEV.

The WHO recommend various types of surveillance for JE in order to understand the geographic distribution and at-risk populations and to inform vaccine policy and evaluate vaccine effectiveness. These range from minimal surveillance such as sentinel hospital surveillance with laboratory confirmation to enhanced surveillance such as using national data on AES with laboratory confirmation of JE. The case definition of AES is used to identify a suspected case of JE. However, in the absence of laboratory testing a definition of 'probable JE' is only provided if there is a geographic or temporal link to a laboratory-confirmed case of JE during an outbreak, otherwise the case is classified as 'AES unknown' (World Health Organization, 2018b). A study conducted in Nepal showed that seasonal peaks of incidence of JE coincided with those of AES therefore the authors proposed that the patterns of AES could act as an early-warning tool for JE (Robertson *et al.*, 2013).

JEV IN VIETNAM

JEV was first isolated in Vietnam in 1951 with a continued high incidence of JE in northern Vietnam between 1969 and 1974 (Okuno, 1978). Between 1978 and 1980, epidemics were reported in northern Vietnam with 93% of cases occurring from May-July with a peak in June (Le, 1986). Reports from 1985 to 1993 in northern Vietnam showed incidence of AES, reported as JE to be highest in the provinces of the Red River Delta (Yen *et al.*, 2010). Reports from 1976-1992 documented year-round cases of AES in southern Vietnam with JEV being isolated from human sera and mosquitoes. The highest incidence occurred in the Mekong Delta (Do, 1995). A number of studies of the vectors which transmit JEV have been conducted in Vietnam. In 2003 a study found Culex to be the predominant species of mosquito in Ha Tay province, northern Vietnam and in a later study, found *Cx. tritaeniorhynchus* to be positive for JEV (Hasegawa *et al.*, 2008; Kuwata *et al.*, 2013; Nguyen-Tien, Lundkvist and Lindahl, 2019). A study in Can Tho city in southern Vietnam found that there was a positive association between the number of *Cx. tritaeniorhynchus* and pigs in close

vicinity (Lindahl *et al.*, 2012b). This finding is supported by the presence of anti-JEV antibodies in 100% of pigs sampled inside the city (Lindahl *et al.*, 2013).

A study of patients with AES admitted to Bach Mai Hospital, Hanoi, northern Vietnam from June to August 1995 found that 31 of 46 paediatric patients (67%) and 2 of 33 adults patients (6%) had JE. The median age of the paediatric patients was 6 years (range 6 months to 16 years) and adults, 33 years (range 19-58 years). It was also noted that 15% of paediatric patients without AES had evidence of past JEV infection as measured by anti-IgG compared to 40% of adults (Lowry *et al.*, 1998). A seaparate study conducted at the provincial level found that in five provinces: Bac Giang, Hai Duong, Hai Phong, Thai Binh and Thanh Hoa, 52% of the cases of AES tested for JEV were IgM were positive. This proportion was highest in Thai Binh (71%) and lowest in Bac Giang (17%) and highest in the age group 11-15 years (65%). The authors recommended expansion of JE testing and collection of further information to better understand the epidemiology of JE (Yen *et al.*, 2010).

'Viral encephalitis' is a notifiable disease in Vietnam (Binh *et al.*, 2013) with JE notificable since 2017 (personal communication, Pham Thai Quang). Since 1997, a domestically produced inactivated mouse brain-devired vaccine against JEV has been included in the Expanded Programme on Immunization (EPI). Vaccination commenced in twelve high-risk districts in northern Vietnam before being rolled-out to other districts (Yen *et al.*, 2010). By 2015, all districts except Cao Bang were receiving the vaccine as part of the EPI but with varying degrees of coverage (data from the National Institute of Hygiene and Epidemiology, Hanoi). Children aged 1-5 years receive two doses of vaccine 1-2 weeks apart followed by a booster dose the following year (Yen *et al.*, 2010). Despite a reduction in the proportion of cases of AES which are due to JE from approximately 60% of cases in children aged over 5 years prior to 1999 to less than 27% since 2002 (Van Tu *et al.*, 2007) concerns about the immunisation programme remain. A study conducted in Ha Tay province in northern Vietnam showed that incidence remained high, especially amongst children aged 5-9 years and therefore trialled a model immunization programme compared to the EPI which showed a significant reduction in the risk of JE (Yen *et al.*, 2015).

DENGUE VIRUS (DENV)

TRANSMISSION AND EPIDEMIOLOGY

Dengue virus (DENV) is a single-stranded positive-sense RNA virus of which there are four seroptypes (DENV1-4) (Simmons *et al.*, 2012). It is transmitted by the bite of an infected *Aedes aegypti* mosquito, the primary vector or *Aedes albopictus* mosquitoes, considered to be secondary vectors (Brady *et al.*, 2013) with female mosquitoes having a preference for feeding on humans (Ferreira-de-Lima and Lima-Camara, 2018; Harrington, Edman and Scott, 2001). *Ae. aegypti* tends to be distributed only in tropical and subtropical climates however, the eggs of *Ae. albopictus* can survive in more temperate regions (Brady *et al.*, 2013). Outbreaks have also been attributed to other species including *Aedes polynesiensis* and those of the *Aedes scutellaris* complex (World Health Organization, 2020c). The EIP of DENV is 8 to 12 days and is faster than higher temperatures (Gubler, 1998; Guzman *et al.*, 2010; Watts *et al.*, 1987).

Both *Ae. aegypti* and *Ae. albopictus* originated in forests with the former in Africa and the latter in South Asia (Tedjou *et al.*, 2019). The *Ae. aegypti* mosquito has adapted to live in urban areas and often breeds in water storage containers (Schmidt *et al.*, 2011; Simmons *et al.*, 2012). *Ae. albopictus* can also inhabit urban areas but less frequently than *Ae. aegypti* and in further proximity to humans (Brady *et al.*, 2013). *Ae. aegypti* feeds only on humans whereas *Ae. albopictus* feeds on humans and animals (Kraemer *et al.*, 2015). However, unlike JEV, humans are not a dead-end host and mosquitoes become infected after biting viraemic people (Duong *et al.*, 2015). Given the short flight distance of *Aedes* of just over 500m (Harrington *et al.*, 2005) the population density and contact patterns between humans play an important role in the transmission of the virus (Padmanabha *et al.*, 2012; Simmons *et al.*, 2012). In forested areas in Southeast Asia and Africa, DENV may also have a sylvatic lineage and can be transmitted between non-human primates, with humans rarely becoming infected (Cardosa *et al.*, 2009; Brady and Hay, 2020). The *Aedes* mosquitoes are mostly active during daylight hours, often with peaks of activity in the early morning and late afternoon (Reinhold, Lazzari and Lahondere, 2018). The timing of the peaks of activity depends on the season (Yasuno and Tonn, 1970). Similar to *Culex*, the activity of *Aedes* mosquitoes is dependent on many climatic factors. Development of

the immature stages of *Ae. aegypti* from egg, to larvae and pupae occurs between 16°C and 34°C (Reinhold, Lazzari and Lahondere, 2018) and *Ae. albopictus* occurs at a wider range of 10.4°C to 29.7°C (Delatte *et al.*, 2009). Flight times of *Ae. aegypti* are reduced at temperatures lower than 10°C and higher than 35°C (Rowley and Graham, 1968) however, the impact of temperature on the flight activity of *Ae. albopictus* is less well known (Reinhold, Lazzari and Lahondere, 2018). In addition to temperature, humidity is correlated with the propagation of DENV within *Ae. aegypti*, with a preferred humidity of over 60% (Thu, Aye and Thein, 1998) and a reduced longevity of adult *Ae. albopictus* mosquito at lower humidities (Waldock *et al.*, 2013). However, the effect of dessication has been shown to be higher on the mortality of *Ae. albopictus* eggs compared to *Ae. aegypti* eggs (Juliano *et al.*, 2002). Although adequate rainfall is required to provide suitable environments for breeding and development of the larvae, heavy rainfall can risk the larvae being washed away (Koenraadt and Harrington, 2008; Seidahmed and Eltahir, 2016). The geographical distribution of both vectors has expanded due to the impact of climate change and increasing travel patterns of humans (Ebi and Nealon, 2016). Nepal saw the introduction of cases of dengue fever more recently, thought to be due to the movement of viraemic people from northern India (Dumre *et al.*, 2013), and now has evidence of the presence of vectors in high mountain regions (Dhimal *et al.*, 2015). Figure 1.3 shows the predicted distribution of *Ae. aegypti* and figure 1.4, the predicated distribution of *Ae. albopictus* based on records of the occurrence of these vectors (Kraemer *et al.*, 2015).

Figure 1.3 Global map of the predicted distribution of *Ae. aegypti* (Kraemer *et al.*, 2015).

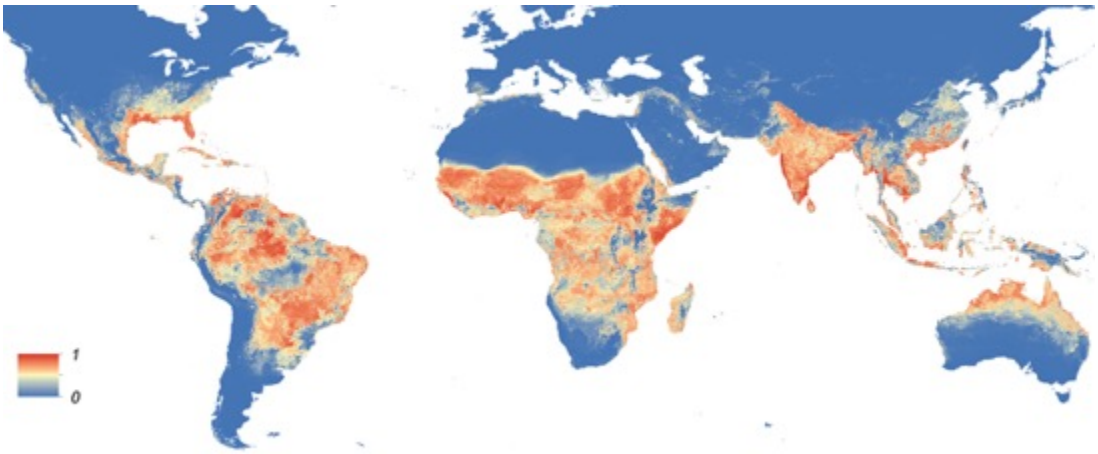
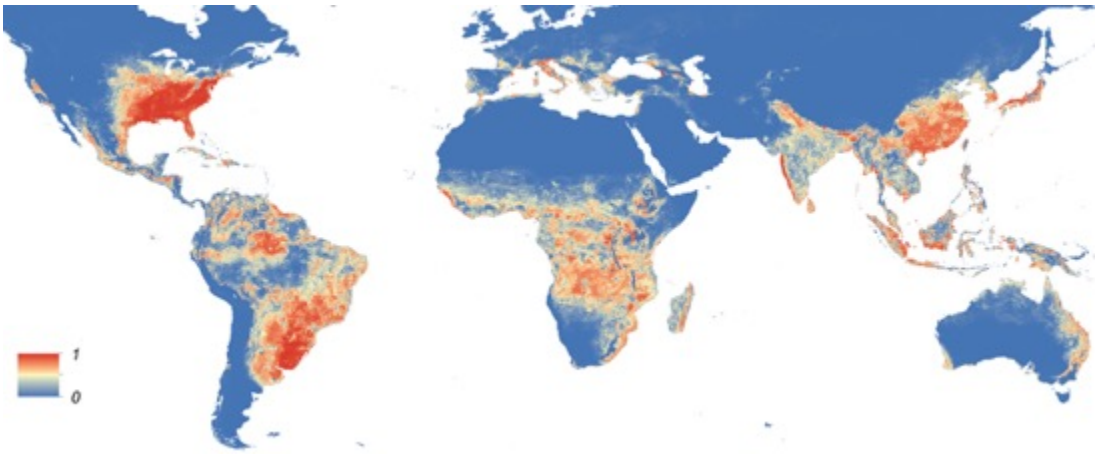


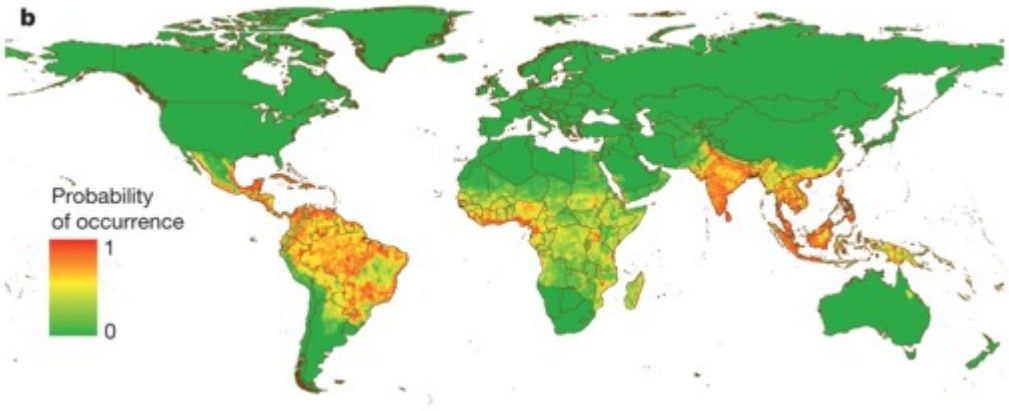
Figure 1.4 Global map of the predicted distribution of *Ae. albopictus* (Kraemer *et al.*, 2015).



Globally, there are an estimated 390 million infections of DENV annually with approximately 100 million developing symptoms and 10,000 dying. The majority of cases occur in Asia and the Americas with fewer in Africa, although this estimate is thought to be an under-estimation and very few in Oceania (Bhatt *et al.*, 2013). Autochthonous cases are now also seen in a number of countries in Europe

(Tomasello and Schlagenhauf, 2013). Figure 1.5 shows the probability of the occurrence of cases based on records of the occurrence of dengue (Bhatt *et al.*, 2013). Studies from southeast Asia showed that up to 20% of those admitted to hospital with an encephalitis-like illness had dengue (Chokephaibulkit *et al.*, 2001; Kankirawatana *et al.*, 2000; Srey *et al.*, 2002). This percentage was higher in south America with 26% in Puerto-Rico (Garcia-Rivera, Vorndam and Rigau-Perez, 2009) and 47% in Brazil (Soares *et al.*, 2011).

Figure 1.5 The probability of occurrence of dengue (Bhatt *et al.*, 2013).



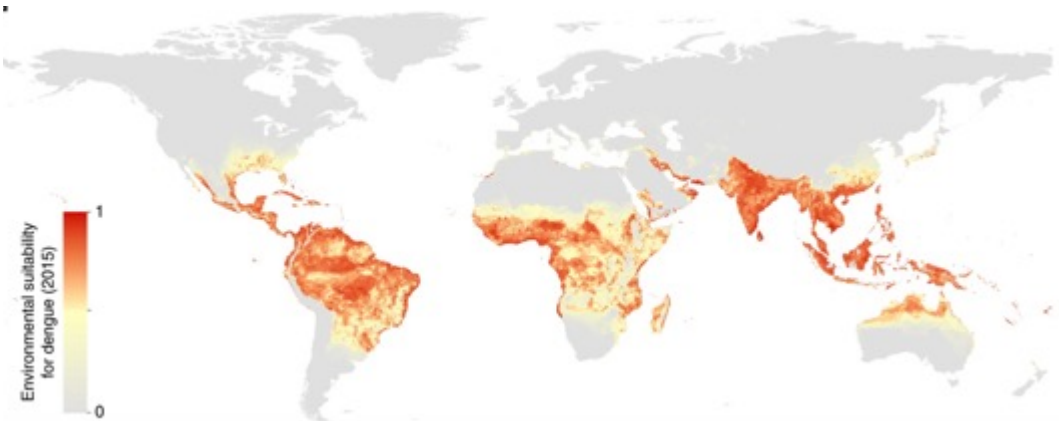
The risk of dengue is associated with a number of factors. In terms of climate, a high level of rainfall and a temperature suitable for transmission of the virus are associated with an increased risk (Bhatt *et al.*, 2013). A number of countries therefore see seasonal peaks in cases of dengue fever in the warm, wet months (Morales *et al.*, 2016; Phanitchat *et al.*, 2019; Zhang *et al.*, 2019; Lowe *et al.*, 2011) with the effect of the El Niño Southern Oscillation (ENSO) predicting the epidemiology in Ecuador (Stewart-Ibarra *et al.*, 2014; Petrova *et al.*, 2019) and India (Kakarla *et al.*, 2019) amongst others (Gagnon, Bush and Smoyer-Tomic, 2001). However, in addition to climate the behaviour and travel patterns of humans should also be taken into account when understanding seasonality (Sippy *et al.*, 2019). In addition to annual seasonal peaks of cases of dengue fever, epidemics occur where the incidence is higher than that seen over previous years which may be due to climatic factors (Vincenti-Gonzalez

et al., 2018; Stewart-Ibarra and Lowe, 2013; Kanakaratne *et al.*, 2009; Descloux *et al.*, 2012) but also human movements (Wesolowski *et al.*, 2015b).

Ae. aegypti is both an endophilic (shelters within homes) and endophagic (feeds inside houses) vector. However, it also moves between inside and outside. *Ae. albopictus* however, is considered to be exophagic (bites outside) but can also be endophilic (Reinhold, Lazzari and Lahondere, 2018). Low-income urban and peri-urban areas (Bhatt *et al.*, 2013) and those which are densely populated are at particular risk (Koyadun, Butraporn and Kittayapong, 2012). However, there is a suggestion that the risk may also be high in rural areas where there is lack of piped water supply (Schmidt *et al.*, 2011). Living in proximity to breeding sites including unclean swamps or sewage have also been shown to be risk factors for disease (Swain *et al.*, 2020; Toan *et al.*, 2015).

Figure 1.6 shows the environmental suitability for dengue based on temperature, precipitation, minimum relative humidity, gross domestic product (GDP) per capita, human population density and the environmental suitability for *Ae. aegypti* and *Ae. albopictus* (Messina *et al.*, 2019).

Figure 1.6 The environmental suitability for dengue (Messina *et al.*, 2019).



CLINICAL SYMPTOMS AND SIGNS AND PROGNOSIS

A diagnosis of dengue fever is suspected based on the presence of a high fever (40°C) with at least two of the symptoms of a severe headache, retro-orbital pain, myalgia and arthralgia, nausea, vomiting, rash and swollen glands (World Health Organization, 2020b). Most patients will have mild, self-terminating disease which doesn't require hospital admission. A minority will develop severe dengue. Severe dengue normally occurs 3-7 days after the onset of symptoms, often coincident with a decrease in fever. Symptoms and signs of severe dengue include persistent vomiting, breathlessness, severe abdominal pain, fatigue, haematemesis, bleeding gums and restlessness (World Health Organization, 2020b). The WHO classify severe dengue as any of 1) plasma leakage which causes dengue shock syndrome or respiratory distress, 2) severe bleeding or 3) severe organ impairment (World Health Organization, 2009; Wilder-Smith *et al.*, 2019b). In areas which have high rates of endemicity for the virus, initial exposure usually occurs during childhood and therefore symptomatic dengue is seen most commonly in older children and young adults. Where endemicity rates are lower, it is seen more commonly in adults than children including in the elderly (Wilder-Smith *et al.*, 2019b; Rowe *et al.*, 2014).Severe dengue has been associated in some instances with a second infection with a heterologous serotype leading to antibody dependent enhancement (ADE) (Halstead, 2007). ADE causes in increases in the number of cells infected with the virus and later, increases in the number cytokines which cause vascular permeability, possible leading to shock and death (Guzman, Alvarez and Halstead, 2013).

DENV can cause a wide range of CNS manifestations and in the case of CNS infections, an encephalitis, meningitis or meningoencephalitis as a result of the virus or an immune-mediated syndrome including an ADEM. Those with a meningitis or meningoencephalitis may present with a reduction in the conscious level, headache, seizures, disorientation, dizziness and changes in behaviour (Carod-Artal *et al.*, 2013; Araujo *et al.*, 2012; Domingues *et al.*, 2008). 30% of those with neurological involvement in a study in Pakistan died (Wasay *et al.*, 2008) and in Vietnam, 29% had neurological sequelae at discharge (Solomon *et al.*, 2000b).

DIAGNOSTICS

A diagnosis of DENV in the first few days of infection can be made via detection of the NS1 antigen using a rapid diagnostic test. Alternatively, RNA can be detected in the blood using reverse transcriptase PCR (RT-PCR). ELISA can be used to detect IgM antibodies from approximately 4-7 days after the onset of symptoms until three months (World Health Organization, 2020b; Wilder-Smith *et al.*, 2019b). As for JEV there is a risk of cross-reactivity of the antibodies with other flaviviruses giving a false positive result (Nunes *et al.*, 2011). The presence of the NS1 antigen, DENV RNA or anti-DENV IgM in the CSF can be used to make a diagnosis of a CNS infection caused by DENV however, compared to the serum, the sensitivity of these tests is low (Carod-Artal *et al.*, 2013; Soares *et al.*, 2006).

TREATMENTS

There are no treatments for dengue virus and management is supportive with the use of analgesia such as paracetamol (World Health Organization, 2020b). Severe dengue should be managed with careful fluid resuscitation (Wilder-Smith *et al.*, 2019b). A number of randomised trials have been conducted to evaluate the effect of drugs with antiviral properties including chloroquine, balapiravir, celgosivir and lovastatin however, there has been no evidence of a benefit (Tricou *et al.*, 2010; Nguyen *et al.*, 2013; Low *et al.*, 2014; Sung *et al.*, 2016; Whitehorn *et al.*, 2016). Additionally, no benefit was seen in a randomised of oral prednisolone, an immunosuppressant (Tam *et al.*, 2012).

CONTROL

Prevention of DENV can be achieved by eliminating mosquito breeding sites and the WHO advocate for integrated vector management (World Health Organization, 2009). Environmental measures might include covering or emptying water storage containers, disposing of waste which can hold water and adding screens to windows; chemical measures using mosquito repellents, residual spraying with insecticides, larvacides, or biological methods such as using larvivorous fish and copepods to eliminate larvae (Wilder-Smith *et al.*, 2019b; World Health Organization, 2020b). New approaches to the control of *Ae. aegypti* include the use of *Wolbachia*, an endosymbiotic bacteria which can render infected mosquitoes incompetent (Dorigatti *et al.*, 2018) or the Release of Insects carrying Dominant Lethal genes (RIDL) (Wilder-

Smith *et al.*, 2019b). A vaccine against dengue 'Dengvaxia' was licensed in 2015 (Wilder-Smith *et al.*, 2016) however, safety data released in 2018 showed an increased risk of severe dengue in recipients who were seronegative at the time of vaccination (Sridhar *et al.*, 2018). As a result of this, it was recommended that the vaccine only be given to those who were dengue-seropositive (Wilder-Smith *et al.*, 2019a).

DENV IN VIETNAM

DENV-1 and DENV-2 are the predominant circulating serotypes in Vietnam, with detection of DENV-3 and DENV-4 occurring more recently (Do et al., 2014, (Ha and Ninh, 2000). Cases of dengue fever occur across the country however, there are regional differences in the seasonality. Transmission occurs throughout the year in southern Vietnam but with a peak during the rainy season from July to September. In northern Vietnam and the central highlands, there are few cases in the winter with Hanoi seeing most cases occurring in the autumn months (Do *et al.*, 2014). Major outbreaks occur almost every 10 years including in 1987 (Tran *et al.*, 2010; Lee *et al.*, 2017a), 1998 (Ha *et al.*, 2000; Lee *et al.*, 2017a), 2009 (Minh An and Rocklov, 2014; Lee *et al.*, 2017a) and most recently, 2017 (Sun *et al.*, 2017). The outbreaks of 1987, 1998 and 2010 are thought to have coincided with increased activity of El Niño and La Niña (Minh An and Rocklov, 2014). *Ae. aegypti* predominates in urban areas whereas *Ae. albopictus* is found urban and suburban areas at a lower density (Nguyen-Tien, Lundkvist and Lindahl, 2019). DENV has been detected by PCR in female *Ae. aegypti* in both Hanoi and HCMC (Kim Lien *et al.*, 2015; Anders *et al.*, 2015).

DENV was the second most common viral aetiology after JEV in a study of adults with CNS infections admitted to the Hospital for Tropical Diseases (HTD) in HCMC, southern Vietnam, accounting for 6.5% of cases (Tan le *et al.*, 2014) and the third most common viral aetiology amongst children with encephalitis admitted to Children's Hospital Number One (CH1) in HCMC, accounting for 4.6% of cases (Le *et al.*, 2010). In a multicentre study of CNS infections across Vietnam, DENV was the most common viral aetiology with HSV in adult patients (4%) but the third most common in children with HSV (2%) (Ho Dang Trung *et al.*, 2012). In 1995, a study of the neurological manifestations of dengue infection amongst patients admitted to the Centre for Tropical Diseases in HCMC with suspected CNS infections found that 4.6%

had evidence of DENV with over half having an encephalitis and 44% a neurological manifestation which coincided with severe dengue (Solomon *et al.*, 2000b).

Dengue is a notifiable disease in Vietnam with control of the disease organized by the Vietnam National Dengue Control Program established in the late 1990s (Quyen *et al.*, 2018; Lee *et al.*, 2017a; Pham *et al.*, 2011). The programme was established with the aim of focusing on vector control such as reducing mosquito breeding sites, spraying insecticide and treating symptomatic cases (Lee *et al.*, 2017a; Quyen *et al.*, 2018).

The Dengvaxia vaccine was trialed in Vietnam but has not been locally licensed (Quyen *et al.*, 2018). The burden of disease is high with dengue and severe dengue leading causes of hospitalisation, morbidity and (Pham *et al.*, 2011; Lee *et al.*, 2017a; Do *et al.*, 2014) however, case fatality rates have declined (Anders *et al.*, 2011; Lam *et al.*, 2013).

ORIENTIA TSUTSUGAMUSHI (SCRUB TYPHUS)

TRANSMISSION AND EPIDEMIOLOGY

Scrub typhus is caused by *O. tsutsugamushi*, a gram-negative obligate intracytosolic bacterium (Paris *et al.*, 2013; Xu *et al.*, 2017). *O. tsutsugamushi*, is transmitted by the bite of an infected trombiculid or *Leptotrombidium delicense* mite (chigger) during its larval stage (Traub and Wisseman, 1974; Xu *et al.*, 2017; Lv, Guo and Jin, 2018). The mite lives in wild, overgrown areas such as jungle or scrub in damp soil or detritus (Seong, Choi and Kim, 2001) and feeds on vertebrates such as rodents and also humans (Paris *et al.*, 2013; Lin *et al.*, 2014). Humans are a dead-end host for the bacteria and it is thought that rodents are too given that only a small proportion of mites become infected after feeding on the animals (Paris *et al.*, 2013; Frances *et al.*, 2000).

L. delicense is mainly distributed in tropical, subtropical and temperature zones in Asia and the Pacific. As with mosquitoes, the development of the chigger mite is dependent on climatic factors. Studies have shown the optimum temperature for development and reproduction was 18-28°C and 18-30°C for transmission of disease. A relative humidity of 95-100% was most suitable for development and reproduction (Lv, Guo and Jin, 2018; Traub and Wisseman, 1974; Xu and Chen, 1960). The mite therefore tends to be found at lower altitudes (Lv, Guo and Jin, 2018). Figure 1.7 shows the distribution of *L. delicense* (Lv, Guo and Jin, 2018).

Figure 1.7 The geographical distribution of L. delicense (Lv, Guo and Jin, 2018).

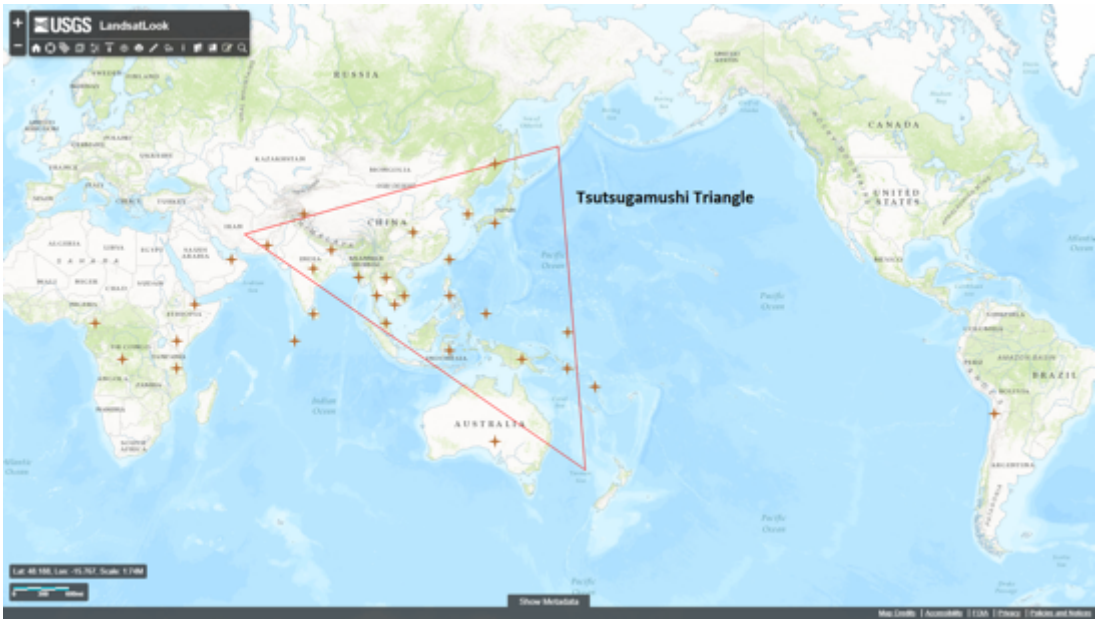
One billion people are at risk of scrub typhus with one million becoming ill each year Determining the incidence of scrub typhus is challenging as established surveillance system exist only in five countries: Japan, Thailand, the Republic of Korea, China and Bhutan (Bonell *et al.*, 2017; Xu *et al.*, 2017). In these countries, the estimated annual incidence ranged from 1.22/100,000 in China (Wu *et al.*, 2016) to 17.7 in the Republic of Korea (Park *et al.*, 2015). However, within-country differences in incidence over

time were seen in China and the Republic of Korea (Park *et al.*, 2015; Wei *et al.*, 2014; Yang *et al.*, 2015b). Separate seroprevalence studies showed an estimated seroprevalence ranging from 9.3% in Indonesia (Richards *et al.*, 2003) to 27.9% in Papua New Guinea (Spicer, Taufa and Benjamin, 2007).

Cases of scrub typhus are generally found within the "*tsutsugamushi* triange" region from northern Japan and eastern Russia to northern Australia and Pakistan and Afghanistan (Lv, Guo and Jin, 2018; Xu *et al.*, 2017). However, cases have also been reported outside of the triangle in the United Arab Emirates (UAE), Chile and a number of countries in Africa (Xu *et al.*, 2017; Izzard *et al.*, 2010; Balcells *et al.*, 2011; Thiga *et al.*, 2015; Ghorbani *et al.*, 1997; Osuga *et al.*, 1991; Horton *et al.*, 2016; Groen *et al.*, 1999). Figure 1.8 shows the countries with reported cases of scrub typhus (Xu *et al.*, 2017).

Figure 1.8 The countries reporting cases of scrub typhus (Xu *et al.*, 2017).

Human cases are shown by a star.



Studies have shown neurological complications in up to 15% of patients (Silpapojakul *et al.*, 1991; Mahajan *et al.*, 2010). A more recent study in Lao PDR diagnosed *O.*

tsutsugamushi in 12% of CSF samples taken from patients with suspected CNS infection (Dittrich *et al.*, 2015) and another in India, showed that 31.8% of cases were positive for anti-IgM against scrub typhus (Jain *et al.*, 2017). Studies demonstrating a meningitis, encephalitis or meningoencephalitis have been described in a number of locations including India (Jamil *et al.*, 2015; Misra, Kalita and Mani, 2015), the Republic of Korea (Kim *et al.*, 2011), Thailand (Silpapojakul *et al.*, 1991) and Nepal (Adhikari *et al.*, 2018).

The incidence of scrub typhus is seasonal, the months which see the highest number of cases depending on the climate of the country. For example, in China, most cases are seen in June and July, whereas in Japan and the Republic of Korea, most cases occur in November and October and November respectively. In India the peak is seen between August and October (Xu *et al.*, 2017).

Those working outside, particularly in rural areas are at higher risk of scrub typhus (Kweon *et al.*, 2009; Suputtamongkol *et al.*, 2009) with risk of infection often highest amongst middle-aged and older adults (Bonell *et al.*, 2017; Xu *et al.*, 2017). Those working in farming are at increased risk as demonstrated in a number of studies (Bonell *et al.*, 2017; George *et al.*, 2018). In some studies males have been shown to be at increased risk yet, in others the risk for females is higher (Bonell *et al.*, 2017). A study in the Republic of Korea hypothesised that the squatting position adopted by female farmers increases their risk of infection (Kweon *et al.*, 2009). Studies in Lao PDR and Nepal showed that those living in rural or peri-urban areas were at increased risk of infection (Vallee *et al.*, 2010; Gautam, Parajuli and Sherchand, 2019). Increased risk of infection has also been described in northern India with storing firewood indoors and handling cattle fodder (Thangaraj *et al.*, 2018), and in southern India with living in proximity to other homes (Trowbridge *et al.*, 2017), having a water body or bushes close to the house or cooking outside the house (Rose *et al.*, 2019).

CLINICAL SYMPTOMS AND SIGNS AND PROGNOSIS

The clinical presentation of scrub typhus can range from a mild illness to severe and even fatal illness. Patients initially develop an eschar, which is an area of necrosis of the skin found at the site of the bite of the chigger mite and is accompanied by regional

lymphadenopathy. This is followed by a range of symptoms including fever, myalgia, cough, rash, gastrointestinal symptoms, headache, transient hearing loss and generalised lymphadenopathy (Paris *et al.*, 2013; Vallee *et al.*, 2010; Xu *et al.*, 2017; Premaratna *et al.*, 2006). In severe cases, multi-organ failure may develop with acute respiratory failure, gastrointestinal bleeding, acute renal failure, coagulopathy and shock (Paris *et al.*, 2013; Wang *et al.*, 2007).

In a case series of 37 patients admitted to a tertiary care teaching hospital in North India and referred to the neurology service with a febrile illness which was attributed to scrub typhus 84% had a change in consciousness and 24%, seizures (Misra, Kalita and Mani, 2015). The case fatality rate from meningitis/meningoencephalitis in a study in India was 5.8% (Abhilash *et al.*, 2015) and in Lao PDR, 14% (Dittrich *et al.*, 2015). A separate study in India demonstrated that 11% of those with a neurological manifestation of scrub typhus had a disability on discharge but all recovered. Those with significant disability were of older age and had a prolonged stay in hospital (Misra, Kalita and Mani, 2015). In addition to meningitis, encephalitis and meningoencephalitis other clinical manifestations of scrub typhus have been described including a cerebellitis, cranial nerve pathology, Guillain-Barré syndrome, peripheral neuropathy, transverse myelitis and cerebrovascular accidents amongst others (Mahajan and Mahajan, 2017).

DIAGNOSTICS

The indirect immunofluorescence assay (IFA) is the gold standard for scrub typhus diagnosis however, it is expensive, complicated to perform and doesn't detect antibodies in the early stages of infection (Xu *et al.*, 2017). In resource-limited settings, ELISA and immunochromatographic tests are therefore more commonly used (Gautam *et al.*, 2020). Other serological tests for scrub typhus include the Weil-Felix agglutination test and the indirect immunoperoxidase assay (IIP) (Xu et al., 2017). Finally, PCR can be used to detect the DNA of *O. tsutsugamushi* (Tantibhedhyangkul *et al.*, 2017), including in CSF samples to diagnose a CNS infection (Dittrich *et al.*, 2015).

TREATMENTS

The standard treatment for scrub typhus is the antibiotic doxycycline however, rifampicin can be used in those who do not respond well to doxycycline (Xu *et al.*, 2017; Watt *et al.*, 2000).

Chloramphenicol or azithromycin are recommended in pregnant women and children where doxycycline is contraindicated (Xu *et al.*, 2017; Lee *et al.*, 2017b).

CONTROL

Despite attempts to develop a vaccine against *O. tsutsugamushi* no current vaccine exists (Chattopadhyay and Richards, 2007). Control is therefore centred around other measures to prevent infection. Those working outdoors are advised to wear long-sleeved clothing to prevent bites, avoid lying on the ground and removing the clothes and washing on returning home. Controlling rodents by for example, poisoning or trapping and improving sanitation and clearing vegetation helps to remove the habitat for the chigger mites (Xu *et al.*, 2017).

O. TSUTSUGAMUSHI IN VIETNAM

Case reports of scrub typhus in Vietnam date back to the 1960s and 1970s, with many American military personnel infected (Berman and Kundin, 1973). However, recent studies in northern Vietnam found a low seroprevalence amongst the general population (1.1%) (Trung *et al.*, 2017) and 3.5% (n=251) of hospital admissions over a two-year period with either fever with an eschar, lymphadenopathy and rash, or fever and no signs of another infection. 55.7% of those with scrub typhus were farmers and incidence was highest in the summer months when rice farming was more common (Nadjm *et al.*, 2014). However, Nadjm et al. 2014 noted that microbiological confirmation of the pathogen was challenging. A separate study, also conducted in northern Vietnam found that the majority of patients with scrub typhus were from rural areas. Although fever and headache were commonly reported, the authors do not mention whether any had evidence of CNS infection (Trung *et al.*, 2019). Similarly, studies of the aetiology of CNS infections in Vietnam did not report any cases of scrub typhus however, it is possible that the diagnostics used were insufficient to detect this (Ho Dang Trung *et al.*, 2012; Taylor *et al.*, 2012).

HERPES SIMPLEX VIRUS (HSV)

TRANSMISSION AND EPIDEMIOLOGY

HSV belongs to family *Herpesviridae* and is a double-stranded DNA virus (Ludlow *et al.*, 2016b). There are two types of herpes simplex virus, herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). HSV-1 is typically acquired during childhood causing a lifelong infection (World Health Organization, 2020f). It is transmitted via oral secretions such as saliva or orolabial ulcers with both symptomatic and asymptomatic transmission occurring (Pebody *et al.*, 2004; World Health Organization, 2020f). Most commonly, the virus is transmitted via oral to oral contact however, oral to genital transmission contact can occur and rarely, vertical transmission during labour (Pebody *et al.*, 2004; World Health Organization, 2020f; Lafferty *et al.*, 2000; Corey and Wald, 2009). HSV-2 is normally transmitted via the genital route (Whitley, 2006) with prevalence increasing with age and incidence highest amongst adolescents (World Health Organization, 2020f). Data from 2016, estimated that 67% of the global population had an HSV-1 infection with prevalence being highest in Africa (88%) and lowest in the Americas (45%). Globally, 13% of those aged 15-49 years were living with HSV-2, with prevalence highest in Africa (44% in women compared to 25% men) (World Health Organization, 2020f).

Herpes viruses can become latent with certain cell types (Ludlow et al., 2016b). HSV-1 is neurotropic, becoming latent in sensory neurones and following replication in oral or genital mucosa (Nicoll, Proenca and Efstathiou, 2012) where HSV-2 is thought to become latent in the sacral ganglia (Berger and Houff, 2008). Despite many people being infected with HSV-1, only a small proportion will develop encephalitis (Xu *et al.*, 2006). Encephalitis caused by HSV occurs either as a consequence of primary infection or reactivation of the latent virus with HSV-1 causing up to 90% of Herpes Simplex encephalitis (HSE) and HSV-2 being more likely to cause a meningitis (Ludlow et al., 2016b, (Berger and Houff, 2008). Initial studies suggested that in the majority of patients, encephalitis occurred in those where there was reactivation of a latent virus compared to a primary infection (Nahmias *et al.*, 1982). A study of the clinical manifestations of genital HSV infection found that 13% of men and 36% of women with primary genital HSV-2 infection had symptoms of meningitis (Corey *et al.*, 1983).

Herpes simplex encephalitis is the most common cause of viral encephalitis in high-income countries with an annual incidence of 1 in 250,000 to 500,000 (Whitley, 2006) also the virus is also a known cause of encephalitis in low and middle-income countries including in south and southeast Asia (Kalita *et al.*, 2016; Rathore *et al.*, 2014).

Unlike the vector-borne diseases, HSE is not thought to have a seasonal pattern (Kalita *et al.*, 2016). Individual risk factors for developing HSE include immune-compromising diseases, diabetes mellitus and malignancy (Ludlow *et al.*, 2016b) with the majority of cases occurring in those aged 50 years and over with a higher incidence in those less than 3 years of age and males and females being equally affected (Bradshaw and Venkatesan, 2016).

CLINICAL SYMPTOMS AND SIGNS AND PROGNOSIS

Patients with HSE develop a prodromal phase of fever, malaise and headache and less frequently, nausea and vomiting. At the same time, a change in consciousness includes behavioural changes and confusion may occur (Sabah, Mulcahy and Zeman, 2012). Seizures are particularly common as the virus affects the temporal lobes (Ludlow *et al.*, 2016b). In some cases, patients develop focal neurological signs such as unilateral weakness (Sabah, Mulcahy and Zeman, 2012). Without treatment, over 70% of those with HSE may die and of those who survive many have neurological sequelae such as seizures, memory impairment, personality change and motor impairment (Ludlow *et al.*, 2016b; Khandaker *et al.*, 2016). Mortality rate of up to 15% are seen in those who are treated and of those who survive, the majority suffer disability and are unable to return to work (Martinez-Torres *et al.*, 2008).

Those with HSV meningitis develop fever, headache, nausea and vomiting and meningeal signs and symptoms such as neck stiffness and photophobia and in some, additional symptoms associated with primary genital infection (Logan and MacMahon, 2008; Mommeja-Marin *et al.*, 2003). One-third of cases develop a sacral radiculomyelitis with constipation, paraesthesia and urinary retention (Corey *et al.*, 1983; Logan and MacMahon, 2008). Unlike HSE, mortality rates are lower and most infections are self-limited (Mommeja-Marin *et al.*, 2003). However, 20-50% of cases

may develop a recurrence of meningitis which is more common in women with a primary infection (Logan and MacMahon, 2008).

DIAGNOSTICS

Diagnosis of both encephalitis and meningitis is confirmed by PCR of the CSF for viral DNA (Solomon *et al.*, 2012; Mommeja-Marin *et al.*, 2003). It should be noted that PCR might be negative during the initial period of illness but should be positive on a second sample take 3-7 days later (Solomon *et al.*, 2012). Changes to the medial temporal lobe and cingulate gyrus seen on magnetic resonance imaging (MRI) of the brain are seen in approximately 90% of patients with HSE within 48 hours of hospital admission (Solomon *et al.*, 2012).

TREATMENTS

Intravenous aciclovir (an antiviral) is used to treat HSE and should be started empirically if HSE is suspected. If the patient has renal impairment, the dose should be reduced (Solomon et al., 2012). Compared to an alternative antiviral, vidarabine, treatment with aciclovir is associated with lower mortality and improved outcome in survivors in randomised controlled trials (Skoldenberg *et al.*, 1984; Whitley *et al.*, 1986). The benefit of using steroids for the treatment of HSE is unknown. There is currently a trial conducted by the University Hospital, Grenoble and the University of Liverpool to look at outcome of HSE following four days of dexamethasone compared to no dexamethasone (ClinicalTrials.gov, 2018). There is variation in the treatment of HSV-2 meningitis with clinicians using antivirals and others not (McGill, Zuckerman and Solomon, 2015). However, no trial has been conducted to evaluate the efficacy of aciclovir in this condition (McGill, Zuckerman and Solomon, 2015; Logan and MacMahon, 2008). Early treatment with aciclovir however, results in a much lower death rate, with 15% being recorded in a multicentre study (Raschilas *et al.*, 2002).

CONTROL

To prevent transmission, those with oral or genital herpes, should avoid oral and genital contact, respectively. Condoms can reduce the risk of infection of genital herpes but not prevent it (World Health Organization, 2020f). Vaccine candidates are

currently being studied as is the use of topical microbicides (World Health Organization, 2020f; Awasthi and Friedman, 2014; Kizima *et al.*, 2014).

HSV AND ITS NEUROLOGICAL MANIFESTATIONS IN VIETNAM

A study conducted in multiple countries amongst women aged 15 years and older to determine the performance of ELISA found that 100% of those in Hanoi and 98% of those in HCMC had antibodies to HSV-1 (Ashley-Morrow *et al.*, 2004). A meta-analysis of reports of HSV-1 reports in Asia showed a pooled mean seroprevalence of 76.5% in adults (Khadr *et al.*, 2019) compared to a study in Europe which showed the seroprevalence in England and Wales amongst those more than 39 years old to be 23% (Pebody *et al.*, 2004).

A cross-sectional study conducted amongst ever married women found that seroprevalence of HSV-2 was 30.8% in those from HCMC compared to 8.8% in those from Hanoi (Le *et al.*, 2009). A separate study found HSV-2 antibodies in 27.7% of female sex workers from border provinces in Vietnam. Prevalence was higher in southern and central provinces compared to those in northern Vietnam (O'Farrell *et al.*, 2006).

4% (n=22) of adults and 2% (n=14) of children had CNS infections due to HSV in a multi-centre study conducted between 2007 and 2010 (Ho Dang Trung *et al.*, 2012) and 3% (n=12) adults in a study in Hanoi from 2007 to 2008 (Taylor *et al.*, 2012).

ENTEROVIRUSES (ENTEROVIRUS 71)

TRANSMISSION AND EPIDEMIOLOGY

Enteroviruses are single-stranded non-enveloped RNA viruses belonging to the *Picornaviridae* family (Ludlow *et al.*, 2016b; Pons-Salort, Parker and Grassly, 2015). Enteroviruses are primarily transmitted via the shedding of the virus via the faecal-oral route and also via the respiratory route between humans (Li et al., 2013a; Ludlow et al., 2016a; Pons-Salort, Parker and Grassly, 2015). However, transmission via contact with infected surfaces or fomites can also occur (Solomon *et al.*, 2010). The virus replicates in the respiratory and gastrointestinal tract before disseminating to

other organs via the bloodstream (Ludlow *et al.*, 2016b). There are over 100 serotypes of human enteroviruses which were originally classified into coxsackieviruses A and B, echoviruses and polioviruses but are now numbered instead (Solomon *et al.*, 2010). Enteroviruses exhibit seasonal changes in incidence, predominantly in temperate climates with peaks in the summer and early autumn (Pons-Salort, Parker and Grassly, 2015).

A number of different enteroviruses can cause an aseptic meningitis or encephalitis following compromise of the blood brain barrier facilitating entry to the CNS (Chen *et al.*, 2020). However, only enterovirus 71 will be described in detail given its ability to cause severe neurological manifestations and hence a public health threat in the Asia-Pacific region (Chen *et al.*, 2020).

Enterovirus A71 (EV-A71) was first isolated in 1969 in the United States of America (USA), though it was likely circulating prior to this (Solomon *et al.*, 2010). The virus is found in a number of countries however, it causes cyclical major outbreaks of hand, foot and mouth disease (HFMD) in the Asia-Pacific region (Cardosa *et al.*, 2003; Solomon *et al.*, 2010; Sabanathan *et al.*, 2014). A number of countries are implementing national surveillance and making the pathogen a notifiable disease (Solomon *et al.*, 2010). In Japan and Malaysia, outbreaks tend to occur every 2-3 years which is thought to be due to the presence of a sufficient population of children susceptible to infection to sustain transmission (Sabanathan *et al.*, 2014). More frequent (annual) epidemics have been seen in China (Xing *et al.*, 2014). Seroprevalence of EV-A71 is high amongst neonates however, declines after maternal antibody wanes. It then increases with age with a systematic review and meta-analysis in China showed that 70% of those at 5 years of age had antibodies (Yang *et al.*, 2015a). Similar trends were seen in a study in Thailand with 80% of those aged over 6 years having antibodies (Linsuwanon *et al.*, 2014).

Children and infants are most commonly affected with transmission often occurring in crowded settings and outbreaks associated with nurseries and primary schools (Sabanathan *et al.*, 2014). However, household transmission is also frequent but with adults less frequently infected (Chang *et al.*, 2004). The incidence of EV-A71 has been shown to correlate with temperature with a study in Taiwan showing an increase

in infections at temperatures above 13°C but a decrease at temperatures greater than 26°C and an increase in incidence with relative humidity (Chang *et al.*, 2012). Similar patterns were also seen in the Republic of Korea (Kim *et al.*, 2016).

CLINICAL SYMPTOMS AND SIGNS AND PROGNOSIS

Those infected with EV-A71 may have a self-limiting illness, may be asymptomatic, or may be severely affected and even die (Sabanathan *et al.*, 2014; Solomon *et al.*, 2010). HFMD is generally a brief and mild illness characterised by fever, oral ulcers and papulovesicular rash on the palms and soles (Ooi *et al.*, 2010). A range of neurological manifestations can occur with an aseptic meningitis, encephalitis, acute flaccid paralysis and encephalomyelitis occurring most commonly. A study conducted in Sarawak, Malaysia conducted over several epidemics showed that 10-30% of children admitted to hospital with HFMD developed a neurological manifestation with 62% developing an encephalitis and 36% an aseptic meningitis (Ooi *et al.*, 2010).

Brainstem encephalitis is the most common neurological complication in children (Wang *et al.*, 1999). Those with brainstem involvement tend to develop myoclonic jerks however, prolonged and recurrent seizures are less common than in other viral encephalitides (Ooi *et al.*, 2010). They may also develop autonomic dysregulation with hypertension and tachycardia with some developing pulmonary oedema which is thought to be neurogenic (Ooi *et al.*, 2010; Sabanathan *et al.*, 2014). A study from Malaysia found that in addition to mouth ulcers and fever, younger age, vomiting, breathless, poor urine output and cold limbs were risk factors for CNS manifestations (Ooi *et al.*, 2007). A study conducted in Taiwan found that 14.3% of children who had brainstem encephalitis had a residual deficit including both cognitive and motor defects with cerebellar dysfunction being most common in the latter (Huang *et al.*, 2006). A separate study also conducted in Taiwan found that at a median follow-up of 2.9 years, all patients with an aseptic meningitis made a complete recovery however, 20% of those with encephalomyelitis and 64% of those with cardiopulmonary failure had unilateral limb weakness and atrophy.

DIAGNOSTICS

A diagnosis of EV-A71 is confirmed by PCR of a sample from an ulcer, throat or rectal swab, serum, urine, vesicular fluid. Virus is detected in only 0-5% of CSF samples in those with neurological manifestations. Detection of IgG and IgM antibodies by ELISA

is complicated due to cross-reactivity with other enteroviruses, particularly in older children (Ooi et al., 2010).

TREATMENTS

There are no known antivirals which are effective against EV-A71. Intravenous immunoglobulin (IVIG) is often used as a treatment of severe EV-A71 and is part of the national treatment guidelines in Taiwan and widely used in Vietnam. However, trials are needed to determine its efficacy (Ooi *et al.*, 2010; Sabanathan *et al.*, 2014). A study with historical controls in Taiwan and one small randomised control trial in Vietnam have shown a reduction in mortality of children with pulmonary oedema in those treated with the cyclic nucleotide phosphodiesterase inhibitor milrinone compared to standard care (Wang *et al.*, 2005; Chi *et al.*, 2013).

CONTROL

During outbreaks, public health education messages including frequent hand washing, disinfecting surfaces and disposal soiled nappies are often implemented (Sabanathan *et al.*, 2014). Additionally, social distancing measures such as closing school and childcare facilities may be implemented (Sabanathan *et al.*, 2014; Ang *et al.*, 2009). Inactivated vaccines have been licensed in China (Mao *et al.*, 2016; Lin, Kung and Shih, 2019). Despite a vaccination campaign in 2016-2017 in Beijing, the vaccine is not included in the EPI as post-licensure effectiveness is still to be determined (Wang *et al.*, 2019) and has not been implemented outside of China (Nhan *et al.*, 2018). Vaccine trials have also been undertaken in Taiwan and Singapore (Lin, Kung and Shih, 2019).

ENTEROVIRUS 71 AND ITS NEUROLOGICAL MANIFESTATIONS IN VIETNAM

EV-A71 was first isolated in Vietnam in 2003. Prior to this, an increase in viral encephalitis amongst those aged less than 4 years and a reduction in cases due to JEV was noticed (Van Tu *et al.*, 2007). However, severe epidemics were not seen until after 2011. HFMD is a notifiable disease, with hospitals reporting cases on a weekly basis (Thao *et al.*, 2017) and a clinical grading system implemented by the Ministry of Health used to guide management (Khanh *et al.*, 2012). The outbreak in 2011 resulted in 113,121 cases and 170 deaths with the majority of both cases and

deaths occurring in the southern provinces and deaths in boys aged 3 years or younger (Nguyen *et al.*, 2014). Although the virus circulates all year in Vietnam, seasonality is seen with peaks from March to May and September to December. There are approximately 50,000 to 100,000 cases annually with over 60% occurring in southern Vietnam (World health Organization, 2020e). A study conducted in HCMC found that 9.3% (n=18) children admitted with encephalitis had an enterovirus of whom six had confirmed or probable EV-A71 with a 50% mortality rate. All patients were aged under 3 years and the majority were male (Le *et al.*, 2010; World health Organization, 2020e).

STREPTOCOCCUS SUIS

TRANSMISSION AND EPIDEMIOLOGY

S. suis is a gram-positive bacterium and zoonotic pathogen with a natural habitat in the gastro-intestinal, respiratory and genital tract of pigs (Nghia *et al.*, 2011). Humans become infected following the consumption of undercooked pig blood or intestine, occupational exposure to raw pig products and the preparation of raw pork in the presence of skin lesions (Nghia *et al.*, 2011; Huong *et al.*, 2015). Additional risk factors for acquisition of the bacteria include being male or consuming excessive amounts of alcohol (Nghia *et al.*, 2011; Wertheim *et al.*, 2009b). In pigs, Porcine Respiratory and Reproductive Syndrome (PRRS) is associated with secondary bacterial infections such as *S. suis* (Wertheim *et al.*, 2009b). Therefore, living in or adjacent to an area with PRRS is also a risk factor for *S. suis* (Huong *et al.*, 2015).

The prevalence of *S. suis* is highest in east and southeast Asia with the first reports of the pathogen coming from Hong Kong in 1984 (Kay, Cheng and Tse, 1995). Awareness of the pathogen increased after an outbreak in China in 2005 (Horby *et al.*, 2010; Yu *et al.*, 2006). Although cases are seen in Europe and North America, the prevalence is lower and mainly confined to butchers, pig breeders and those working in abattoirs *(Huong et al., 2014a)*. There is no clear estimate of the global burden of *S. suis* infection in humans, however a systematic review of 177 publications found the cumulative prevalence rate to be highest in Thailand (8.21 cases/million population) followed by Vietnam (5.40/million). The pooled mean age of the cases

was 51.4 years (95%CI 49.5-53.2%, 8.0% (4.6-13.7%) had diabetes mellitus, and meningitis was the main clinical syndrome with a pooled rate of 68.0% (95%CI 58.9%-75.8%) *(Huong et al., 2014a)*. Again, the global burden of *S. suis* meningitis is unknown however, a separate systematic review and meta-analysis of twenty-four studies between 1980 and 2015 identified 913 cases from studies performed in Thailand, Vietnam, Hong Kong, the Netherlands, China and Japan. Risk factors for meningitis were same as those for acquisition of infection with pathogen (van Samkar *et al.*, 2015).

Some studies have suggested a possible temporal pattern of infection of *S. suis*. Higher numbers of patients were admitted to hospitals in China and Thailand during the rainy season from June to September (Wertheim *et al.*, 2009a). In northern Vietnam, the incidence has been shown to peak between May and July (Wertheim *et al.*, 2009b) However, in southern Vietnam there was no clear effect of seasonality with cases occurring throughout the year (Nghia *et al.*, 2011). A further study conducted in northern Vietnam suggested that a possible cluster of infections between March and August 2010 corresponded with the peak of a PRRS outbreak (Huong *et al.*, 2016).

CLINICAL SYMPTOMS AND SIGNS AND PROGNOSIS

After meningitis, the most common clinical syndromes associated with *S. suis* are sepsis, arthritis, endocarditis, and endophthalmitis (Huong et al., 2014). 31% of patients have dermatological manifestations including petechiae, purpura and ecchymoses, skin necrosis, haemorrhagic bullae and gangrene of the peripheries (Wertheim *et al.*, 2009a). A systematic review and meta-analysis of *S. suis* meningitis reported that the most common presentations included fever (97%), headache (95%), neck stiffness (93%) and nausea or vomiting (65%). 31% had altered consciousness, 2.9% died and 64% were left with sequelae with the most common being hearing loss (53%) (van Samkar *et al.*, 2015). This mortality rate is lower than the pooled case-fatality rate (CFR) from a separate systematic review of all *S. suis* infections which was 12.8% and showed a negative correlation between the CFR and meningitis (Huong et al., 2014). Hearing loss is a known outcome of infection with *S. suis* with reported rates varying between 6 and 100% (Huong et al., 2014). A case-control study conducted at the National Hospital for Tropical Diseases (NHTD) in Hanoi found significantly increased rates of hearing loss and vestibular dysfunction amongst

meningitis cases compared to controls at hospital discharge, with an improvement in occurring only within the first three months after discharge and vestibular dysfunction showing little recovery (Huong *et al.*, 2018).

DIAGNOSTICS

The pathogen can be cultured from CSF or blood but is often mis-reported as alternative *Streptococcal* species, *Enterococcus faecalis* or *Aerococcus viridans* (Wertheim *et al.*, 2009a; Donsakul, Dejthevaporn and Witoonpanich, 2003). As cultures can be negative if antibiotics have been used prior to admission PCR may be a preferable method of diagnosis (Wertheim *et al.*, 2009a).

TREATMENTS

Empirical treatment of bacterial meningitis with the antibiotic ceftriaxone will adequately treat *S. suis*. Penicillin G has also been used successfully to treat *S. suis* (Wertheim *et al.*, 2009a). Dexamethasone is given to adult patients in southern Vietnam who have a short disease onset following a randomised controlled trial which found a significant reduction in mortality in those with bacterial meningitis and a lower rates of hearing loss in those with *S. suis* meningitis who were given dexamethasone compared to a placebo (Wertheim *et al.*, 2009a).

CONTROL

There is no vaccine available against *S. suis* in humans (Wertheim *et al.*, 2009a; Hopkins *et al.*, 2019). Autogenous vaccines may be used but the effectiveness is unknown (Hopkins *et al.*, 2019; Prufer *et al.*, 2019). Preventative measures therefore include hand-washing after or wearing gloves during the handling of raw pork meat. It is also advised that pork is cooked thoroughly (Wertheim *et al.*, 2009a).

S. SUIS AND ITS NEUROLOGICAL MANIFESTATIONS IN VIETNAM

S. suis was detected in Vietnam in 1997. However, national guidelines for diagnosis and treatment of the disease were not implemented until 2007 following a report identifying *S. suis* as the most common cause of bacterial meningitis at the National Hospital for Tropical Diseases, Hanoi (Horby *et al.*, 2010). Since 2011, *S. suis* has

been a notifiable communicable disease in Vietnam with health facilities required to report culture confirmed *S. suis* within twenty-four hours. By 2015, over five-hundred cases of *S. suis* had been reported in Vietnam however, this number is likely to be an under-estimate of the true burden potentially due to the absence of or underutilisation of microbiology in hospital or use of antibiotics prior to admission (Huong *et al.*, 2019). Based on modelling approaches, the annual incidence in Vietnam in 2014 was estimated to be 0.249 per 100,000 population with a DALY of 1401 (Huong *et al.*, 2019). A study conducted in HCMC found that *S. suis* was the most common pathogen causing bacterial meningitis accounting for 33.6% (n=151) of cases however, mortality was lower than for other pathogens (CFR=2.6%). There was no evidence of clustering of cases in time and place (Mai *et al.*, 2008).

1.4 PATHOGENS WHICH ARE FOUND IN VIETNAM AND ITS NEIGHBOURING COUNTRIES BUT ARE LESS COMMON CAUSES OF CNS INFECTIONS

HERPESVIRIDAE

VARICELLA ZOSTER VIRUS (VZV)

Primary disease with VZV causes chickenpox, a disease characterized by fluid-filled blisters, fever, fatigue, headache and loss of appetite. The virus transmitted by respiratory droplets or direct contact with the blisters of an infected person or if the virus particle from the blister becomes airborne (Centers for Disease Control and Prevention, 2016). In temperate countries, children are most commonly affected whereas in tropical regions infection is less common and may occur at a later age. Incidence in temperate climates peaks in the winter, whereas in tropical climates this may differ by country. For example, in India, Sri Lanka and Thailand outbreaks are more common in the cooler months. However, in Singapore there appears to be no seasonal effect (Lee, 1998). Neurological manifestations including a varicella cerebellitis may occur with chickenpox, but this is rare. Reactivation of the virus which has become latent in the sensory ganglia is known as herpes zoster or shingles and is more likely to cause neurological manifestations including meningitis and particularly in those who are immunocompromised, encephalitis (Ludlow *et al.*, 2016b). Studies of adults admitted to hospitals in HCMC and Hanoi with a CNS infection, found that 1.7% (n=5) and 1.4% (n=5) had VZV (Tan le *et al.*, 2014; Taylor

et al., 2012). A vaccine against VZV has been available since 1974 *(World Health Organization, 2015c)* however, it is recommended mainly higher-income countries (Gabutti *et al.*, 2019) and is not part of the EPI in Vietnam (Binh *et al.*, 2013).

CYTOMEGALOVIRUS (CMV)

CMV is transmitted via bodily fluids with many people becoming infected as children. The virus then becomes latent with an ability to reactivate later in life (Centers for Disease Control and Prevention, 2020). A study in Beijing showed no evidence of seasonality of acute infection (Chen *et al.*, 2019). Most healthy people have no symptoms or only mild symptoms (Centers for Disease Control and Prevention, 2020; Pass, 1985; Krishna, Wills and Sinclair, 2019). However, primary infection or reactivation during pregnancy can result in congenital CMV which can result in neurological sequelae (Lim and Lyall, 2017). Alternatively, CMV-induced neurological disorders, including an encephalitis amongst others are most commonly seen in immunosuppressed patients as a result of reactivation of the virus (Ludlow *et al.*, 2016b). In studies conducted in patients with CNS infections in HCMC, only one adult and one child had CMV (Tan le *et al.*, 2014).

EPSTEIN-BARR VIRUS (EBV)

EBV is transmitted via contact with oral secretions. Infants and children are normally asymptomatic or have nonspecific symptoms however, adolescents and adults can develop an infectious mononucleosis with fever, lymphadenopathy and pharyngitis (Cohen, 2000). Studies in the United States have shown no clear evidence of seasonality of infection. (Odumade, Hogquist and Balfour, 2011). Neurological manifestations of can occur as a result of primary infection, reactivation of the virus or a chronic infection and can include a meningitis or encephalitis amongst others (Ludlow *et al.*, 2016b). Despite evidence of past infection of EBV in a study of 24 adults with CNS infection, none had an acute infection (Tan le *et al.*, 2014). There is no vaccine against EBV and prevention is through avoiding sharing contact with the saliva of those infected (Centers for Disease Control and Prevention, 2018).

PARAMYXOVIRIDAE

MEASLES VIRUSES

Measles virus is transmitted between humans via respiratory droplets and is highly contagious. Patients have a fever and erythematous maculopapular rash. Since the introduction of vaccination there has been a reduction in deaths from measles however, cases still remain in countries where vaccine coverage is lower. Despite a reduction in cases in Vietnam, outbreaks still occur (Choisy *et al.*, 2019; Nmor, Thanh and Goto, 2011). Annual seasonal peaks are seen in the winter and early spring in temperate climates with more irregular cycles seen in tropical regions (Moss, 2017). One in 1000 develop an acute demyelinating encephalitis (ADEM) and one in 1700 to one in 3300 of those under 5 years of age develop a subacute sclerosing panencephalitis (SSPE) 4-10 years after infection (Ludlow *et al.*, 2016a). Cases of acute measles encephalitis (AME) have been described during an outbreak of measles in 2008 in northern Vietnam (Fox *et al.*, 2013).

MUMPS VIRUS

Mumps virus is transmitted by respiratory droplets, direct contact or contaminated fomites (Hviid, Rubin and Muhlemann, 2008). Mumps has also been shown to be seasonal with the highest incidence in the spring and summertime in Taiwan (Liu *et al.*, 1998; Ho *et al.*, 2015). Initial symptoms include fever, malaise, headache and parotitis with 1-10% developing a meningitis and 0-1%, an encephalitis (Hviid, Rubin and Muhlemann, 2008) Vaccines against mumps exist as monovalent, bivalent (with measles) and trivalent (with measles and rubella (MMR)) however, mumps vaccine is not part of the EPI in Vietnam (Jit *et al.*, 2015). Mumps virus accounted for 0.7% (n=2) of cases of adults admitted to a hospital in HCMC with CNS infections (Tan le *et al.*, 2014).

NIPAH VIRUS

Nipah virus is an emerging pathogen which caused an outbreak of severe encephalitis in Malaysia from 1998-1999, with many cases occurring in those who had had exposure to pigs (Chua *et al.*, 2000). The virus was later isolated in *Pteropus* bats which were found to be a natural host for the pathogen (Chua *et al.*, 2002). During outbreaks in Bangladesh, the consumption of date palm sap which had been

contaminated with bat excreta was suggested as a potential source of transmission of the virus (Islam *et al.*, 2016; Rahman *et al.*, 2012). Seasonality of cases was evident with illness onset occurring from December to May coinciding with the harvesting of date palm sap (Luby *et al.*, 2009). Evidence of person to person transmission has also been shown in outbreaks in West Bengal, India (Chadha *et al.*, 2006); Bangladesh (Gurley *et al.*, 2007; Homaira *et al.*, 2010) and Kerala, India (Chatterjee, 2018). A study looking at the seroprevalence of Nipah virus in Vietnam found henipavirus antibody in 49.1% of bats specimens from Hoa Binh province in northern Vietnam. However, it was thought that 'Nipah-like' viruses rather than Nipah or Hendra viruses may be circulating in these bat populations given that there were no increases in reports of encephalitis where the bats were captured (Hasebe *et al.*, 2012). Nipah virus has been found in bats in Cambodia (Cappelle *et al.*, 2020). There are no known published reports of Nipah virus in humans in Vietnam. A study conducted in Hanoi, tested eighty patients with CNS infections for the virus but none were positive (Taylor *et al.*, 2012).

TOGOVIRIDAE

RUBELLA VIRUS

Rubella is transmitted by respiratory droplets normally affecting children and young adults (World Health Organization, 2020i). A mild, self-limiting disease occurs in most cases of infection of rubella virus with adults being more likely to develop fever and a rash compared to children (Lambert *et al.*, 2015; Banatvala and Brown, 2004). However, infection during pregnancy, this can result in miscarriage or congenital rubella syndrome (Lambert *et al.*, 2015). A SSPE can occur in those with congenital rubella syndrome (Weil *et al.*, 1975; Banatvala and Brown, 2004) and rarely, a progressive rubella panencephalitis is seen in older children with congenital rubella syndrome or those infected post-natally (Frey, 1997). However, a meningoencephalitis can also be seen in young adults (Aguado *et al.*, 1993). Outbreaks of rubella were seen frequently in Vietnam however, since 2014, vaccination has been included in the routine immunisation programme (Vynnycky *et al.*, 2016). A study in Mexico showed evidence of regional differences in seasonality however, the effect of seasonality on the virus in Vietnam is unknown (Vynnycky *et al.*, 2016; Metcalf *et al.*, 2011). In a study conducted in HCMC between 1996 and

2008, 2% of adults (n=6) had a CNS infection due to rubella. However, the authors only tested those with a rash and therefore this number is likely to be an underestimate (Tan le *et al.*, 2014). Rubella virus was not tested for in other studies of the aetiology of CNS infections in Vietnam (Le *et al.*, 2010; Taylor *et al.*, 2012; Ho Dang Trung *et al.*, 2012).

ORTHOMYXOVIRIDAE

INFLUENZA VIRUS

Influenza virus typically affects the respiratory system. CNS involvement is the most common extra-respiratory complication including encephalitis and meningitis (Ludlow *et al.*, 2016b). A study in Japan described most cases being associated with Influenza A (Morishima *et al.*, 2002) and in a study of children admitted to a hospital in HCMC, Vietnam, there was one case of encephalitis caused by influenza A which was the highly pathogenic influenza A (H5N1) (Le *et al.*, 2010; de Jong *et al.*, 2005). A study of adults admitted to a hospital in HCMC with CNS infections found that none were caused by Influenza A or B (Tan le *et al.*, 2014). Influenza A peaks in the winter in temperate regions when there is a reduction in absolute humidity (Shaman and Kohn, 2009). However, in tropical climates such as Vietnam, influenza-like illness and absolute humidity are positively correlated (Thai *et al.*, 2015). A seasonal influenza vaccine against the strain A/H1N1, A/H3N2 and B was licensed in Vietnam in 2019 (PATH, 2019).

FLAVIVIRIDAE

ZIKA VIRUS (ZIKV)

ZIKV is transmitted via the bite of infected *Aedes* mosquitoes however, humans can be infected via other routes (Petersen, Jamieson and Honein, 2016). These include vertical transmission during pregnancy (Calvet *et al.*, 2016), sexual transmission (Davidson *et al.*, 2016; Moreira *et al.*, 2017) and through the transfusion of blood products or organ transplantation (Motta *et al.*, 2016; Williamson *et al.*, 2017; Nogueira *et al.*, 2017).

The first confirmed human infection occurred in 1962 in Uganda with a few cases occurring Sub-Saharan Africa and Southeast Asia since (Baud *et al.*, 2017) and outbreaks in the western Pacific in 2007 (Duffy *et al.*, 2009) and 2013 (Musso, Nilles and Cao-Lormeau, 2014). An epidemic in Brazil in 2015 spread through Latin America and the Caribbean with outbreaks also occurring in Cape Verde, Singapore and Florida (Baud *et al.*, 2017). Modelling has suggested that the transmission of the virus is driven by the climatic conditions caused by El Niño and that seasonality depends on the region in world. For example in South America, peaks in the reproduction ratio are seen in the winter and spring and in Asia, the summer and autumn (Caminade *et al.*, 2017).

An association between infection during pregnancy, particularly during the first trimester, and subsequent microcephaly in infants, has been shown in Brazil and French Polynesia where ZIKV outbreaks coincided with an increase in congenital CNS malformations (Cauchemez *et al.*, 2016; de Araujo *et al.*, 2016; Kleber de Oliveira *et al.*, 2016). There have also been case reports of encephalitis in adults which are secondary to ZIKV (Soares *et al.*, 2016; Ugarte *et al.*, 2017; Mehta *et al.*, 2018). Since 2016, cases of ZIKV have been were reported in central and southern Vietnam and in travellers returning from Vietnam (Bui *et al.*, 2018; Chu, Ngoc and Tao, 2017; Hashimoto *et al.*, 2017). One case of microcephaly occurred in Dack Lak province in the Central Highlands (Moi *et al.*, 2017). However, serological evidence suggests that ZIKV was circulating at very low levels in southern Vietnam in 2013 (Quyen *et al.*, 2017) and neutralising antibodies against ZIKV were found in northern Vietnam in 1954 (Pond, 1963). There are no known published reports of Zika encephalitis in Vietnam.

RHABDOVIRIDAE

RABIES VIRUS

Rabies virus is normally transmitted via the saliva of an infected animal through contamination of a wound (e.g. bite or scratch) or mucus membrane. The virus can be carried either by a carnivore (known as terrestrial rabies) or bat (bat rabies) and 95% of cases occur in Asia and Africa (World Health Organization, 2018d). A study in in Lao PDR showed that the number of cases of rabies in dogs was higher during the dry season which corresponded with other studies showing increases in the

number of cases in the spring to coincide with increased contact between animals (Douangngeun *et al.*, 2017).

80% of cases of rabies present in an encephalitic form including autonomic dysfunction, hydrophobia, hyperexcitability and aerophobia (Leung, Davies and Hon, 2007). Pre and/or post-exposure vaccination and post exposure immunoglobulin are used to prevent rabies in humans (World Health Organization, 2018e) however, without these the disease is fatal (Jackson *et al.*, 2003). Post-exposure prophylaxis is available in Vietnam (Nguyen *et al.*, 2018) however, the virus still kills more than seventy people each year (World Health Organization, 2020h).

BACTERIA

STREPTOCOCCUS PNEUMONIAE

S. pneumoniae, a Gram-positive bacterium asymptomatically colonises the upper respiratory tract. It can result in an acute otitis media or pneumonia if it infects the middle-ear space and terminal airways respectively. It is transmitted between humans via contact with infected secretions with strains carried for weeks to months before being cleared (Kadioglu *et al.*, 2008). A study conducted on the Thailand-Myanmar border found that transmissibility of the virus in infants was highest during the cool and dry months (Numminen *et al.*, 2015). Those at highest risk of invasive disease include young children and those aged over 50 years, those with underlying medical conditions and use of drugs which compromise the immune system or those with damage to the naso and oropharyngeal mucosae for example due to a viral infection or local pneumococcal infection (Mook-Kanamori *et al.*, 2011). A prospective case series of adults with pneumococcal meningitis in the Netherlands found that 84% had a headache, 81% neck stiffness, 84% a fever and 85%, an altered mental state with a 30% CRF (Weisfelt *et al.*, 2006). Since 2009, polysaccharide-protein conjugate vaccines have been on the market and the WHO recommend the use of pneumococcal vaccines (PCV) in childhood immunisation programmes globally (World Health Organization, 2019g). The PCV has not yet been introduced in Vietnam (Le Polain De Waroux *et al.*, 2018). *S. pneumoniae* accounted for 6% (n=35) of CNS infections in adults and 6% (n=37) of children in a multi-centre study in Vietnam (Ho

Dang Trung *et al.*, 2012) and 2% (n=7) of CNS infections in adults in Hanoi (Taylor *et al.*, 2012).

NEISSERIA MENINGITIDIS

N. meningitidis is a Gram-negative bacteria which is a commensal in the nasopharynx of humans and is transmitted by aerosol or secretions (Rosenstein *et al.*, 2001) particularly during mass gatherings (World Health Organization, 2018c). Meningitis occurs when the bacteria spread to the CNS with young children and young adults most commonly affected (World Health Organization, 2018c). Symptoms include headache, fever and neck stiffness, with nausea, vomiting, photophobia and altered mental status occurring on occasion and in some cases, a meningococcal septicaemia with a petechial or purpuric rash (Rosenstein *et al.*, 2001). There are twelve serogroups of which six cause epidemics (A, B, C, W, X and Y). The largest burden of disease occurs in the meningitis belt in sub-Saharan Africa during the dry season from December to June. CFR in treated patients is 8-15%, rising to 50% in those untreated. Vaccines against serogroups A, C, W and Y are available and used both as part of routine immunisation schedules and during outbreaks with conjugate vaccines used in the former and polysaccharide in the latter. Vaccination against serogroup B is generally used during outbreaks. Antibiotics chemoprophylaxis may also be given to close contacts of cases (World Health Organization, 2018c). In Vietnam which has suffered a number of epidemics of meningococcal disease there is a requirement to notify all cases however, only a polysaccharide vaccine is available (Phan *et al.*, 2019). *N. meningitidis* was the cause of CNS infections in 0.6% (n=4) of adults and 1% of (n=6) children in the study by Ho Dang Trung et al., 2012 and 0.6% of adults (n=2) in the study by Taylor et al., 2012.

HAEMOPHILUS INFLUENZAE TYPE B

H. influenzae type b (Hib) is a Gram-negative bacterium (Robbins *et al.*, 1973). It is transmitted via oral and respiratory secretions (Murphy *et al.*, 1989) with invasive disease including meningitis mainly occurring in children. A study in Taiwan found that most cases of Hib meningitis occurred during the winter months (Shao *et al.*, 2004). Incidence has reduced globally greatly since the introduction of vaccination (World Health Organization, 2020d). The Hib vaccine was initiated in 2010 in Vietnam (Le *et al.*, 2015). Despite this, in the study by Ho Dang Trung et al. 2012 Hib was the most

common pathogen in children less than one year old (n=27, 41%) and accounted for 6% (n=39) of all infections in children. No infections were seen in adults.

MYCOBACTERIUM TUBERCULOSIS (MTB)

MTB is an intracellular bacteria transmitted via aerosolised droplets which can affect many organ but is primarily causes a pulmonary disease (tuberculosis (TB)) (Smith, 2003). Millions of people are infected with MTB every year with the highest burden in Asia and Africa (World Health Organization, 2020j). Peaks in incidence of TB are seen in northern Vietnam in the summer months with an association between sunlight and TB incidence at a lag of 6 months which has been seen in other countries (Bonell *et al.*, 2020).

1% of cases are tuberculous meningitis (TBM), with patients often presenting with a subacute meningitis starting with a low-grade fever and headache (Thwaites, van Toorn and Schoeman, 2013). TBM is thought to account for more than 100,000 new cases per year globally with a CFR of nearly 50% (Wilkinson *et al.*, 2017). The Bacillus Calmette-Guérin (BCG) vaccine has been shown to be efficacious in preventing childhood MTB (Trunz, Fine and Dye, 2006). The use of the BCG vaccine in the prevention of MTB in adults in unknown (Brancusi, Farrar and Heemskerk, 2012). In the study by Ho Dang Trung et al, 2012 MTB accounted for 5% (n=34) cases of CNS infections in adults and 2% (n=11) in children. In the study by Taylor et al., 2012 it accounted for 3% of the cases (n=9).

LEPTOSPIRA SPECIES

Leptospirosis, caused by the spirochaete *Leptospira* is a zoonotic disease. The pathogen is transmitted to humans via the urine of infected mammals, particularly rodents (Adler and de la Pena Moctezuma, 2010). The geographical distribution of leptospirosis is broad (Costa *et al.*, 2015) however, it has more recently emerged in Thailand (Wuthiekanun *et al.*, 2007) and Sri Lanka (Agampodi, Peacock and Thevanesam, 2009). Those who are resource-poor, including farmers from tropical regions are particularly at risk as are those who come into contact with animal reservoirs or contaminated environments (Costa *et al.*, 2015). However, epidemics are also reported in urban slums which provide environmental conditions for rodents

(Felzemburgh *et al.*, 2014; Ko *et al.*, 1999). Leptospirosis case numbers have been shown to positively correlate with rainfall in Thailand (Chadsuthi *et al.*, 2012) however, this is not always the case, as was demonstrated in Lao PDR (Dittrich *et al.*, 2015). The clinical manifestations of leptospirosis typically include fever, headache, myalgia, conjunctivitis and occasionally, a rash. Severe cases may result in renal failure which may be accompanied by hepatic failure and/or pulmonary haemorrhage (Adler and de la Pena Moctezuma, 2010; Costa *et al.*, 2015). CNS manifestations of leptospirosis have been reported in studies in Brazil and the Philippines (Romero, Blanco and Yasuda, 2010; Watt, Manaloto and Hayes, 1989) and accounted for 12% of adults and children in Lao PDR in whom a lumbar puncture was taken (Dittrich *et al.*, 2015). Of these patients, 45% had a meningoencephalitis, 23% meningitis without AES and 7% AES without meningitis (Dittrich *et al.*, 2015). A study in southern Vietnam found a seroprevalence of leptospirosis in 12.8% of children (Thai *et al.*, 2006) and a study in the Mekong Delta found a seroprevalence of 18.8% in those aged 15-60 years (Van *et al.*, 1998). However, the contribution of leptospirosis to CNS infections in Vietnam is unknown possibly because the pathogen is not frequently tested for.

RICKETTSIA TYPHI

Murine typhus caused by *R. typhi*, is transmitted through the faeces of infected fleas, either through bites to or an abrasion of the skin or via mucous membranes or he conjunctiva (Azad, 1990; Gasem *et al.*, 2009). The rodents, on which the fleas live, provide a reservoir (Azad, 1990). Although many patients present with fever, murine typhus can also cause a meningoencephalitis and contributed to up to 11% of cases of suspected CNS infection in a study in Laos (Dittrich *et al.*, 2015). Unlike scrub typhus, cases of murine typhus normally occur in urban areas (Vallee *et al.*, 2010; Hamaguchi *et al.,* 2015a) and a study in Lao PDR showed no effect of seasonality (Dittrich *et al.*, 2015). A study conducted in northern Vietnam found evidence of murine typhus in one-third (n=193) of patients with suspected rickettsiosis with 52.9% living in Hanoi and 72% being male (Hamaguchi *et al.,* 2015a). The contribution of *R. typhi* to CNS infection in Vietnam in unknown.

1.5 PATHOGENS WHICH ARE NOT KNOWN TO BE FOUND IN VIETNAM OR ITS NEIGHBOURING COUNTRIES BUT ARE PARTICULARLY IMPORTANT CAUSES OF CNS INFECTIONS GLOBALLY

CHIKUNGUNYA VIRUS (CHIKV)

CHIKV is an alphavirus of the family *Togoviridae*. It is transmitted to human by the bites of infected *Aedes* mosquitoes (Pialoux *et al.*, 2007). The first outbreak was reported in Tanzania from 1952-1953 (Robinson, 1955) followed by a large outbreak in India in 1964 (Myers *et al.*, 1965) and Thailand (Halstead *et al.*, 1969). However, since 2004 there has been an ongoing global epidemic in tropical and sub-tropical areas (Petersen and Powers, 2016). CHIKV also shares similar symptoms and signs with DENV including fever, myalgia, headache and rash. However, those with CHIKV are more likely to suffer from arthralgia (Staples, Breiman and Powers, 2009). Despite usually being a self-limiting illness, severe infection can occur with multi-system complications (Tandale *et al.*, 2009). Cases of CHIKV encephalitis have been described from La Réunion Island (Gerardin *et al.*, 2016), Tonga (Nelson *et al.*, 2014) and Brazil (Pereira *et al.*, 2017). A recent seroprevalence study conducted in Vietnam showed evidence of previous CHIKV transmission in central and southern Vietnam but no evidence of sustained transmission (Quan *et al.*, 2018). In a similar way to DENV, CHIKV also shows seasonality with transmission rates increasing in warmer and wetter weather (Johansson, 2015).

TICK-BORNE ENCEPHALITIS VIRUS (TBEV)

TBEV, a flavivirus is found throughout Asia and Europe and is maintained in an enzootic cycle between wild mammalian hosts such as rodents and Ixodid ticks (Charrel *et al.*, 2004; Ludlow *et al.*, 2016a; Wengler, Wengler and Gross, 1978; Mandl *et al.*, 1997). The infected tick transmits the virus whilst feeding on the host (Mansfield *et al.*, 2009) with the rodent host acting as a reservoir for the virus but also amplifying it (Suss, 2003) whereas humans and larger mammals such as deer and horses are a dead-end hosts (Mansfield *et al.*, 2009; Gritsun, Lashkevich and Gould, 2003). In addition to being infected via the bite of a tick, humans can also become infected via consumption of unpasteurized milk (Dumpis, Crook and Oksi, 1999; Ludlow *et al.*, 2016a). An increase in the number of cases of tick-borne encephalitis in Europe and Russia is thought to be due to a combination of climate change with the ticks moving to higher altitudes and latitudes and socio-political changes (Suss, 2008; Suss, 2011).

In Europe tick activity peaks from April until July (Randolph *et al.*, 2000) with cases of tick-borne encephalitis (TBE) seen in the summer months (Rezza *et al.*, 2015). 70% of infections are asymptomatic with a few developing a flu-like illness and of these, some progressing to a meningitis, encephalitis or meningoencephalitis (Ludlow *et al.*, 2016b).

WEST NILE VIRUS (WNV)

West Nile virus (WNV) is a flavivirus transmitted in an enzootic cycle between birds by *Culex* mosquitoes, transmitted to and other mammals through mosquito bites (Petersen, Brault and Nasci, 2013). The first described case occurred in Uganda in 1937 (Hayes, 2001). Epidemics of WNV which occurred in north America are thought to be influenced by the accumulation of ground water which provided breeding sites for the mosquitoes (Shaman, Day and Komar, 2010). 1 in 140 develop an encephalitis or meningitis with incidence higher in the elderly population (Ludlow *et al.*, 2016b).

1.6 NON-INFECTIOUS CAUSES OF CNS INFECTIONS

AUTOIMMUNE

A multicentre study in England found that 21% of patient with encephalitis (n=42) had an acute immune-mediated encephalitis. Of these, twenty-three had an ADEM, nine had antibodies against the N-Methyl D-Aspartate (NMDA) receptor, seven antibodies against voltage-gated potassium channels (VGKC) and the remaining were secondary to systemic vasculitis, multiple sclerosis or paraneoplastic (Granerod *et al.*, 2010a). ADEM normally occurs following a viral infection or vaccination, more commonly in children, which results in demyelination involving the brain and spinal cord (Tenembaum, 2013). A study in the US showed that more cases occurred in the winter and spring (Murthy *et al.*, 2002).

Patients with anti-NMDA receptor encephalitis typically present with headache, fever and psychiatric symptoms such as agitation, paranoia and hallucinations. A number also develop seizures and a reduction in conscious level (Dalmau *et al.*, 2008). Women account for approximately 80% of cases of whom around half have an underlying tumour which is frequently an ovarian teratoma (Kayser and Dalmau,

2011). A study in the US found that 78% of paediatric cases of anti-NMDA receptor encephalitis occurred from April-September.

Anti-VGKC encephalitis has since been divided in three categories. Those with leucine-rich glioma-inactivated1 (LGI1) antibodies often present with a limbic encephalitis with hyponatraemia and seizures whereas those with contactin-associated protein like 2 (Caspr2) may also have a limbic encephalitis but hyponatremia is rare. In both cases, patients are more likely to be male and of and older age. However, in the third group which are LGI1 and Caspr2 negative, half are male, all ages are affected, and presentation may include psychiatric symptoms, pain syndrome and cognitive decline (van Sonderen *et al.*, 2016).

In a study conducted in the Hospital for Tropical Diseases, HCMC over a period of eighteen months, twenty-four patients met the inclusion criteria for autoimmune encephalitis out of ninety-nine admitted with all-cause encephalitis. Nine patients tested positive for anti-NMDA receptor encephalitis. The median age of patients was 28 and 55.6% were female. All had prior admission to a mental health hospital, two had fever and seven had seizures. The authors suggested that this was an important differential diagnosis in patients admitted to hospital with encephalitis in Vietnam (Yen *et al.*, 2010) .

LYCHEE TOXICITY

Encephalitis thought to be related to harvesting lychees in May-July has been described in children in northern Vietnam where incidence of AES was independently associated with the proportion of lychee plantation surface (Paireau *et al.*, 2012b). A case-control study was conducted in northern India where an increased incidence of a neurological syndrome coincided with the lychee harvesting season. The study found that those who had eaten lychees were at higher odds of having an encephalopathy compared to those who had not and this effect was increased in those who had not consumed an evening meal. The encephalopathy was thought to be due to the effect of the hypoglycin A or methylenecyclopropylglycine (MCPG) toxins (Shrivastava *et al.*, 2017).

1.7 SUMMARY

There are multiple causes of CNS infections globally which are both infectious and non-infectious. This review gives an overview of the most common causes in Vietnam and neighbouring countries including Cambodia, Thailand and Lao PDR. Although clinical symptoms and signs may overlap, the epidemiology of the aetiologies differs greatly. Some pathogens are transmitted by mosquitoes and other arthropod vectors, with incidence showing seasonal patterns, some have a zoonotic reservoir and others are transmitting only from human to human. There is variation in the age distribution of cases and risk factors for acquisition of the pathogen. The aetiology remains unknown in many cases however, by understanding this epidemiology, we can start to consider the potential pathogens which may contribute to these cases which will form the basis of the results chapters.

CHAPTER 2 THE SPATIO-TEMPORAL DISTRIBUTION OF AND RISK FACTORS ASSOCIATED WITH AES AND MENINGITIS IN VIETNAM: AN ECOLOGICAL STUDY

2.1 INTRODUCTION

SPATIAL EPIDEMIOLOGY

Spatial epidemiology is defined as “the description and analysis of geographic variations in disease with respect to demographic, environmental, behavioural, socioeconomic, genetic and infectious risk factors” (Elliott and Wartenberg, 2004). In addition to considering place, the epidemiology of diseases also often considers time, referred to as spatio-temporal epidemiology (Waller and Gotway, 2004; Meliker and Sloan, 2011). Spatial statistics uses Tobler’s First Law of geography “everything is related to everything else, but near things are more related than distant things" (Tobler, 1970). This defines ‘spatial autocorrelation’ where pairs of spatially close observations are more similar than those which are far apart. The presence of spatial correlation in the residual of a model is a risk for the under-estimation of standard errors and too narrow CIs, thereby giving misleading results (Mohebbi, Wolfe and Jolley, 2011; Saas and Gosselin, 2014).

SPATIAL SCALES

Spatial models may use data from different scales (Meliker and Sloan, 2011). This might include data which are aggregated into units such as administrative zones (Bivand, Pebesma and Gómez-Rubio, 2013; Meliker and Sloan, 2011). The components of spatial data which have distinct properties and locations are referred to as ‘features’ where an administrative zone would be an ‘area’, the geographic location of a house, a ‘point’ and roads, a ‘line’ (Waller and Gotway, 2004). Where data is missing, the value may be obtained using measurements from surrounding locations, a process known as interpolation. A number of methods exist for interpolation with the most common including inverse-distance interpolation and Kriging (Waller and Gotway, 2004).

SOURCES OF SPATIAL DATA

Spatial data may be obtained from a variety of sources. Health data may come from notifiable disease surveillance systems, disease registries, death certificates and health surveys (Waller and Gotway, 2004). However, to predict where and when disease, or the potential for disease may occur, we may need to include explanatory variables such as environmental factors including temperature, precipitation or land use which may influence the survival of, for example, mosquito vectors (Ostfeld, Glass and Keesing, 2005; Keiser *et al.*, 2005). These may include remotely sensed data from satellite images, environmental and natural resource data such as air and water quality and climate and census data relating to counts of the population and housing (Waller and Gotway, 2004).

SPATIO-TEMPORAL DYNAMICS

Dynamic maps can be used to describe spatial patterns of disease over time, for example, how the incidence of disease changes within regions and where there may be disease hotspots (Meliker and Sloan, 2011). However, analytical methods may be used to investigate the spread of infection, known as “spatio-temporal dynamics” which can help to guide disease control interventions (Bayles *et al.*, 2017). Whilst not specific to spatio-temporal dynamics, these might be divided into statistical models, mathematical or mechanistic models and empirical or machine learning-based models. Statistical models might include regression (frequentist and Bayesian) and time series (Siettos and Russo, 2013, Lessler *et al.*, 2016, Aswi *et al.*, 2018, Raghavan *et al.*, 2014). Wavelet analysis is one form of time series analysis used to characterise the periodicity of non-stationary epidemiological time series (Grenfell, Bjornstad and Kappey, 2001) allowing it to detect sudden changes in periodic patterns of time series (Cazelles, Cazelles and Chavez, 2014). Examples of wavelet analysis include the evaluation of change in the periodicity of incidence of DENV in time and space and also its relationship with climate and the El Niño-Southern Oscillation (ENSO) (Thai *et al.*, 2010); the seasonality of influenza in Uganda (Yang *et al.*, 2018) and Brazil (Alonso *et al.*, 2007) and the movement of measles epidemics from urban to rural areas in United Kingdom in the 1950s and 60s areas (Grenfell, Bjornstad and Kappey, 2001). Mathematical models commonly include the deterministic or stochastic susceptible-infectious-recovered (SIR) model or other variations of it such as the susceptible-exposed-infectious-recovered (SEIR) model. Examples of these

models have been used to predict the transmission of, most recently, SARS-CoV-2 (Kucharski *et al.*, 2020) but also severe acute respiratory syndrome (SARS) (Riley *et al.*, 2003) and measles (Becker et al., 2016). Other mathematical models include individual-based models (IBMs) such as agent-based models (ABMs) which have been used to look at the effect of social mixing patterns and mobility on vaccine-preventable diseases and how those might, for example, affect vaccination programmes (Willem *et al.*, 2017). Other examples of mathematical models used in infectious disease dynamics include complex networks and stochastic Markov Chain (Siettos and Russo, 2013). Finally, machine learning-based models might include web-based data mining or surveillance networks (Siettos and Russo, 2013). An example of a machine learning method includes the use of search queries on the Google platform to detect symptoms of influenza to detect an early epidemic (Ginsberg *et al.*, 2009).

REGRESSION MODELS

Regression models which are used for non-spatial data such as logistic, linear and Poisson regression can be applied to spatial analysis provided these take into account spatial autocorrelation if this demonstrated in the residuals. As health data normally includes counts, or the presence or absence of a disease, Poisson or negative binomial or logistic regression may be used respectively. In a paper by Saas and Gosselin (2014) which compared regression methods for spatially autocorrelated count data found that non-spatial Poisson generalised linear models (GLMs) performed the worst with generalised linear mixed models (GLMMs) and generalised additive mixed models (GAMMs) with independent location-specific random effects and quasi-Poisson non-spatial GLMs showing a better fit however, the optimal models are those which had spatially-structured location-specific random effects. The spatial dependencies in these models included neighbourhood-based correlated random effects or distance-based random effects (Saas and Gosselin, 2014). The GLMM and GAMM can include Gaussian random effects however, these may not allow for hierarchical structure (Waller and Gotway, 2004). Instead, methods used in Bayesian statistics can be applied such as Markov chain Monte Carlo (MCMC) algorithms and Integrated Nested Laplace Approximation (INLA) with the latter being computationally faster (Saas and Gosselin, 2014).

CLUSTERING

The analysis of spatial point patterns is used to identify locations where there may be clusters of e.g. cases with a particular disease (Waller and Gotway, 2004). This is particularly important in detecting outbreaks (Unkel *et al.*, 2012) but also used to understand the spatio-temporal transmission of disease (Ruiz-Moreno *et al.*, 2010). A common method for determining clusters is the kernel density estimation which either estimates a 'density function' (the probability of observing an event at a location) or the probability of the number of events expected per unit area at a location (the 'intensity function'). Other methods for detecting clusters include Poisson cluster processes, Cox processes and contagion/inhibition processes (Waller and Gotway, 2004).

THE SPATIO-TEMPORAL MODELLING OF CNS INFECTIONS

The spatio-temporal modelling of CNS infections can be used to help guide where and when to implement disease prevention strategies and allocate public health and diagnostic resources. Examples of these studies are given below.

Data from JE cases in Nepal, confirmed using an anti-JE IgM antibody capture ELISA was obtained from the AES surveillance unit of the WHO Programme for Immunization Preventable Disease (IPD). This was used to determine the temporal pattern and geographic distribution of cases including where there was evidence of clustering. Spatial interpolation of the incidence of JE in each district was performed using an inverse distance weighing (IDW) method. The IDW method creates a smoothed surface which gives increased weighting to closer locations. Spatial autocorrelation was assessed using Moran's Index with maps showing clusters of high and low incidence of JE based on Local Indicators of Spatial Autocorrelation (LISA). High-high incidence clusters (where the surrounding areas had a similarly high incidence) and were found in the Terai region, southern Nepal with low-low incidence clusters (those where the surrounding areas also had a low incidence) in the mountainous districts in the northwest (Kumar Pant *et al.*, 2017). A separate study, also conducted in Nepal, used surveillance data to compare the spatio-temporal dynamics of non-JE AES, JE and an unknown viral encephalopathy. In addition to comparing the spatial and temporal patterns of the groups, geographically weighted regression models were used to show associations of JE and AES with landscape

variables. Irrigated land was positively associated with JE and AES and forest, negatively (Robertson *et al.*, 2013).

Many of the spatio-temporal studies of meningitis originate in sub-Saharan Africa. A study conducted in Niger evaluated spatial and spatio-temporal clustering of meningococcal meningitis from 2002 to 2009. The authors initially looked for the presence of geographical clusters occurring within the same year. The Anselin's Local Moran's I test was used to determine spatial autocorrelation between each health centre catchment area (HCCA) and its neighbours which were classified as high-high (high incidence HCCA surrounded by high incidence rate HCCAs) and high-low (high incidence HCCA surround by low incidence rate HCCAs).

The Kulldorff's spatial scan statistic using the SaTScan software was then used to detect clusters of cases of meningococcal meningitis situated together in space and time whilst using the alert threshold for epidemic detection based on the number of cases per 100,000 inhabitants. It was found that most clusters occurred the in the southern districts with epidemics occurring in different HCCAs each year (Paireau *et al.*, 2012a). In a separate study of meningococcal meningitis conducted in Nigeria and Niger between 2013 and 2017, the Global Moran's I statistic and Anselin's local Moran's I to locate clusters to inform the vaccination policy used to manage outbreaks. There was evidence clustering of high incidence of meningococcal meningitis in different districts each year but the authors suggested that there was no need to change their response methods to epidemics for example by lowering the incidence threshold for reporting or vaccinating neighbouring districts (Cooper *et al.*, 2019).

A number of epidemiological studies have been conducted which describe the spatio-temporal distribution of CNS infections in Vietnam. Yen et al., 2010 analysed national surveillance data from 1998 to 2007. Temporal differences were seen between different regions with evidence of seasonality in northern but not southern Vietnam with peaks in incidence in the summer months in northern Vietnam. Mean annual incidence of AES was highest in the provinces of Lai Chau, Son La, Dien Bien, Bac Giang and Lang Son in northern Vietnam and Binh Duong and Bac Lieu in southern Vietnam. In 2004-2005, five provinces in northern Vietnam: Bac Giang, Hai Duong, Hai Phong, Thai Binh and Thanh Hoa also conducted testing for anti-JEV IgM in sixty

percent of cases. It was unknown how these cases were selected however, 52% were positive for JEV ranging from 17% in Bac Giang to 71% in Thai Binh.

A study conducted in three central hospitals in HCMC from 2005-2015 found used GAMM to examine the spatio-temporal distribution and risk factors for paediatric CNS infections excluding TBM as defined by ICD-10 criteria. Data from 9469 cases showed that the highest incidences of both presumed bacterial and non-bacterial infection (BI and non-BI, respectively) were seen in the urban districts close to the hospitals but also some districts further away. Incidence of bacterial infections was highest in the dry season from December to April with a relatively strong positive association with a one-month lag in temperature (RR=1.73, 95%CI 1.22-2.45 and RR=2.83, 95%CI 1.77-4.53, respectively). However, positive associations were also seen between BI and the average monthly rainfall (RR=1.04, 95%CI 1.01-1.06) and between non-BI and rainfall at lags of 1 and 3 months (RR=1.05, 95%CI 1.02-1.09 and RR=0.96, 95%CI 0.93-0.99, respectively). The authors suggested that the findings may explain the smaller peak of BI during the later months of the rainy season but do not comment on the non-BI (Ho *et al.*, 2017).

THE SURVEILLANCE OF COMMUNICABLE DISEASES

Public health surveillance is defined as the 'ongoing systematic collection, collation, analysis and interpretation of data and dissemination in order for action to be taken'. It is serves as an early warning system to identify public health emergencies, to guide public health policy and to understand the impact of an intervention towards public health goals (World Health Organization, 2019d). Different types of surveillance include national active surveillance (accelerated disease control), passive surveillance and sentinel surveillance (World Health Organization, 2019a). Visiting health facilities to review medical records and talk to health-care providers is defined as active surveillance and is normally used when a disease is a target for elimination or eradication (World Health Organization, 2019b). The notification of cases from healthcare providers including healthcare centres and laboratories to a higher administrative level is passive surveillance (World Health Organization, 2019c). Sentinel surveillance requires selected units to report cases of specific diseases which may not be detected using the WHO passive system. This requires a high-quality diagnostic laboratory (World Health Organization, 2019e).

THE SURVEILLANCE OF COMMUNICABLE DISEASES IN VIETNAM

The General Department of Preventive Medicine (GDPM), a department of the Ministry of Health (MoH), Vietnam conducts a number of activities including the prevention and control of communicable diseases and diseases of unknown cause (Ministry of Health Viet Nam, 2018). The surveillance system for notifiable diseases was set up in 1979 and became web-based in 2009 (personal communication, Marc Choisy). There are currently fifty-seven notifiable diseases (Vietnam General Department of Preventive Medicine, 2016) which hospitals and clinics need to be report within twenty-four hours to the provincial preventive medicine centres (PMCs). The PMCs then report these on either a daily, weekly or monthly basis to the GDPM depending on the disease (Binh *et al.*, 2013; Phung *et al.*, 2018). In addition to the known pathogens, severe viral respiratory infections and dangerous emerging diseases of unknown cause must also be reported (Binh *et al.*, 2013). Sentinel site surveillance is undertaken for pathogens including rotavirus (Van Man *et al.*, 2005); influenza virus (Nguyen *et al.*, 2009) and JEV (Yen *et al.*, 2010).

THE SURVEILLANCE OF AES IN VIETNAM

AES is one of the notifiable diseases in Vietnam and for reporting purposes, is called 'viral encephalitis'. It is defined clinically as "Fever great than 38°C and a change in mental status, seizures, abnormal movements, tremor or spastic paralysis". The district health centres reported the number of cases and deaths of AES on a weekly basis to the provincial health authorities who then report these to the regional surveillance centres (northern, central, central highlands and southern) on a monthly basis. The regional surveillance centres then report cases and deaths to the National Institute of Hygiene and Epidemiology (NIHE) and the GPDM annually (Yen *et al.*, 2010). The GDPM reports to do not include the aetiology of AES however, sentinel site surveillance for meningoencephalitis in children aged 15 years and younger exists at two hospitals: the National Children's Hospital in Hanoi and the Children's Hospital No.1 in Ho Chi Minh City. The case definition for suspected meningoencephalitis (ME) includes: a sudden onset of fever (≥38ºC), headache, seizures, vomiting, altered consciousness, a change in behaviour, neck stiffness, a bulging fontanel (in infants less than 12 months of age) and also atypical signs in infants such as "poor sucking" and "not doing well" (WHO, personal communication). Cases of suspected ME are tested for anti-JEV IgM using enzyme-linked immunosorbent assays.

THE SURVEILLANCE OF MENINGITIS IN VIETNAM

The case definition for meningococcal meningitis in Vietnam is “the sudden onset of high fever, severe headache, nausea and vomiting and a stiff neck with possible haemorrhagic lesions” and viral meningitis “a sudden onset of high fever (39-40ºC), headache, disorderly movement and confusion (Binh *et al.*, 2013). The sentinel site surveillance for meningoencephalitis defines cases of probable bacterial meningitis as children less than 5 years old with more than one of the following cerebrospinal fluid findings: appearance turbid or cloudy; white blood cell count >100/mm^3; white blood cell count 10-100/mm^3 and protein>100mg/dl; WBC 10-100/mm^3 and Glucose<40mg/dl. Confirmed bacterial meningitis is defined as a case of probable bacterial meningitis with the following laboratory findings: *H. influenzae*, *S. pneumoniae*, or *N. meningitidis* isolated in the CSF by bacterial culture, latex agglutination or PCR; or *S. pneumoniae* positive by the Binax immuno-chromatographic test for the presence of the antigen.

THE SPATIO-TEMPORAL DISTRIBUTION OF CNS INFECTIONS IN VIETNAM

JUSTIFICATION FOR THE RESEARCH AND QUESTIONS TO BE ADDRESSED

A number of studies describe the spatio-temporal distribution of CNS infections in Vietnam however, none have used the most recent national surveillance data. Additionally, although clinical studies showed that Japanese encephalitis virus (JEV) is the most common cause of CNS infections in children in Vietnam (Ho Dang Trung *et al.*, 2012; Le *et al.*, 2010) and *Streptococcus suis*, the most common cause in adults (Ho Dang Trung *et al.*, 2012; Taylor *et al.*, 2012); the cause remained unknown in half of the cases or more (Ho Dang Trung *et al.*, 2012; Le *et al.*, 2010; Tan le *et al.*, 2014; Taylor *et al.*, 2012).

This study aims to continue the work by Yen *et al.*, 2010 using more recent surveillance data for AES but also to combine this with surveillance data for meningitis and sentinel site data for JE to provide a full spatio-temporal analysis of CNS infections in Vietnam. Additionally, the work uses explanatory variables and

surveillance data from other pathogens or syndromes which have the potential to cause CNS infections to suggest possible causes of CNS infections of unknown aetiology in Vietnam using multivariate mixed models.

The objectives to be addressed are as follows:

1. To compare and contrast the spatio-temporal distribution of AES and meningitis in Vietnam with other syndromes and pathogens.

2. To estimate population level risk factors for AES and meningitis in Vietnam.

3. To determine the potential effect of the JE vaccine on the incidence of AES and meningitis in Vietnam.

4. To use the results from questions 1, 2 and 3 to estimate the aetiology of AES and meningitis in Vietnam.

2.2 METHODS

DATA ACQUISITION

INCIDENCE DATA

Data on monthly incidence of AES and meningitis (using the case definition for meningococcal meningitis) at the provincial level was obtained from the GDPM. The R package 'gdpm' (Choisy and Contamin, 2019b) contains monthly data of selected notifiable diseases at the provincial level from 1st January 1980 to 31st December 2016. The list of diseases is shown in table 2.1

Table 2.1 The list of diseases in the GDPM

Adenovirus	Amoebiasis	Anthrax
Chickenpox	Cholera	Dengue
Diarrhoea	Diphtheria	Dysentery
Encephalitis	H5N1 influenza	Hepatitis
Hand foot and mouth disease (HFMD)	Influenza-like-illness (ILI)	Leptospirosis
Malaria	Measles	Meningitis
Mumps	Neonatal tetanus	Pertussis
Plague	Polio	Rabies
Rubella	Shigella	*S. suis*
Tetanus	Typhoid	

In addition to surveillance data for encephalitis and meningitis, surveillance data for pathogens and syndromes which were known causes of CNS infection in Vietnam or countries within the region and may exhibit some evidence of seasonality which could be compared to cases of AES were also obtained from the GDPM. These included: dengue (Le *et al.*, 2010; Tan le *et al.*, 2014; Ho Dang Trung *et al.*, 2012; Thai *et al.*, 2010), influenza-like-illness (ILI) as a proxy for influenza (Morishima *et al.*, 2002; Thai *et al.*, 2015), *S. suis* (Mai *et al.*, 2008; Wertheim *et al.*, 2009b), measles (Fox *et al.*, 2013; Moss, 2017), leptospirosis (Dittrich *et al.*, 2015; Chadsuthi *et al.*, 2012) and hand, food and mouth disease (enterovirus 71) (HFMD) (Le *et al.*, 2010; Chang *et al.*, 2012; Kim *et al.*, 2016). Other pathogens within the list also have the potential to cause CNS manifestations however, have either been reported less widely as aetiologies in Vietnam or the region or were unlikely to have a seasonal pattern of incidence and so less likely to be contributing to many of the unknown aetiologies of AES.

The case definitions for the each of the pathogens are given below (Binh *et al.*, 2013). *S. suis* is reported based on the confirmation of culture (Huong *et al.*, 2019).

Dengue A high fever above 38ºC for 2-7 days; headache, muscle and joint pain, periorbital pain, congestion, skin rash, signs of bleeding or signs of shock; influenza as a sudden onset of fever (39-40 ºC), severe headache, body, muscle and joint pain, runny nose, sore throat or coughing.

ILI A measured fever of ≥38°C and cough; with the onset within the last 10 days (World Health Organization, 2014)..

Suspected measles Fever with a least one of the following symptoms: coughing, runny nose, conjunctivitis or rash.

Leptospirosis A sudden onset of high fever, headache, chills, malaise, myalgia (especially in the calves and thighs), conjunctival effusion, renal failure, arrhythmias, jaundice or rash.

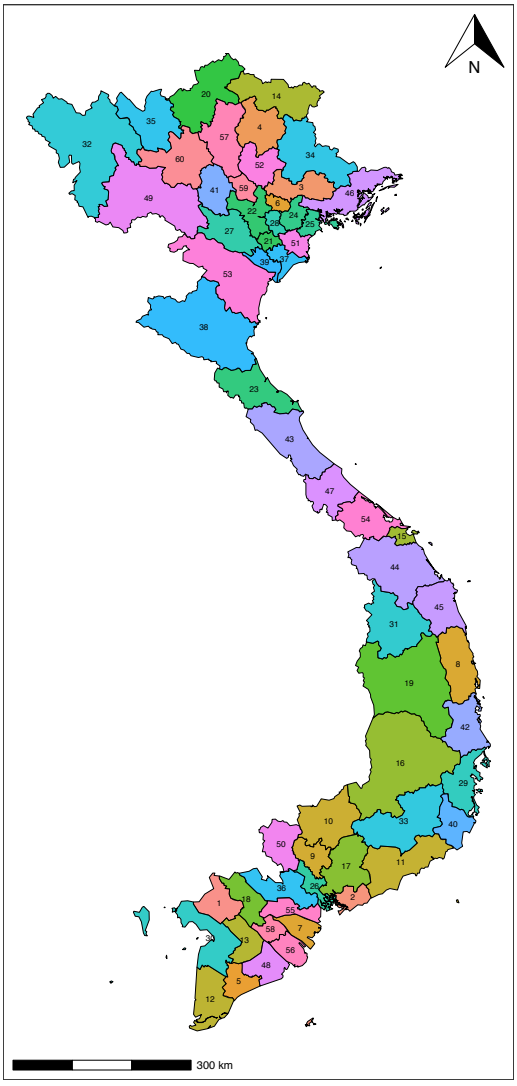
HFMD A brief febrile illness in children accompanied by a typical skin rash, with or without mouth ulcers. The rash is papulo-vesicular occurring on the palms or soles of the feet, or both. In young children or infants, the rash may be maculo-papular without vesicles and may also involve the buttocks, knees or elbows (Nguyen *et al.*, 2014).

Due to missing data in the earlier years, it was decided to include from 1998 onwards. Data was available from 1998 until 2016 for all pathogens/syndromes with the exception of HFMD and *S. suis* where data was available from 2011 only. Sentinel site data for JE and PBM were provided by the Expanded Programme for Immunization, WHO, Hanoi from June 2011 to August 2017. The month and year of onset, the province of the home of the patient, the hospital site and the diagnosis (JE or PBM) were the only data used for the analysis.

THE PROVINCES IN VIETNAM

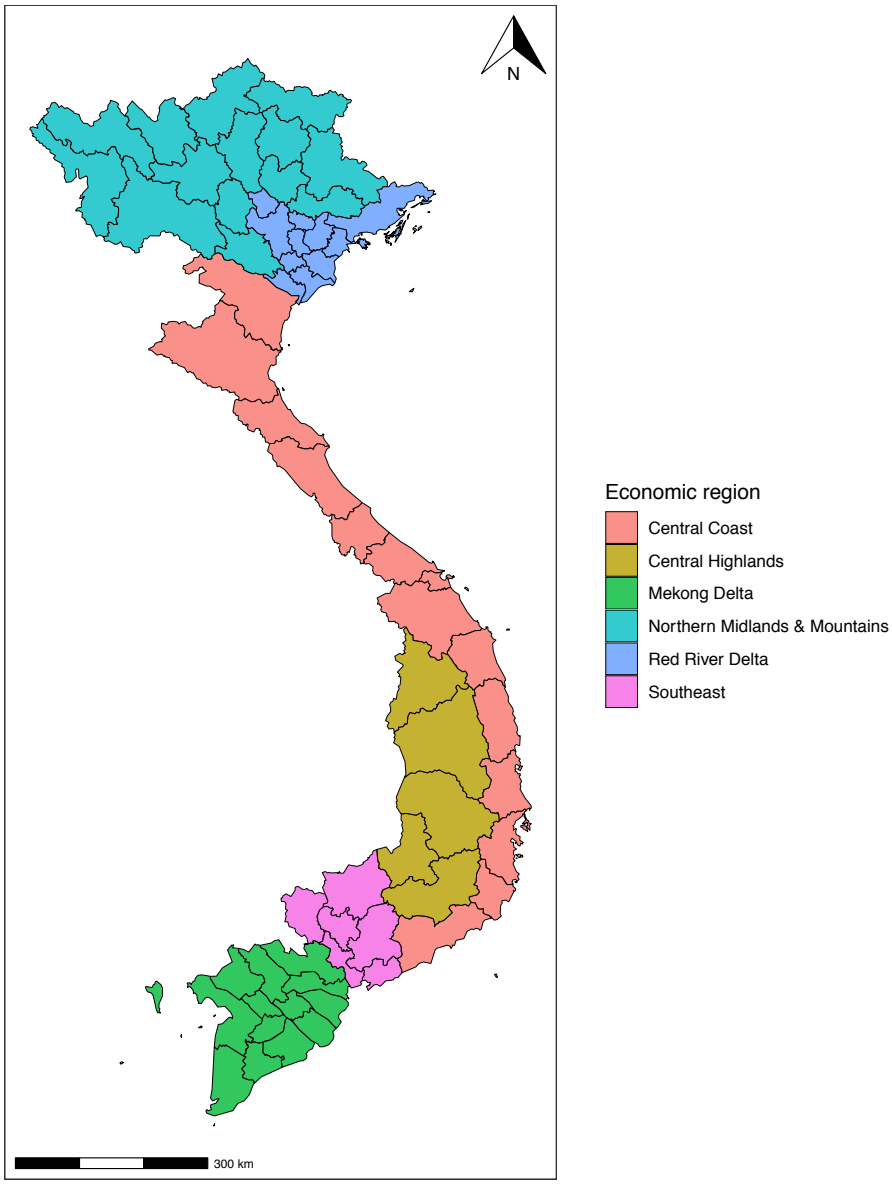
The R package ‘gadmVN’ (Choisy and Contamin, 2019a) was used to obtain the maps of the provinces of Vietnam. As provinces have merged and split over time, the maps used include the provinces in 1998 with Hanoi merged with Ha Tay (figure 2.1). The economic regions of Vietnam are shown in figure 2.2.

Figure 2.1 The provinces of Vietnam in 1998.



An Giang	1	Kon Tum	31
Ba Ria - Vung Tau	2	Lai Chau	32
Bac Giang	3	Lam Dong	33
Bac Kan	4	Lang Son	34
Bac Lieu	5	Lao Cai	35
Bac Ninh	6	Long An	36
Ben Tre	7	Nam Dinh	37
Binh Dinh	8	Nghe An	38
Binh Duong	9	Ninh Binh	39
Binh Phuoc	10	Ninh Thuan	40
Binh Thuan	11	Phu Tho	41
Ca Mau	12	Phu Yen	42
Can Tho	13	Quang Binh	43
Cao Bang	14	Quang Nam	44
Da Nang	15	Quang Ngai	45
Dak Lak	16	Quang Ninh	46
Dong Nai	17	Quang Tri	47
Dong Thap	18	Soc Trang	48
Gia Lai	19	Son La	49
Ha Giang	20	Tay Ninh	50
Ha Nam	21	Thai Binh	51
Ha Noi	22	Thai Nguyen	52
Ha Tinh	23	Thanh Hoa	53
Hai Duong	24	Thua Thien Hue	54
Hai Phong	25	Tien Giang	55
Ho Chi Minh	26	Tra Vinh	56
Hoa Binh	27	Tuyen Quang	57
Hung Yen	28	Vinh Long	58
Khanh Hoa	29	Vinh Phuc	59
Kien Giang	30	Yen Bai	60

Figure 2.2 The economic regions of Vietnam.



Climatic data

Climatic variables have been shown to be correlated with the incidence of Japanese encephalitis in humans with positive correlations seen between JE and temperature and relative and absolute humidity (Lin *et al.*, 2017; Tian *et al.*, 2015). The association with rainfall varies with a study in China showing a positive association of rainfall with the number of JE cases (Tian *et al.*, 2015) however, during an epidemic in Nepal and a different study in China, JE cases were negatively associated with rainfall (Impoinvil *et al.*, 2011; Bai *et al.*, 2014). Similarly, climatic variables have been shown to be correlated with the incidence of other pathogens and syndromes which have the potential to cause CNS infections including dengue (Do *et al.* 2014), leptospirosis (Sumi *et al.*, 2017) and ILI (Thai *et al.*, 2015). In the case of JE and DENV, this is likely to be due to the dependence of the mosquito vectors on temperature and humidity and rainfall for their development and survival (Reisen *et al.*, 2008). The transmission of leptospirosis is associated with flooding as human behaviour such as walking barefoot in stagnant water may increase the risk of exposure to the bacteria (Kawaguchi *et al.*, 2008). ILI in Vietnam is positively associated with absolute humidity (Thai et al., 2015) however, in temperate climates, influenza incidence peaks in the winter possibly due to indoor crowding (Tamerius *et al.*, 2011).

Climatic data was obtained from sixty-seven weather stations situated in Vietnam including monthly means for daily absolute (g/L) and relative (%) humidity, daily minimum and maximum and average temperatures (ºC) and cumulative precipitation (mm) and hours of sunlight (Bonell *et al.*, 2020). In this study, the data from the weather stations was used to impute missing values on a 10,000-cell regular grid using the method of interpolated called Kriging. Kriging, developed by DG Krige in the 1950s is a Gaussian process where interpolation is used to predict unknown values (van Beers and Kleijnen, 2003). The interpolations are weighted averages of neighbouring measures (Bonell *et al.*, 2020). The method for Kriging utilises a variogram, a diagram which shows the variation between two locations in terms of the difference between their measurements (van Beers and Kleijnen, 2003). In this study, Kriging used the function autoKrige() from the R package ‘automap’ which automatically tunes the hyperparameters of the estimation (Hiemstra *et al.*, 2008). As demonstrated in a previous study, this Kriging process, performs well with the

exception of the rainfall data which produces some extreme outliers, most likely due to variation of this variable in space. As a result of this, any climatic variables which were extreme outliers were removed from the dataset.

In order to compute climatic variables which were representative of the distribution of the population within the provinces (polygons), weighted averages of the population density were taken for each grid whereby the magnitude of population density was directly proportion to the climatic variable. The population densities were computed from the WorldPop project 2009 raster file which has a resolution of 100m (Lloyd, Sorichetta and Tatem, 2017) using the R packages 'sptools' (Choisy and Contamin, 2019e), 'magrittr' (Milton Bache and Wickham, 2014), 'purrr' (Henry and Wickham, 2019) and 'raster' (Hijmans, 2019b). Computed versions of the climatic variables were all lagged by one and two months, and rainfall by three months based on the possibility that preceding climatic conditions can influence a later peak in transmission of pathogens (Hsu, Yen and Chen, 2008; Bai *et al.*, 2014). Lag times beyond three months are deemed not biologically plausible for infections caused by vectors (Jacups *et al.*, 2008).

Normalised difference vegetation index (NDVI)

NDVI is used to estimate the density of land which is green. It is calculated from the light reflected by vegetation including visible (VIS) and near-infrared (NIR) (NDVI = (NIR-VIS)/(NIR+VIS) where chlorophyll strongly absorbs VIS and the cell structure of the leaves strongly reflects NIR (NASA: Earth Observatory, 2000). The NDVI ranges from -1 to +1 where the higher the value, the more biomass of photosynthetically active vegetation exists. Negative values refer to non-biomass e.g. clouds, water or snow (Helbich, 2019). The NDVI of rice paddy has been shown be a positively related to both mosquito density and the number of Japanese encephalitis cases in China (Tian *et al.*, 2015).

The NDVI rasters for each month over the analysis time period were obtained from the National Centers for Environmental Information (NOAA) (NOAA: National Centers for Environmental Information, n.d.). The NDVI for each province for each month was extracted and weighted by population density as explained for the climatic variables.

JE Vaccination coverage

Data of coverage of three doses of vaccination against JEV by province and year was provided by the National Institute of Hygiene and Epidemiology (NIHE). Vaccination campaigns provide two doses of the domestically produced, inactivated mouse brain-derived JE vaccine as part of the expanded programme on immunisation (EPI) 1-2 weeks apart followed by a booster dose one year later (Yen *et al.*, 2010).

Poverty, hospitals and number of pigs

Data of the poverty rate, number of hospitals and number of pigs per province, per year was provided by the General Statistical Office of Vietnam (General Statistics Office of Viet Nam, 2010) and extracted using the R packages 'gso' (Choisy and Contamin, 2019c) and 'gdpm' (Choisy and Contamin, 2019b). For the purpose of analysis, missing data was imputed using the R package 'mice' (van Buuren and Groothuis-Oudshoorn, 2011) which imputes the missing data based on the values in the other columns (Multivariate Imputation by Chained Equations) (RDocumentation, n.d.-a); the number of hospitals was given per 1000 km^2 to provide a proxy measure of accessibility to healthcare and the number of pigs per 100,000 human population to provide a proxy measure of proximity between humans and pigs which may be associated with the risk of JE (Liu *et al.*, 2010).

Elevation

The elevation data for each province was obtained from the National Aeronautics and Space Administration (NASA) Shuttle Radar Topographic Mission (SRTM) digital elevation models (DEM) available from the Consortium of International Agricultural Research Centres Consortium for Spatial Information (CGIAR-CSI) GeoPortal (CGIAR-CSI, 2018). The R package 'srtmVN' contains a raster file of the DEM for Vietnam at a resolution of 90m (Choisy and Contamin, 2019f). The mean elevation weighted by population density was extracted for each province.

Table 2.2 The datasets used, their unit of time and source.

Dataset	Unit of time	Spatial unit	Source	Year	Resolution
Number of cases of pathogens/syndromes	Monthly	Province	GDPM	1998-2016	Province
Climatic variables	Monthly	Weather stations	IMHEN	1998-2016	NA
NDVI	Monthly	Pixel	NOAA	1998-2016	0.05°
Elevation	Fixed	Pixel	CGIAR-CSI	2018	90m
Population density	As needed for the covariate for which it is weighted	Pixel	WorldPop	2009	100m
Population	Yearly	Province	GSO	1998-2016	NA
Number of pigs	Yearly	Province	GSO	19998-2016	NA
Number of hospitals	Yearly	Province	GSO	2005-2016	NA
Poverty rate	Yearly	Province	GSO	2006, 2008, 2010, 2012-2016	NA
JE vaccination coverage	Yearly	Province	NIHE	1998-2016	NA

ANALYSIS

All analysis was conducted using R statistical programming software version 3.6.1.

DESCRIPTIVE ANALYSIS

Time series plots showing the national monthly incidence of each of the syndromes/pathogens including AES, meningitis, dengue, ILI, *S. suis*, measles, leptospirosis and hand foot and mouth diseases were plotted using the R functions ts() and plot(). The function ts() is used to create time-series objects for example from a vector (RDocumentation, n.d.-f) and plot() is used for plotting objects to create graphics (RDocumentation, n.d.-b). The times series were decomposed using the function stl() with the features of decomposition extracted using the function feat_stl(). The stl() function decomposes a time series into seasonal, trend and irregular components. This is done using Loess (RDocumentation, n.d.-d). Loess is a locally weighted regression which is based on moving averages for time series (Cleveland and Devlin, 1988). The feat_stl() function extracts the features of the time series including the strength of the trend and the seasonality (RDocumentation, n.d.-e). The strength of both the trend and seasonality are given on a scale of 0 to 1 with zero exhibiting almost no seasonality or trend (Hyndman and Athanasopoulos, 2018).

Given the difference in acquisition of data for JE, a time series plot of the combined incidence of JE at the hospital sites was compared with the incidence of AES for cases arising from the same provinces from 2012 until 2016.

Choropleth maps of the mean monthly incidence of each of the syndromes/pathogens and heatmaps of the mean monthly and yearly incidences by province were constructed using the R packages ‘ggplot2’ (Wickham, 2016), ‘viridis’ (Garnier, 2018), ‘ggspatial’ (Dunnington, 2018) and ‘ggpubr’ (Kassambara, 2019).

UNIVARIATE ANALYSIS

Separate negative binomial generalised linear models (GLM) with log population fitted as an offset were constructed to determine the association between time fitted as a linear continuous predicator (months 1 to 288); month as a categorical covariate and year also as a categorical covariate with the number of cases of AES and meningitis nationally using the R package 'MASS' (Venables and Ripley, 2002).

The same models were run to determine the effect of seasonality on the number of a cases of AES and meningitis using annual, bi-annual and quarterly harmonic terms as covariates including: sin(2*pi*month/12)), sin(4*pi*month/12)) and sin(8*pi*month/12)) respectively.

Spatial autocorrelation amongst the residuals from the models was tested determined using the Monte-Carlo simulation of Moran I using 1000 iterations to allow for convergence using the R package 'spdep' (Bivand, Pebesma and Gomez-Rubio, 2013). The association between year and the number of cases of AES and seasonality and the number of cases at the level of province were shown as choropleth maps where the p value of the estimate was <0.05.

Negative binomial GAM models, again with log population fitted as on offset were then constructed to determine the association of each of the covariates with the number of cases (incidence) of AES and meningitis per month as follows: mean maximum and minimum temperatures (°C), mean absolute and relative humidities (g/m^3 and %, respectively); mean rainfall (mm), mean sunshine (hours) and mean NDVI with a lag of one and two months and in the case of rainfall, three months; mean elevation (m), number of pigs per 100,000 human population, number of hospitals per km^2, poverty rate (%) and coverage with three doses of JE vaccination (%); in addition to the incidence per 100,000 population of meningitis, dengue, *S. suis*, measles, ILI, leptospirosis and HFMD. Month and year (as linear predictors) were fitted as smoothed terms using the 's' function in the R package 'mgcv' to account for non-linearity of these covariates. The 'mgcv' package is used to create GAMs and GAM mixed models (GAMMs) using multiple smoothing parameter estimations (Wood,

2019). The ‘s’ function is a thin plate regression spline which does not have ‘knots’ and is the default smoothing function as it is optimal for any given basis dimension/rank (Wood, 2003; R Documentation, n.d.).

Given the evidence for significant spatial autocorrelation amongst the residuals in each of the univariate GAM models, the analysis was repeated using Integrated Nested Laplace Approximation (INLA) negative binomial models. INLA uses a Bayesian approach whereby the probability of an event is based on prior and current data to derive the posterior distribution (Blangiardo *et al.*, 2013). Linear regression models such as generalised linear mixed models (GLMMs) and GAMMs can be used to produce spatial models which account for spatially autocorrelated data (Waller and Gotway, 2004). These frequentist approaches can do this by for example, using distance-based or neighbourhood-based correlated random effects (Saas and Gosselin, 2014). However, adding these to the model can be a complex process. The R package ‘R-INLA’ (Rue, Martino and Chopin, 2009) eliminates some of this complexity by allowing the construction of spatial neighbourhood matrices (Moraga, 2019). In addition to INLA, Markov Chain Monte Carlo (MCMC) methods can be used to compute spatial Bayesian models where a Markov chain (a sequence of random values) is used to compute the posterior distribution (Waller and Gotway, 2004; Diggle and Ribeiro 2007). However, unlike INLA, MCMC methods can be computationally intensive to perform (Saas and Gosselin, 2014). A study performed by Saas and Gosselin, 2014 compared regression methods for spatially autocorrelated data. They found that Bayesian methods (MCMC and INLA) performed better than the frequentist methods (GLMM and GAMM).

Markov Chain Monte Carlo (MCMC) methods are commonly used to compute Bayesian models however, these can be computationally intensive. INLA which uses a latent Gaussian approach is more efficient (Blangiardo *et al.*, 2013). The formula for the linear predictor included the incidence of AES and meningitis as outcomes and the covariates as described above. To take into account spatial autocorrelation, the neighbouring province was fitted as a random effect using the f() function, the first argument of which is an index vector for which the random effect is applied to each observation and the second is the name of the model (Moraga, 2019) using a Besag-York-Mollie (bym) model (Besag, York and Mollie, 1991). The bym model accounts for over-dispersion by considering both unstructured and structured spatial

components (Riebler *et al.*, 2016). To account for temporal autocorrelation, year and month were added as random effects using the model 'Random Walk of order 1'. The inla() function was then used to fit the model using the family "nbinomial" (negative binomial). The outputs of the model were plotted using the function Efxplot() from the R package 'ggregplot' (Albery, n.d.). These show the estimate (mean) for each covariate with the posterior standard deviation and 2.5% and 97.5% quantiles (the credible interval) (Mtambo, Masangwi and Kazembe, 2015). Those estimates which did not cross zero were deemed to be significant at $p<0.05$.

MULTIVARIATE ANALYSIS

Given the evidence for spatial autocorrelation in the univariate analysis, multivariate models were constructed using only the INLA method described above. Separate correlation matrices were developed to compare the covariance between each of the climatic covariates and between the incidence per 100,000 population of each of the different pathogens/syndromes determined using Pearson's correlation coefficient function rcorr() from the 'Hmisc' package (Harrell Jr, 2019). These were plotted using the function corrplot() from the 'corrplot' package (Wei and Simko, 2017) which creates a correlogram of the correlation matrix where the magnitude of the correlation is determined by the size and colour of a dot.

Based on the results of this different models with an outcome of AES or meningitis were fitted using a combination of covariates. Climatic variables with a correlation coefficient of 0.80 or greater were not included in the same models to reduce the effect of multicollinearity. Incidence of meningitis and *S. suis* were the only syndromes/pathogens included in models with an outcome of the number of cases of AES and incidence of HFMD with an outcome of the number of cases of meningitis as these were the only pathogens/syndromes showing a strong association with the outcomes respectively, in the univariate analyses. Twelve models were therefore constructed as shown in table 2.3.

Table 2.3 Construction of the multivariate models. The boxes shaded indicate the presence of the covariate in the model.

Model	1	2	3	4	5	6	1	2	3	4	5	6
	Outcome = number of cases of encephalitis						Outcome = number of cases of meningitis					
Covariates												
Maximum temperature (°C)	■			■			■					■
Maximum temperature at a lag of minus one (°C)	■			■			■					■
Maximum temperature at a lag of minus two (°C)	■			■			■					■
Minimum temperature (°C)		■			■			■			■	
Minimum temperature at a lag of minus one (°C)		■			■			■			■	
Minimum temperature at a lag of minus two (°C)		■			■			■			■	
Relative humidity (%)	■	■	■	■	■	■	■	■	■	■	■	■
Relative humidity at a lag of minus one (%)	■	■	■	■	■	■	■	■	■	■	■	■
Relative humidity at a lag of minus two (%)	■	■	■	■	■	■	■	■	■	■	■	■
Absolute humidity (g/m^3)			■			■			■			■
Absolute humidity at a lag of minus one (g/m^3)			■			■			■			■
Absolute humidity at a lag of minus two (g/m^3)			■			■			■			■
Rainfall (mm)	■	■	■	■	■	■	■	■	■	■	■	■
Rainfall at a lag of minus one (mm)	■	■	■	■	■	■	■	■	■	■	■	■
Rainfall at a lag of minus two (mm)	■	■	■	■	■	■	■	■	■	■	■	■
Rainfall at a lag of minus three (mm)	■	■	■	■	■	■	■	■	■	■	■	■
Sunshine (hours)	■	■	■	■	■	■	■	■	■	■	■	■
Sunshine at a lag of minus one (hours)	■	■	■	■	■	■	■	■	■	■	■	■
Sunshine at a lag of minus two (hours)	■	■	■	■	■	■	■	■	■	■	■	■
NDVI	■	■	■	■	■	■	■	■	■	■	■	■
NDVI at a lag of minus one	■	■	■	■	■	■	■	■	■	■	■	■
NDVI at a lag of minus two	■	■	■	■	■	■	■	■	■	■	■	■
Elevation (m)	■	■	■	■	■	■	■	■	■	■	■	■
Number of pigs per 100,000 human population	■	■	■	■	■	■	■	■	■	■	■	■
Poverty rate (%)	■	■	■	■	■	■	■	■	■	■	■	■
Number of hospitals per $1000km^2$	■	■	■	■	■	■	■	■	■	■	■	■
JE vaccination coverage with three doses	■	■	■	■	■	■	■	■	■	■	■	■
Incidence of meningitis per 100,000 population				■	■	■						
Incidence of *S. suis* per 100,000 population				■	■	■						
Incidence of HFMD per 100,000 population										■	■	■

As for the univariate analysis, the outputs of the model were plotted using the function Efxplot() from the R package 'ggregplot'. Additionally, the Deviance Information Criterion (DIC) and Watanabe-Akaike information criterion (WAIC) were given to assess the fit of the models. A choropleth map of the estimate of number was constructed for each of the models. Due to the absence of data for *S. suis* prior to 2011, models which included this variable were fitted only for data from 2011-2016.

2.3 RESULTS

DESCRIPTIVE ANALYSIS: PATHOGENS AND SYNDROMES

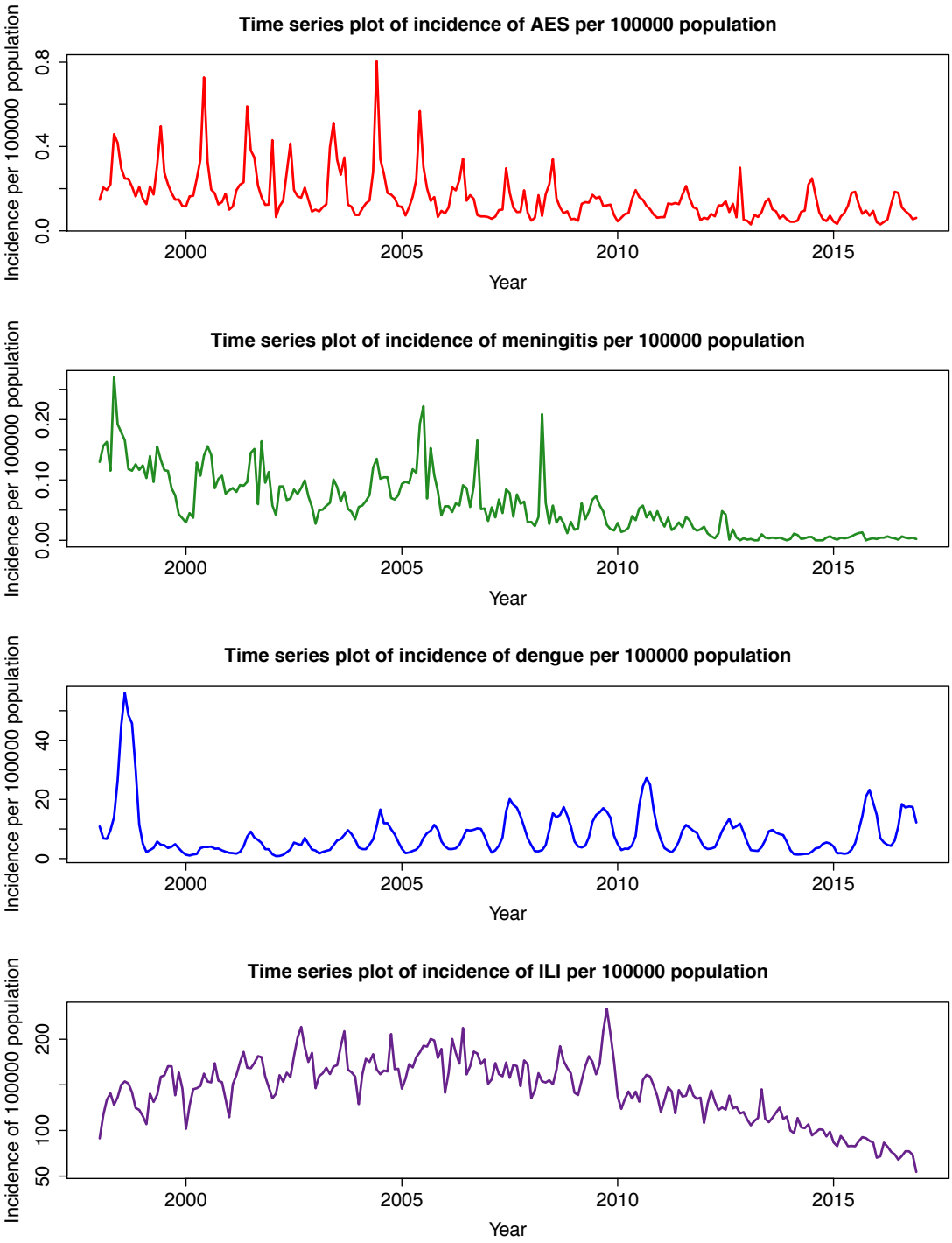
THE DESCRIPTION OF THE INCIDENCE OF AES IN SPACE AND TIME

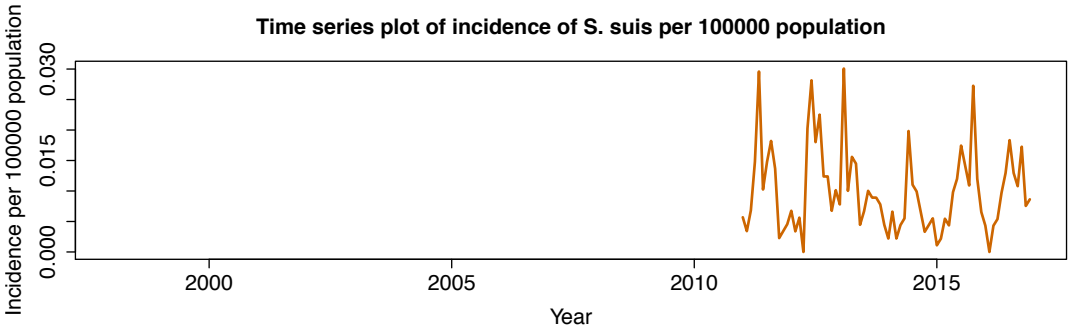
Figures 2.3 (below) and A2.1, appendix 2.1) show an initial increase national monthly incidence of AES in the earlier years of the study and then a decline after a peak in incidence seen in June 2004 (0.80 cases per 100,000 population). The annual incidence of AES reduced from 3.01 cases per 100,000 population in 1998 to 1.06 cases per 100,000 population in 2016 with strong evidence for a negative correlation of incidence of AES with time, particularly after 2005 with a non-linear trend (tables A2.1 and A2.2, appendix 2.1). However, in eight provinces, an increase in incidence over was seen over time. This was highest in the provinces of Son La and Lao Cai in the Northern Midlands and Mountains (an increase of 5.64 cases per 100,000 population and 4.26 cases per 100,000 population, respectively) and Gia Lai in the Central Highlands (4.45 cases per 100,000 population) (figure 2.4).

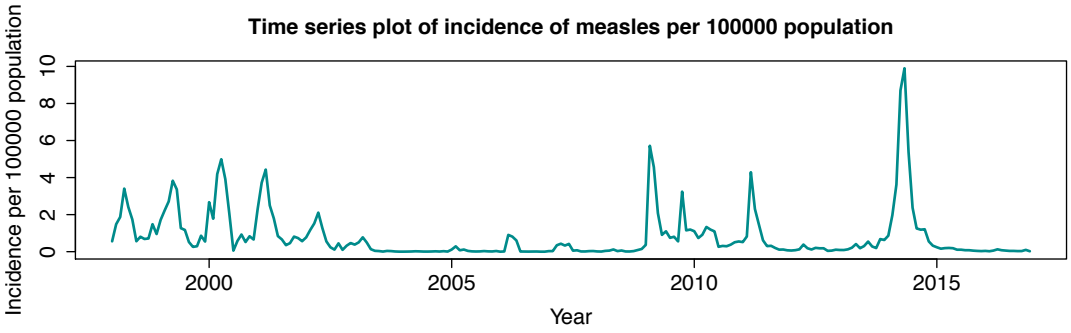
Decomposition of the time series showed a trend strength of 0.604 and seasonal strength of 0.766 (figure A2.1, appendix 2.1). The mean national monthly incidence was highest in June (0.35 cases (standard deviation (SD)=0.21) per 100,000 population) and lowest in February (0.08 cases (SD=0.04) per 100,000 population). May to September were positively correlated with the number of cases of AES compared to January (table A2.3, appendix 2.1).

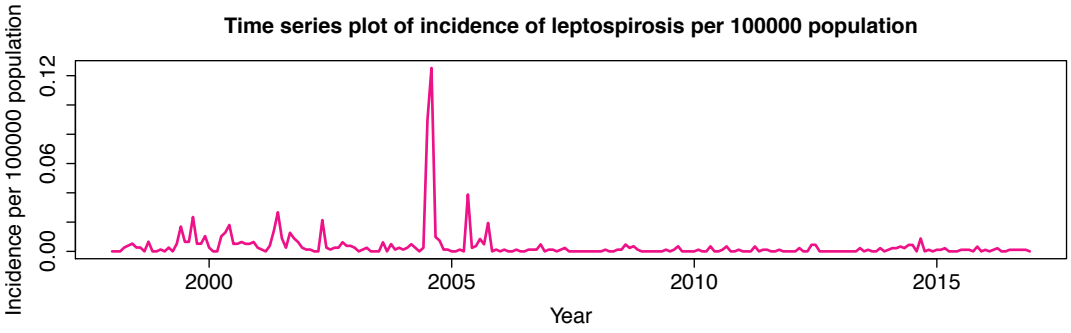
The mean monthly incidence of AES was highest in Son La (0.77 (SD=0.89) cases per 100,000 population) and lowest in Phu Yen on the Central Coast (0.01 (SD=0.05) cases per 100,000 population). However, the highest incidence was seen in January 2002 in Kon Tum in the Central Highlands (69.10 cases per 100,000 population). Nationally, there was evidence of annual seasonality (table A2.4, appendix 2.1) which seen the most in northern Vietnam and Kon Tum province (figure 2.5).

Figure 2.3 Time series plots showing the national incidence of AES, meningitis, dengue, ILI, *S. suis*, measles, leptospirosis and HFMD from 1998-2016.




Time series plot of incidence of S. suis per 100000 population
Incidence per 100000 population
0.000
0.015
0.030
2000
2005
2010
2015
Year


Time series plot of incidence of measles per 100000 population
Incidence per 100000 population
0
2
4
6
8
10
2000
2005
2010
2015
Year


Time series plot of incidence of leptospirosis per 100000 population
Incidence per 100000 population
0.00
0.06
0.12
2000
2005
2010
2015
Year

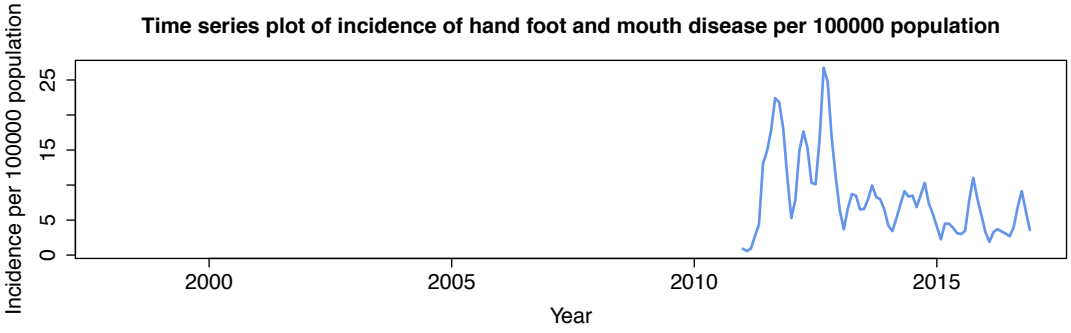
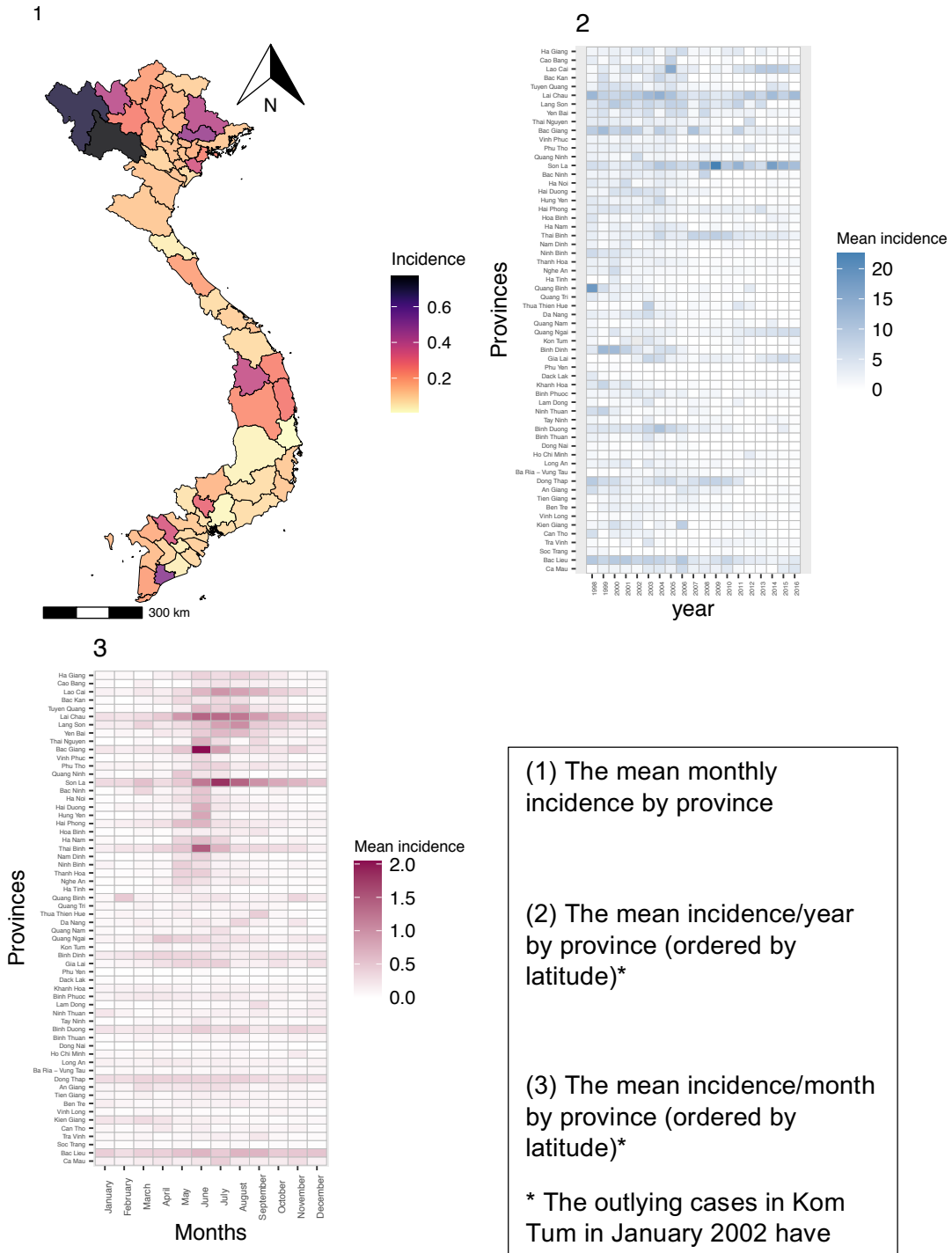

Time series plot of incidence of hand foot and mouth disease per 100000 population
Incidence per 100000 population
0
5
15
25
2000
2005
2010
2015
Year

Figure 2.4 The mean incidence of AES per 100,000 population by province from 1998-2016.



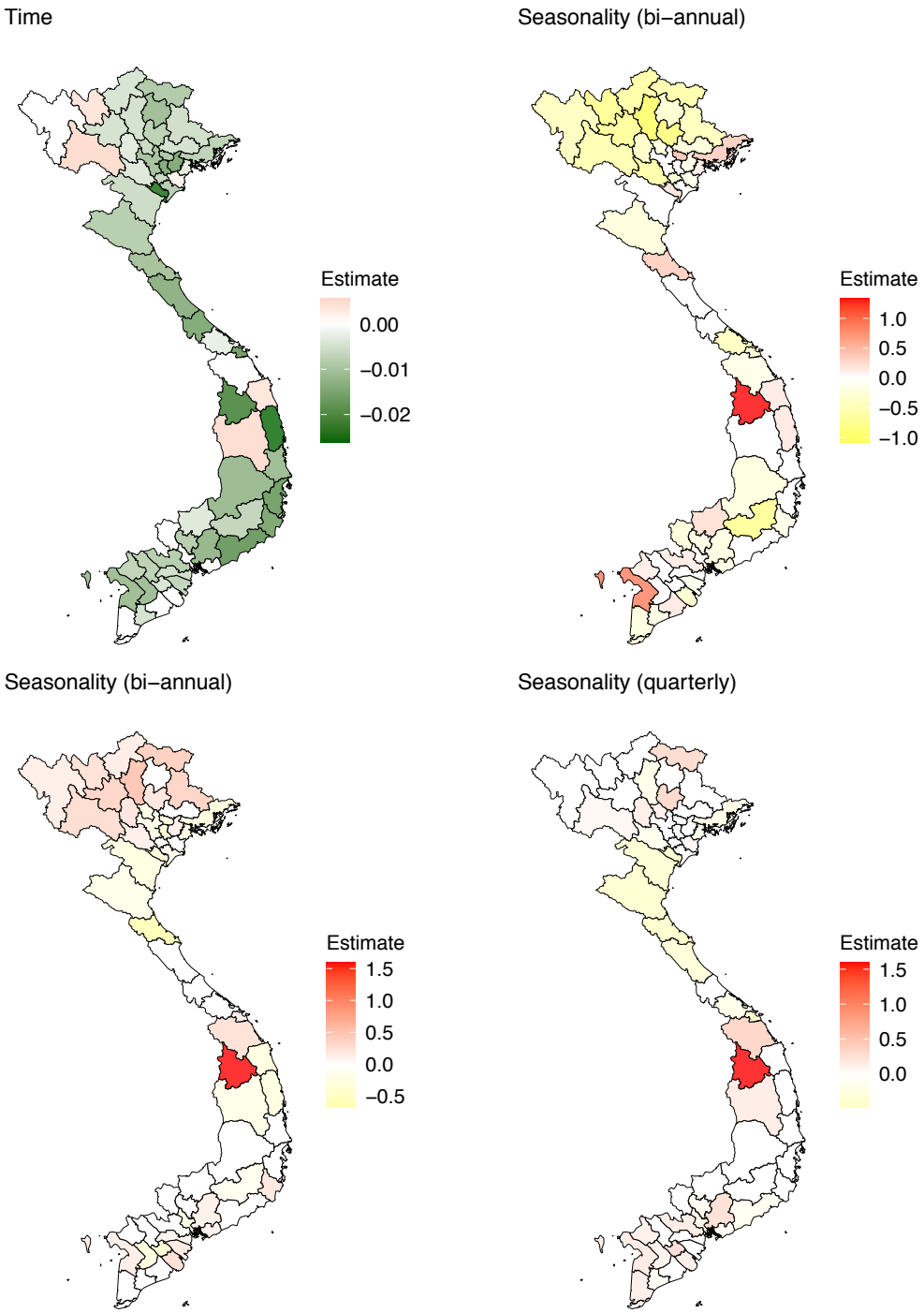
(1) The mean monthly incidence by province

(2) The mean incidence/year by province (ordered by latitude)*

(3) The mean incidence/month by province (ordered by latitude)*

* The outlying cases in Kom Tum in January 2002 have been removed to improve the visualisation of the remaining data

Figure 2.5 Estimates from negative binomial GLMs with an outcome of number of cases of AES and year and harmonic terms fitted as covariates by province.*



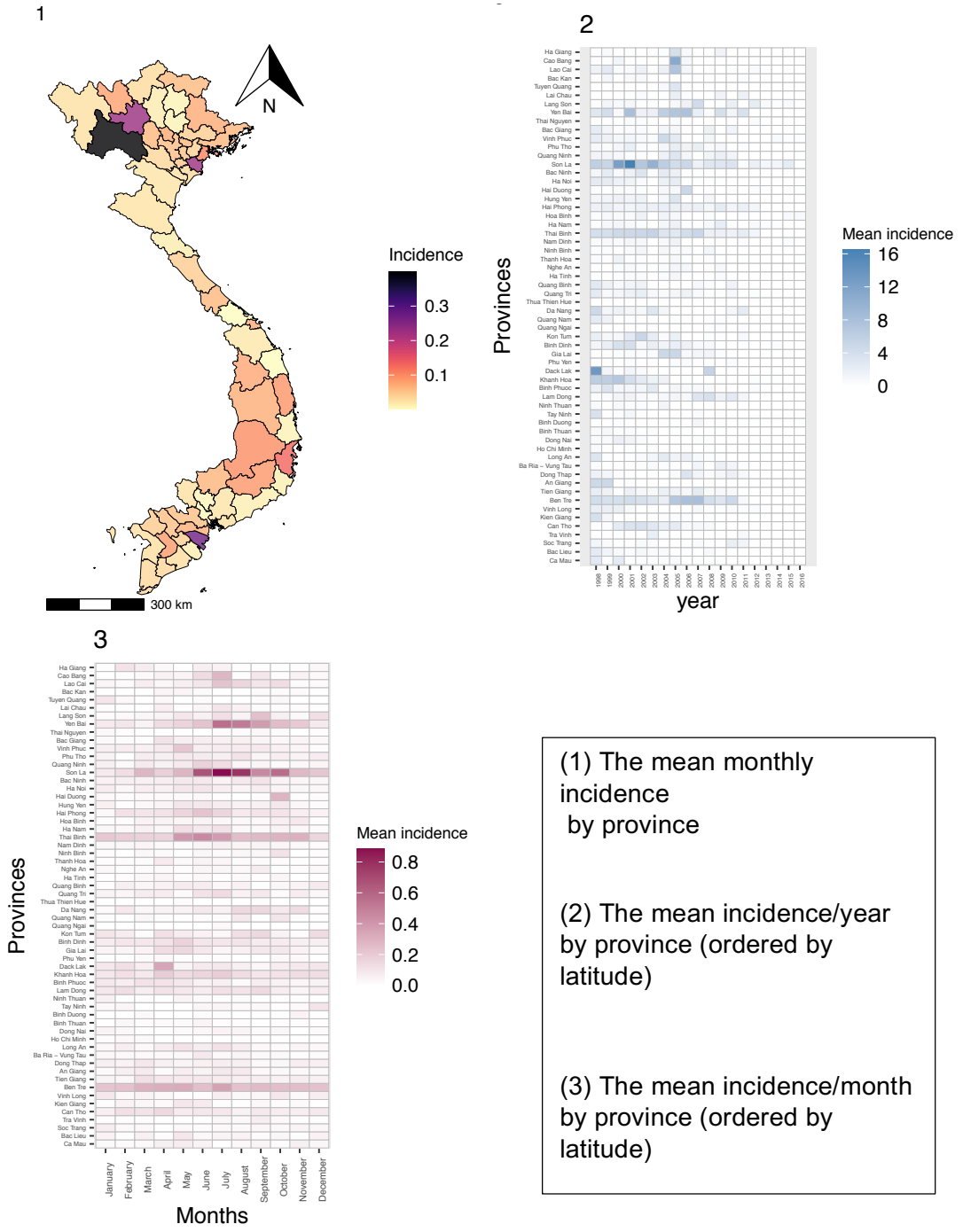
* Time fitted as GAM to allow convergence

THE DESCRIPTION OF THE INCIDENCE OF MENINGITIS IN SPACE AND TIME

The annual incidence of meningitis reduced from 1.85 cases/100,000 in 1998 to 0.05/100,000 in 2016 with a sharp decline since 2010 (figures 2.3 (above) and A2.1, appendix 2.1; and tables A2.5 and A2.6, appendix 2.1). Decomposition of the time series showed a trend strength of 0.823 and a seasonal strength of 0.417 (figure A2.1, appendix 2.1). The mean national monthly incidence was highest in July (0.08 (SD=0.06) cases per 100,000 population) and lowest in December (0.04 (SD=0.04) per 100,000 population). March to October were positively correlated with the incidence of meningitis compared to January (table A2.7, appendix 2.1).

The mean monthly incidence of meningitis was highest in Son La (0.40 (SD=0.73) cases per 100,000 population) and lowest in Thua Thien Hue on the Central Coast ($3.25x10^{-3}$ (SD=0.02) per 100,000 population) with strong evidence for a difference between the two ($p<0.001$). However, the highest incidence was seen in Dak Lak province in the Central Highlands in April 2008 (5.53 cases per 100,000 population). Despite a national decline in annual incidence there was an increase in incidence between 1998 and 2016 in three provinces, with the highest increase in Hoa Binh in the Northern Midlands and Mountains (0.32 cases per 100,000 population) (figure 2.6). Nationally, there was evidence of annual and quarterly seasonality (table A2.8, appendix 2.1) with seasonality greatest in Northern Midlands and Mountains and the Central Highlands (figure 2.7).

Figure 2.6 The mean incidence per 100,000 population of meningitis by province from 1998-2016.

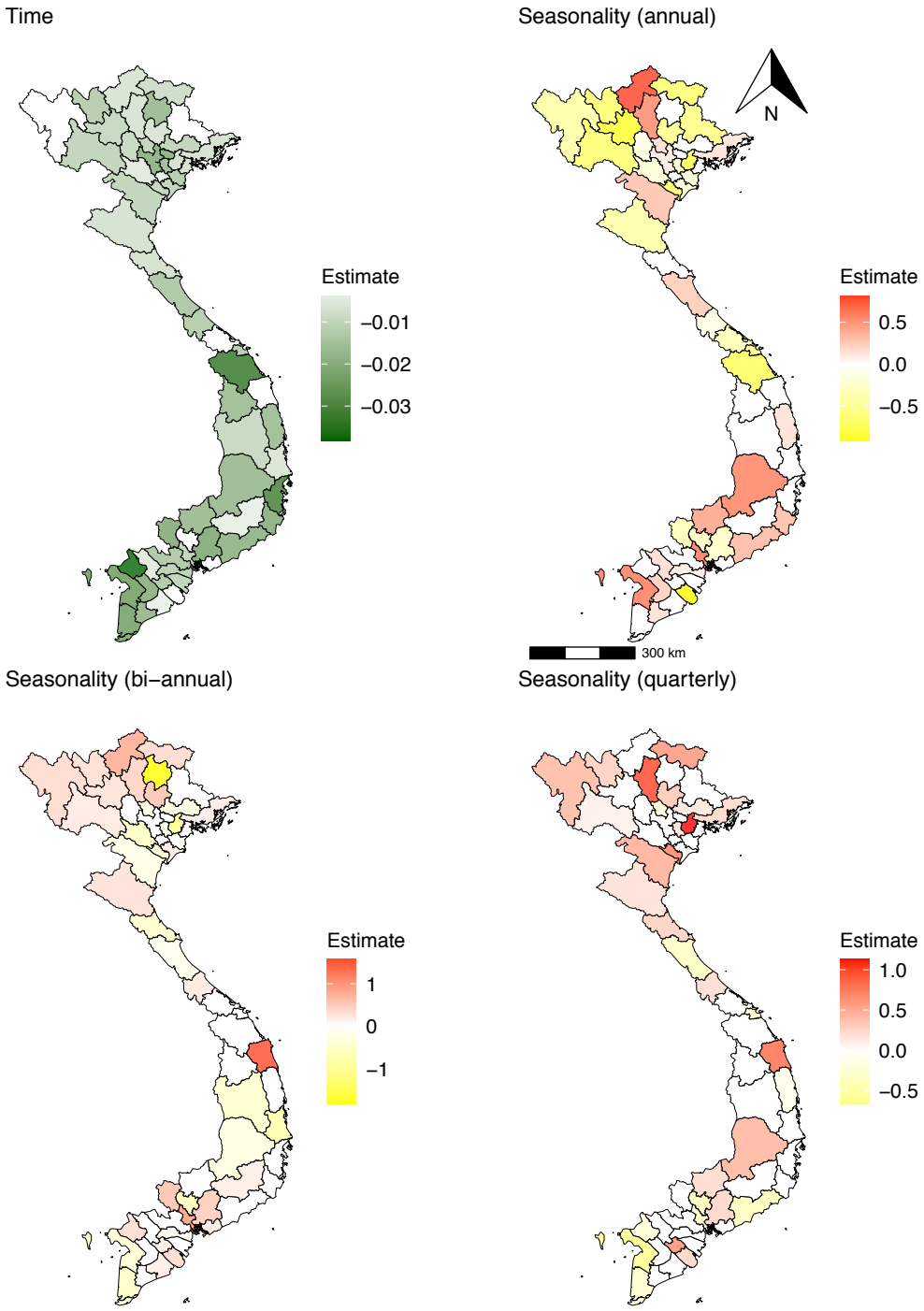


(1) The mean monthly incidence by province

(2) The mean incidence/year by province (ordered by latitude)

(3) The mean incidence/month by province (ordered by latitude)

Figure 2.7 Estimates from negative binomial GLMs with an outcome of number of cases of meningitis and year and harmonic terms fitted as covariates by province.*

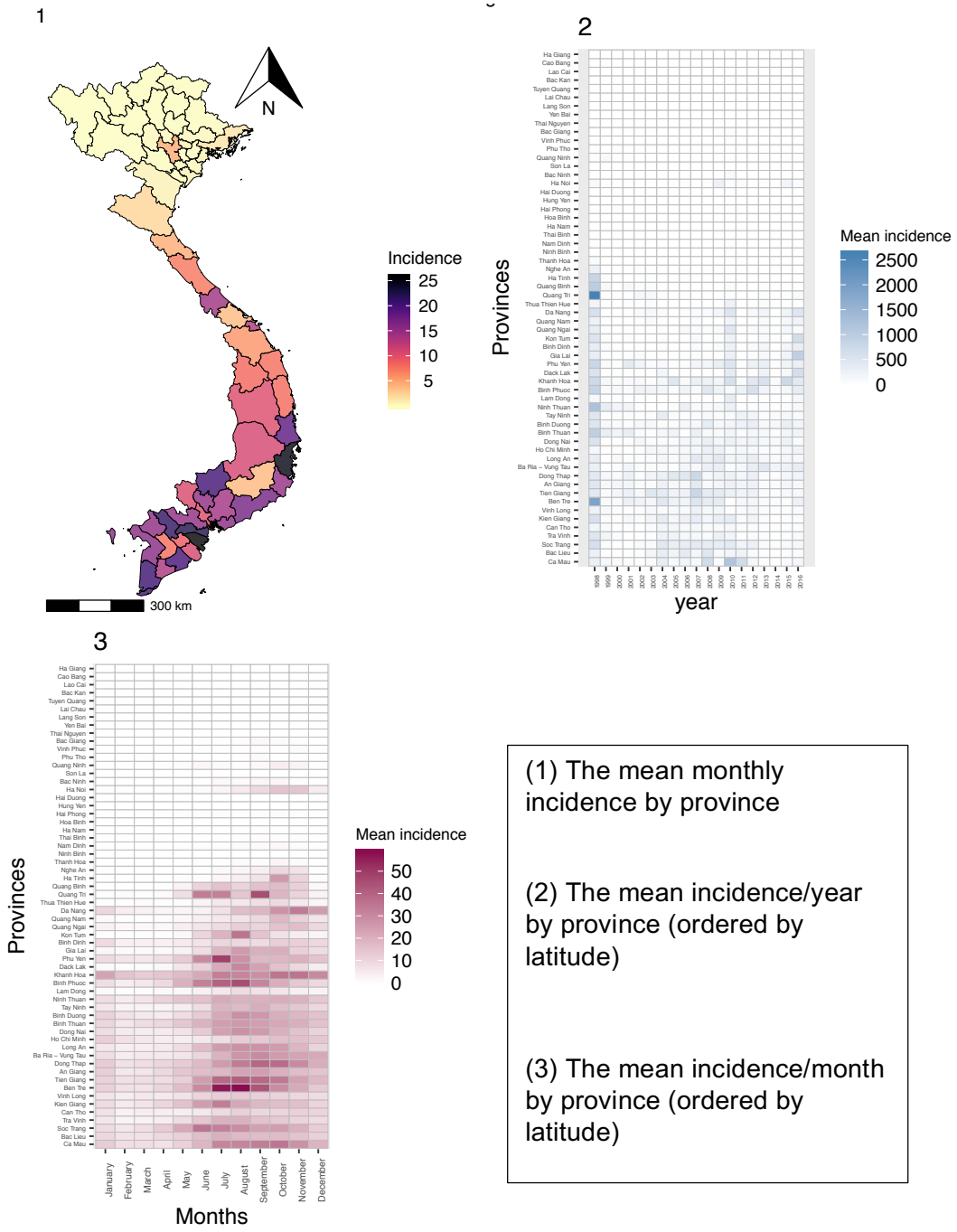


THE DESCRIPTION OF THE INCIDENCE OF DENGUE VIRUS IN SPACE AND TIME

Unlike AES and meningitis, there has not been a general decrease in incidence of dengue (figures 2.3 and A2.1, appendix 2.1). The highest annual incidence was seen in 2010 with 148.03 cases per 100,000 population however, peaks were also seen in 1998 (311.33 cases per 100,000 population) and 2016 (136.07 cases per 100,000 population). The decomposition of the time series gave a trend strength of 0.646, a seasonal strength of 0.584 (figure A2.1, appendix 2.1). The mean national monthly incidence was highest in October (13.3 (SD=9.76) cases per 100,000 population) and lowest in February and March (2.85 (SD=1.66) cases per 100,000 population) and 2.8 (SD=1.39 cases) per 100,000 population, respectively) (figure 2.8).

The mean monthly incidence of dengue was highest in Ben Tre in the Mekong Delta (25.56 (SD=53.94) cases per 100,000 population) and the lowest in Tuyen Quang in the Northern Midlands and Highlands ($1.16x10^{-3}$ (SD=$12.39x10^{-3}$ cases per 100,000 population). However, the highest incidence was seen in Quang Tri on the Central Coast, in September 1998 (766.36 cases per 100,000 population) (figure 2.8).

Figure 2.8 The mean incidence per 100,000 population of dengue by province from 1998-2016.

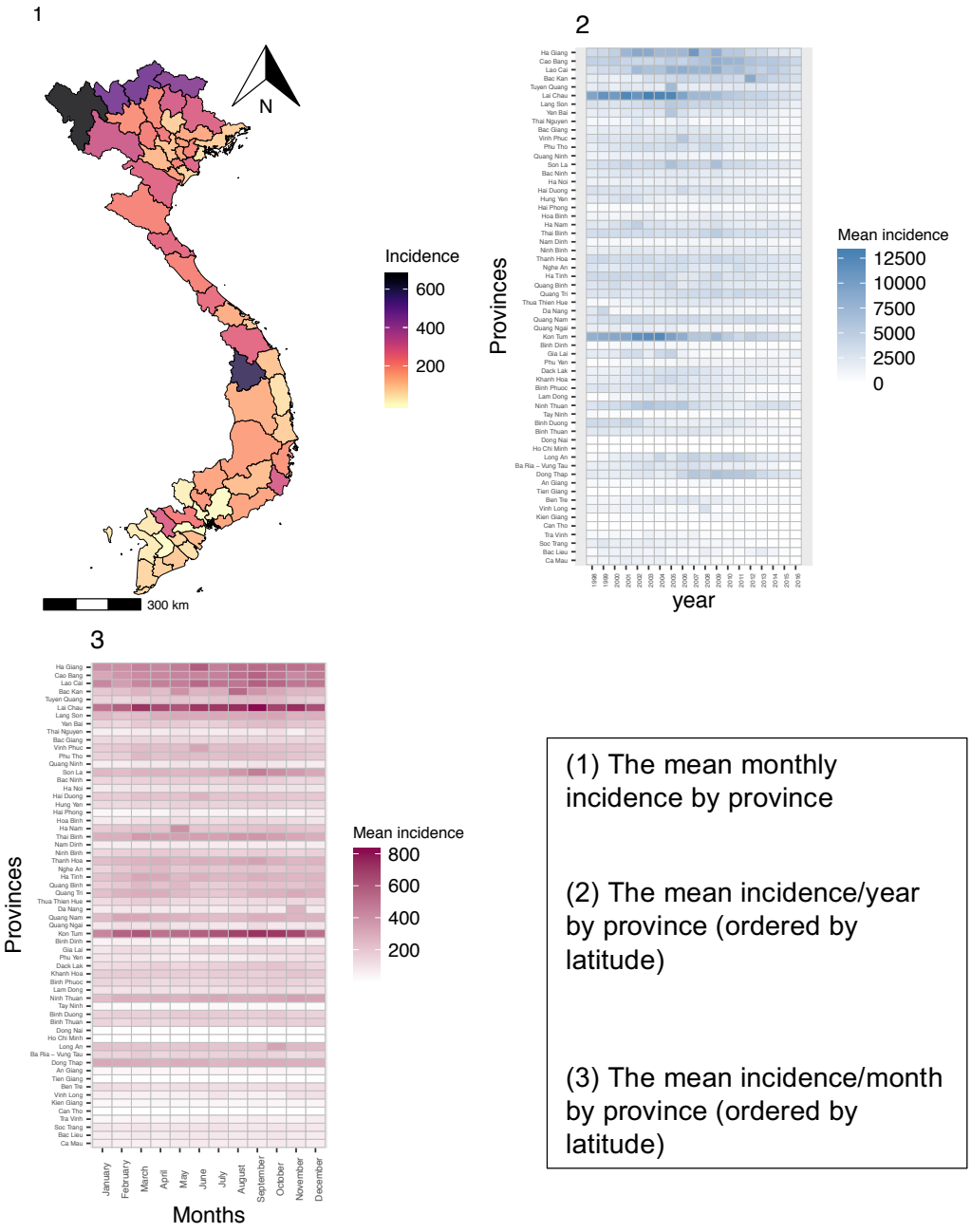


THE DESCRIPTION OF THE INCIDENCE OF INFLUENZA-LIKE-ILLNESS (ILI) IN SPACE AND TIME

Over the study time period, the incidence of ILI increased and then decreased with a peak incidence in 2005 of 2163.04 cases per 100,000 population. In 2016 the incidence was 883.37 cases per 100,000 population (figures 2.3 andA2.1, appendix 2.1). Decomposition of the time series gave a trend strength of 0.92 and seasonal strength of 0.64 (figure A2.1, appendix 2.1). The mean national monthly incidence was highest in September (160.46 (SD=40.70) cases per 100,000 population) and lowest in January (120.20 (SD=22.94) cases per 100,000 population) (figure 2.9).

The mean monthly incidence of ILI was highest in Lai Chau in the Northern Midlands and Mountains (667.30 cases (SD=347.42) per 100,000 population) and lowest in Ho Chi Minh in the Southeast (0.20 (SD=1.90) cases per 100,000 population) (figure 2.9).

Figure 2.9 The mean incidence per 100,000 population of ILI by province from 1998-2016.



(1) The mean monthly incidence by province

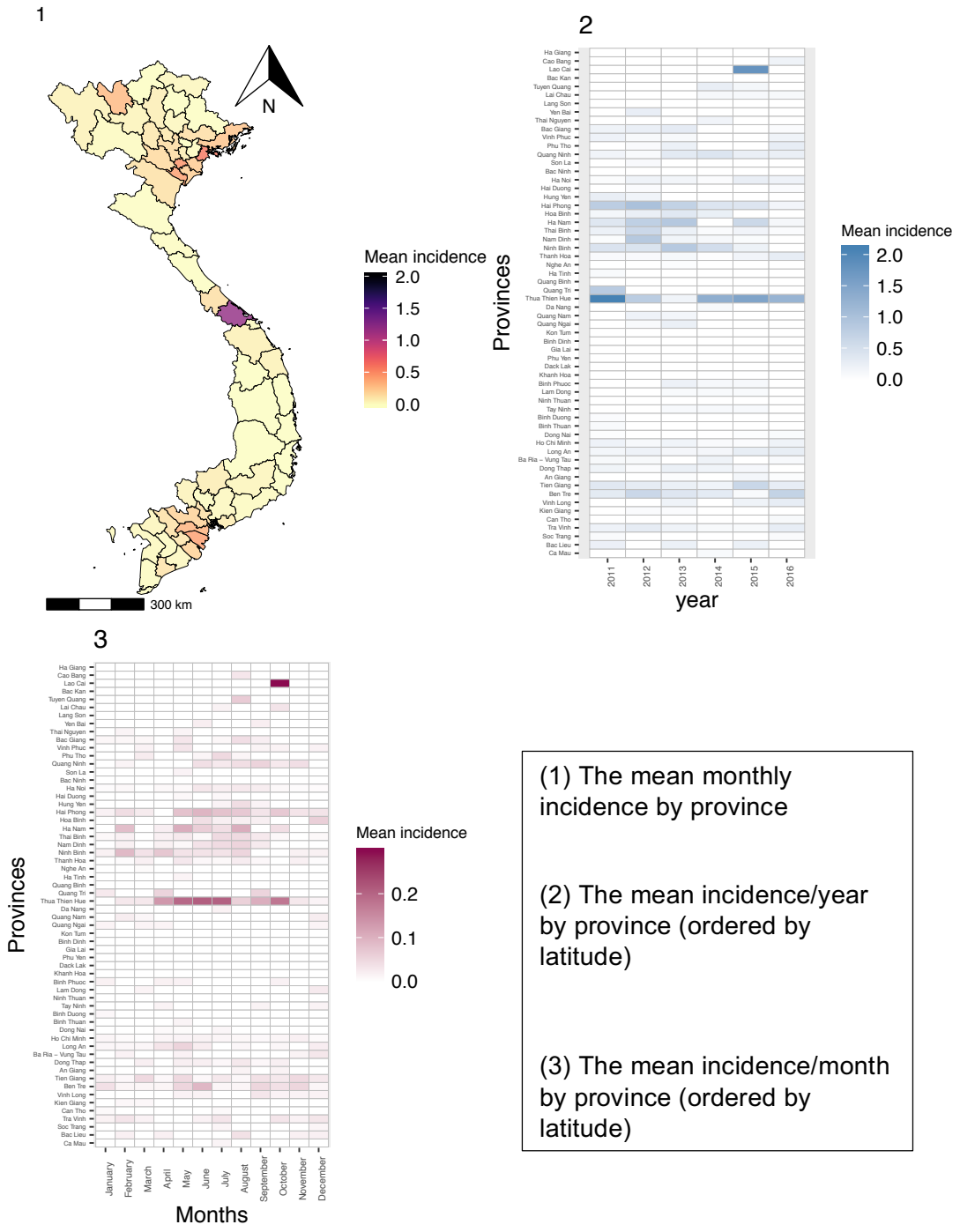
(2) The mean incidence/year by province (ordered by latitude)

(3) The mean incidence/month by province (ordered by latitude)

THE DESCRIPTION OF THE INCIDENCE OF *S. SUIS* IN SPACE AND TIME

Data from 2011 showed the highest incidence of *S. suis* in 2012 (0.15 cases per 100,000 population) and lowest in 2014 (0.08 cases per 100,000 population) (figures 2.3 and 2.4). Decomposition of the time series showed a trend strength of 0.21 and seasonal strength of 0.42 (figure A2.1, appendix 2.1). The mean national monthly incidence was highest in May ($1.49x10^{-2}$ (SD= SD=$8.78x10^{-3}$) cases per 100,000 population) and lowest in January ($4.64x10^{-3}$/100,000, SD=$2.62x10^{-3}$/100,000) (figure 2.10). The mean monthly incidence of *S. suis* was highest in Thua Thien Hue on the Central Coast ($2.17x10^{-2}$/100,000, SD=$3.05x10^{-2}$/100,000) and lowest in Dack Lak in the Central Highlands ($1.07x10^{-4}$, SD=$7.47x10^{-4}$/100,000) (figure 2.10).

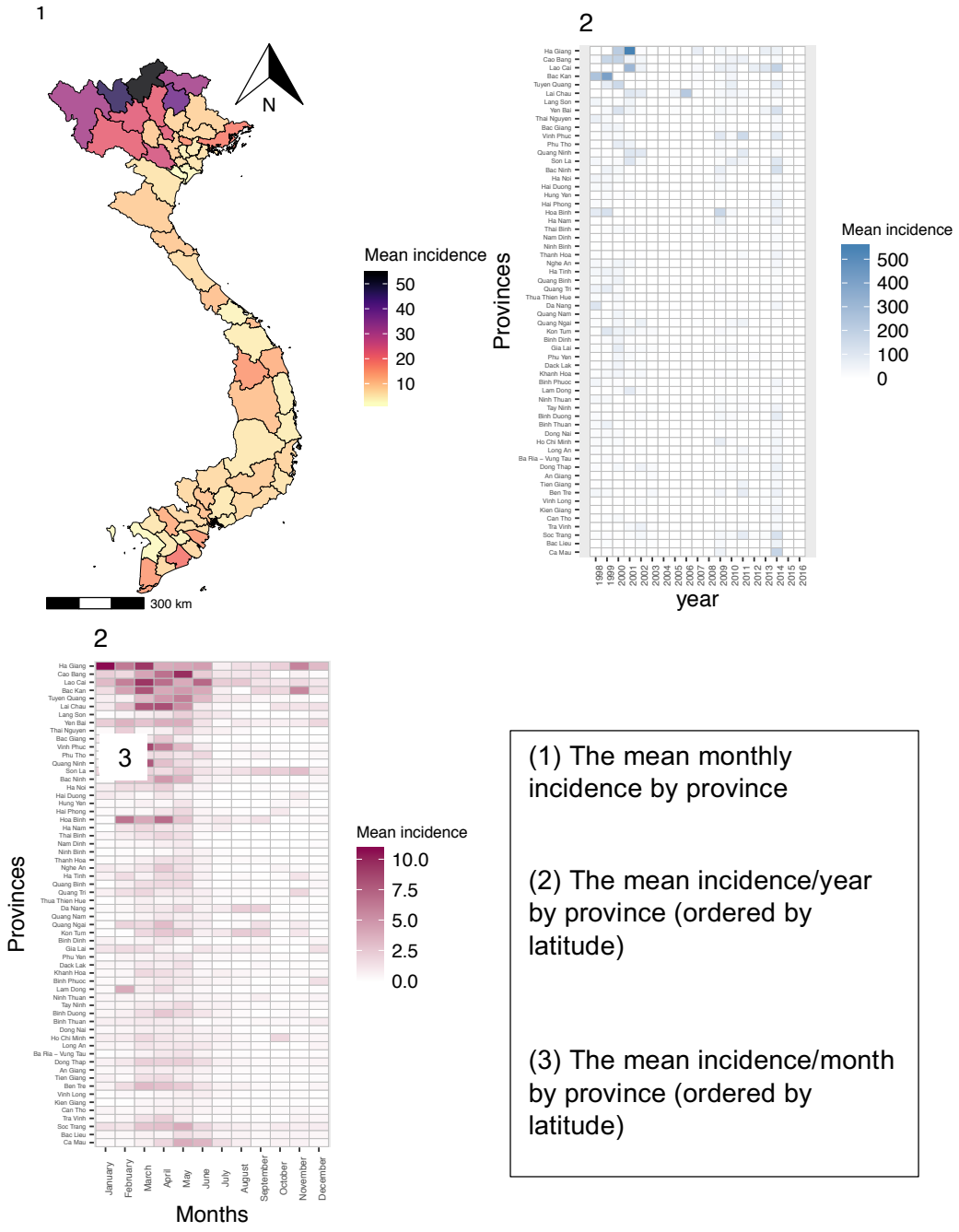
Figure 2.10 The mean incidence per 100,000 population of *S. suis* by province from 1998-2016.



THE DESCRIPTION OF THE INCIDENCE OF MEASLES IN SPACE AND TIME

Yearly incidence of measles generally declined since 2001 however, there were peaks in in 2009 (22.43 cases per 100,000 population) and 2014 (37.35 cases per 100,000 population) (figures 2.3 and A2.1, appendix 2.1). Decomposition of the time series gave a trend strength of 0.56 and seasonal strength of 0.39 (figure A2.1, appendix 2.1). The mean monthly incidence was greatest in April (1.80 (SD=2.23) cases per 100,000) and lowest in September (0.30 (SD=0.34) cases per 100,000 population) (figure 2.12). The mean monthly incidence was highest in Ha Giang in the Northern Midlands and Mountains (4.47 (SD=19.02) cases per 100,000 population) and lowest in Ninh Binh in the Red River Delta (0.19 (SD=0.85) cases per 100,000 population) (figure 2.11).

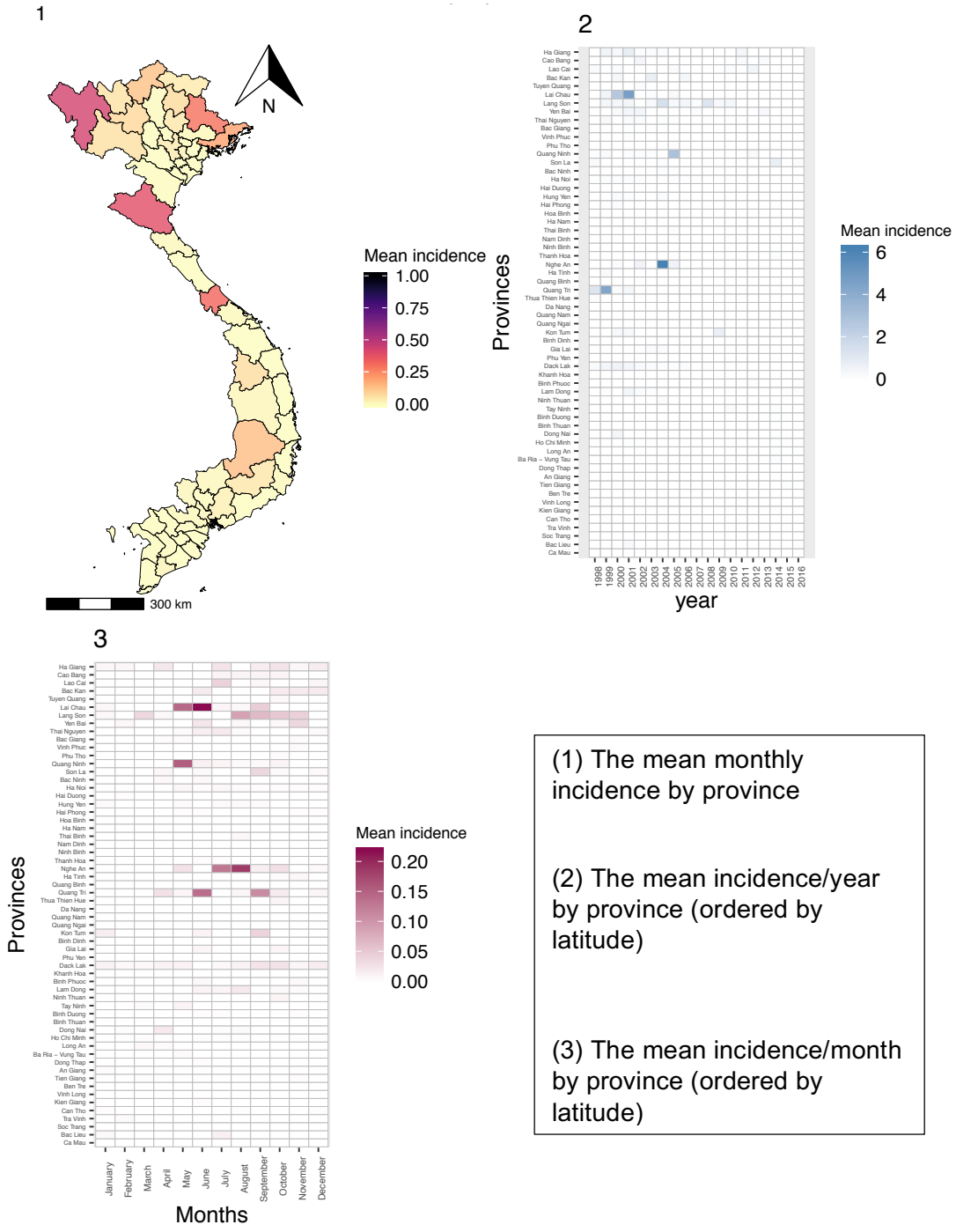
Figure 2.11 The mean incidence per 100,000 population of measles by province from 1998-2016.



THE DESCRIPTION OF THE INCIDENCE OF LEPTOSPIROSIS IN SPACE AND TIME

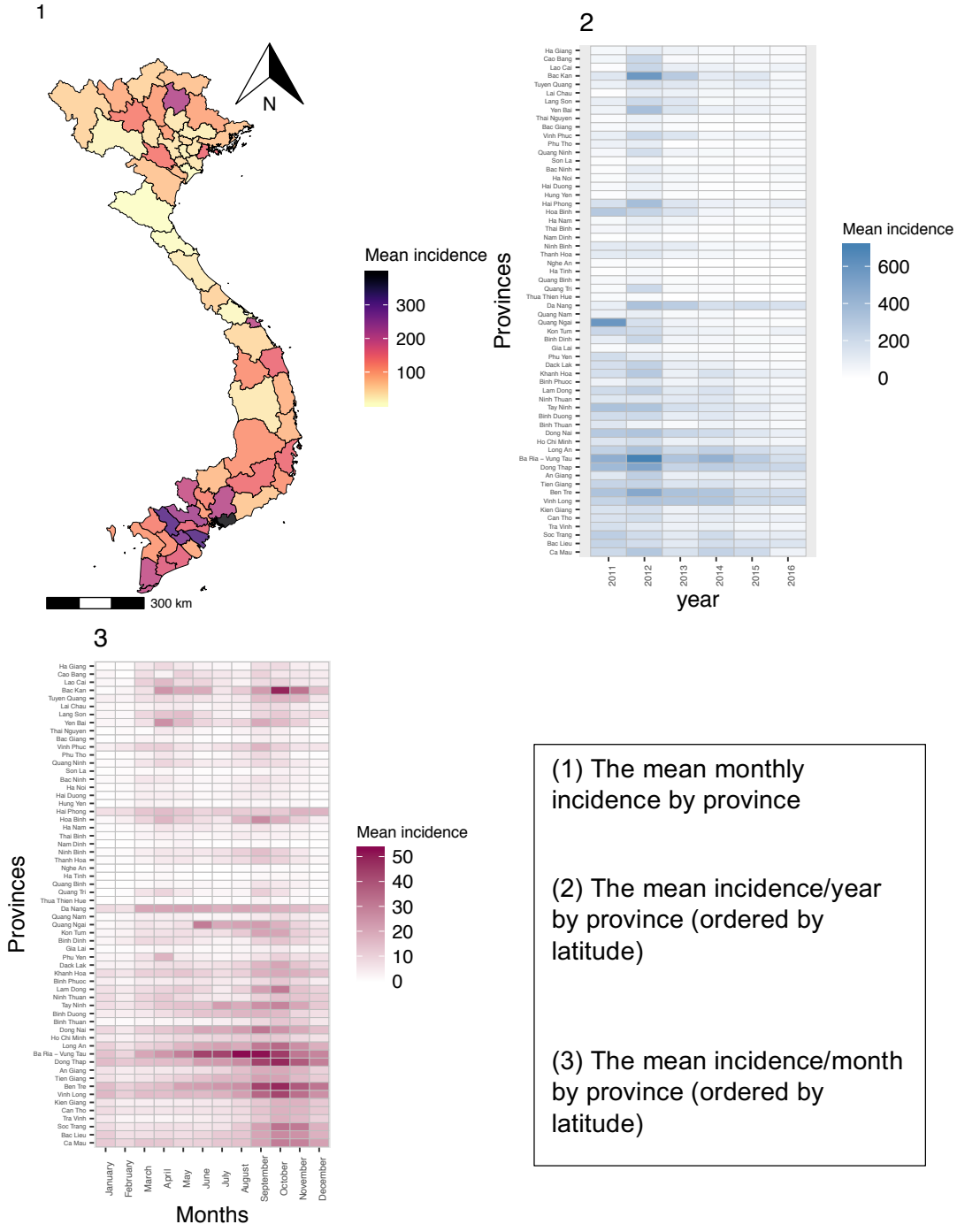
Incidence of leptospirosis remained relatively stable until 2004 when a peak was seen (0.25 cases per 100,000 population). However, by 2016 the incidence had decreased to $9.7x10^{-3}$ cases per 100,000 population in 2016 (figures 2.3 and A2.1, appendix 2.1). The decomposition of the time series showed a trend strength of 0.25 and seasonal strength of 0.22 with a seasonal peak at month 8 and trough at month 2 (figure A2.1, appendix 2.1). The mean national monthly incidence was highest in August ($8.99x10^{-3}$ (SD=$2.83x10^{-2}$) cases per 100,000 population) and lowest in February ($6.22x10^{-4}$ (SD=$8.20x10^{-4}$) per 100,000 population) (figure 2.13). The mean monthly incidence was highest in Lai Chau in the Northern Midlands and Mountains ($3.59x10^{-2}$ (SD=0.28) per 100,000 population). In sixteen provinces, no cases were reported (figure 2.12).

Figure 2.12 The mean incidence per 100,000 population of leptospirosis by province from 1998-2016.



Data from 2011 showed incidence to be highest in 2012 (177.22 cases per 100,000 population) and decreased to 51.17 cases per 100,000 population in 2016 (figures 2.3 and 2.4). Decomposition of the time series showed a trend strength of 0.74 and seasonal strength of 0.68 (figure A2.1, appendix 2.1). Mean incidence was highest in October (14.23 (SD=7.14) cases per 100,000 population) and lowest in February (3.28 (SD=2.52) cases per 100,000 population) (figure 2.13). The mean monthly incidence was highest in Bac Kan in the Northern Midlands and Mountains (12.20 (SD=11.98) cases per 100,000 population) and lowest in Ha Noi in the Red River Delta (0.26 (SD=0.29) cases per 100,000) (figure 2.13).

Figure 2.13 The mean incidence per 100,000 population of HFMD by province from 1998-2016.



(1) The mean monthly incidence by province

(2) The mean incidence/year by province (ordered by latitude)

(3) The mean incidence/month by province (ordered by latitude)

Data for JE was available from 2012 to 2016 from two hospital sites in Hanoi in the Red River Delta and Ho Chi Minh City in the Southeast with the highest number of cases in 2014 (n=97). The number of cases was highest in June (total=179) and lowest in January and February when no cases were seen. The temporal pattern corresponded with that of AES (figure 2.14). The total number of cases was highest in Ha Nam province (n=50) (figure 2.15).

Figure 2.14 Time series plot of the monthly number of cases JE admitted to the National Children Hospital in Hanoi and Children's Hospital No.1 in Ho Chi Minh City (the red line), compared to the number of cases of AES from national surveillance data (the blue line) 2012 to 2016.

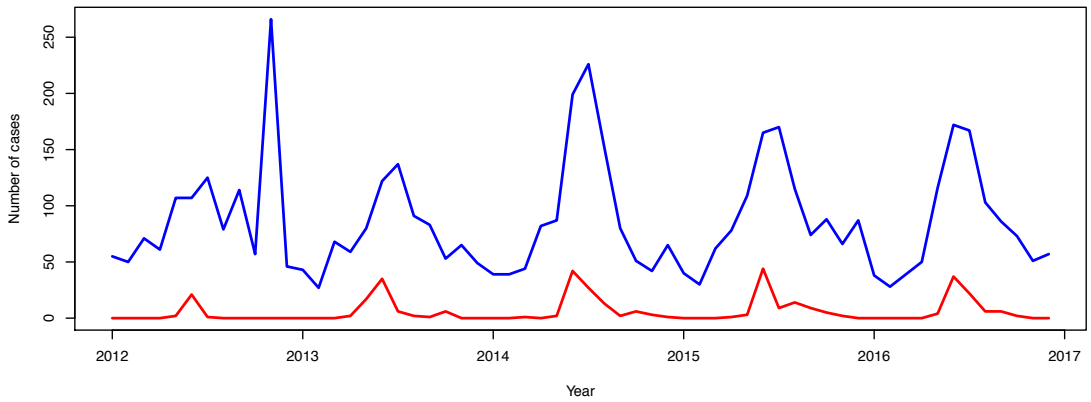
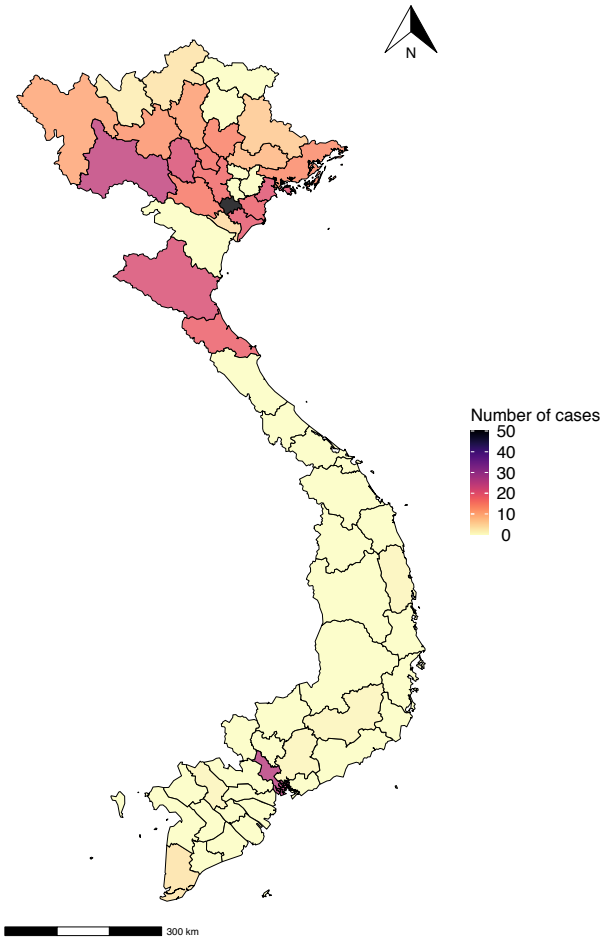


Figure 2.15 The total number of cases JE admitted to the National Children Hospital in Hanoi and Children's Hospital No.1 in Ho Chi Minh City from national surveillance data, from 2012 to 2016.

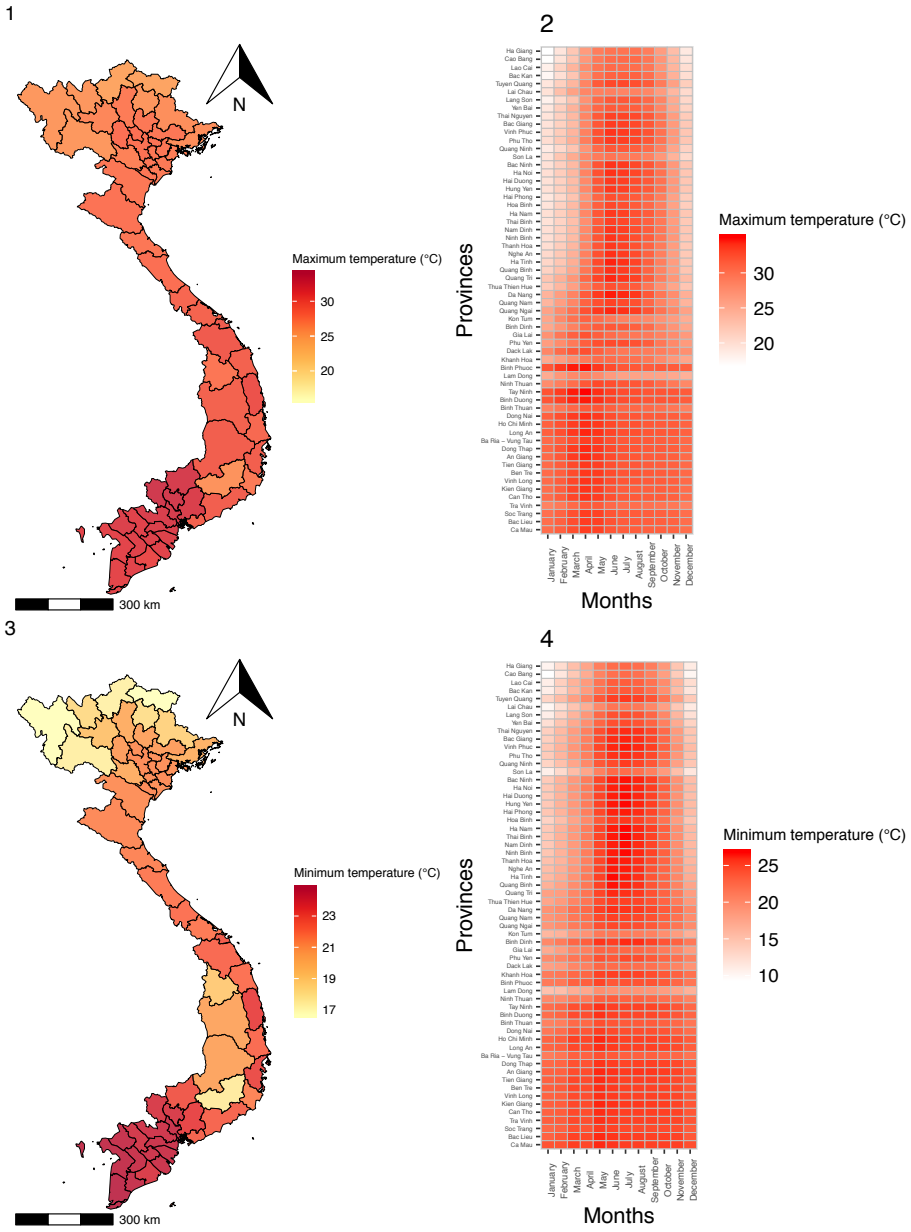


DESCRIPTIVE ANALYSIS: COVARIATES

DESCRIPTION OF THE CLIMATIC VARIABLES IN SPACE AND TIME

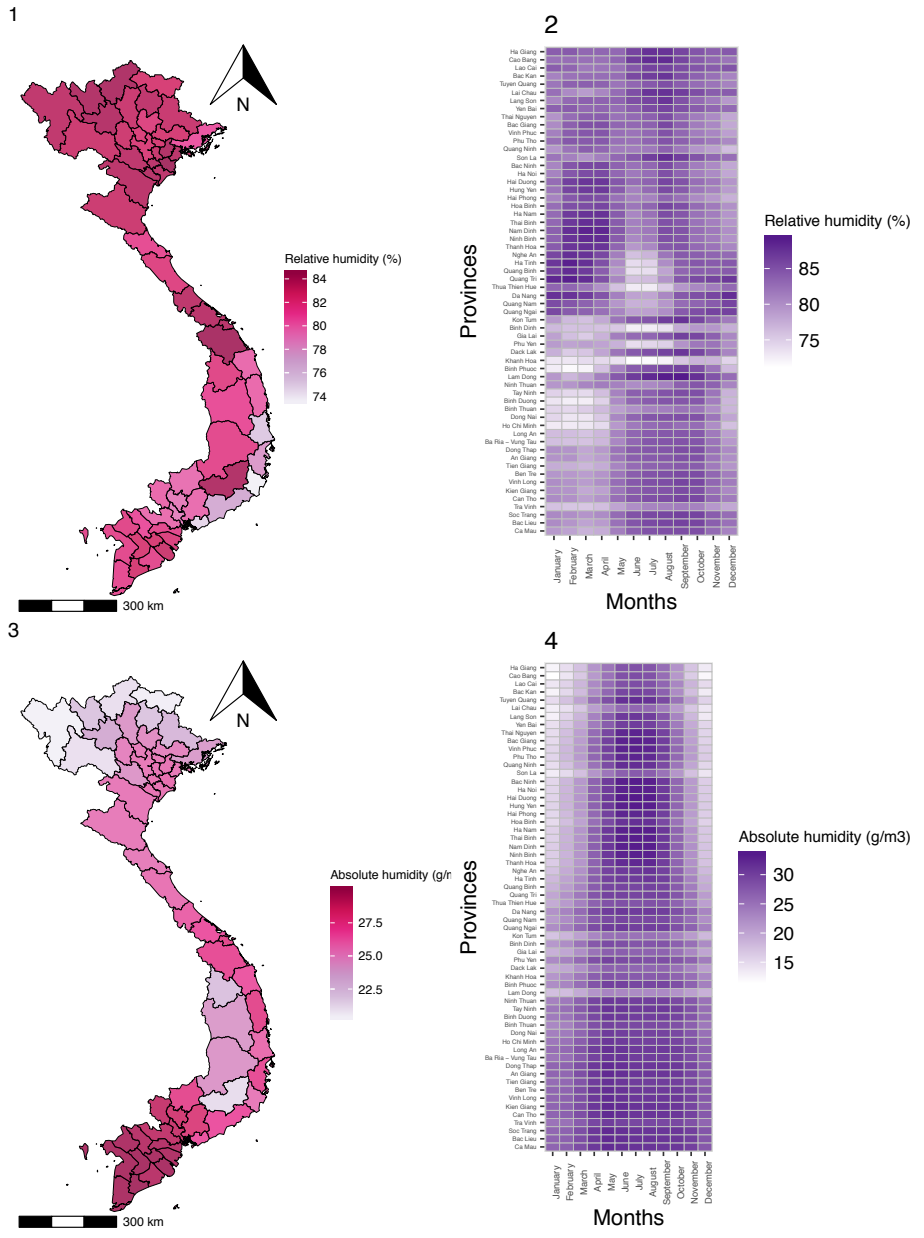
The mean temperatures for the study period were higher in southern compared to northern Vietnam. In the Mekong Delta and the Southeast, the temperatures remained relatively constant throughout the year whereas the northern part of the Central Coast, the Red River Delta and the Northern Midlands and Mountains experienced warmer summers and cooler winters (figure 2.16). The relative humidity remained relatively constant throughout the year in northern Vietnam however, in southern Vietnam and the Central Highlands it was highest in the summer months. In the Central Coastal provinces, it was high throughout the year except in June to August. The absolute humidity remained high throughout the year in southern Vietnam whereas in the Central Coastal provinces and northern Vietnam it was highest in the summer months, with the exception of provinces at higher elevation including Lai Chau and Son La where it remained lower throughout the year with a mild increase in the summer months (figure 2.17). Throughout the country rainfall was greatest in the summer and autumn months with the exception of the provinces on the Central Coast Vietnam when it was highest in the autumn. In northern and Central Coastal Vietnam, there was a marked difference between the hours of sunshine throughout the year with these being greatest in the summer months. In southern Vietnam, there was less of a difference however, the number of hours was greater in the winter and spring (figure 2.18).

Figure 2.16 The mean monthly maximum and minimum temperature by province from 1998-2016.



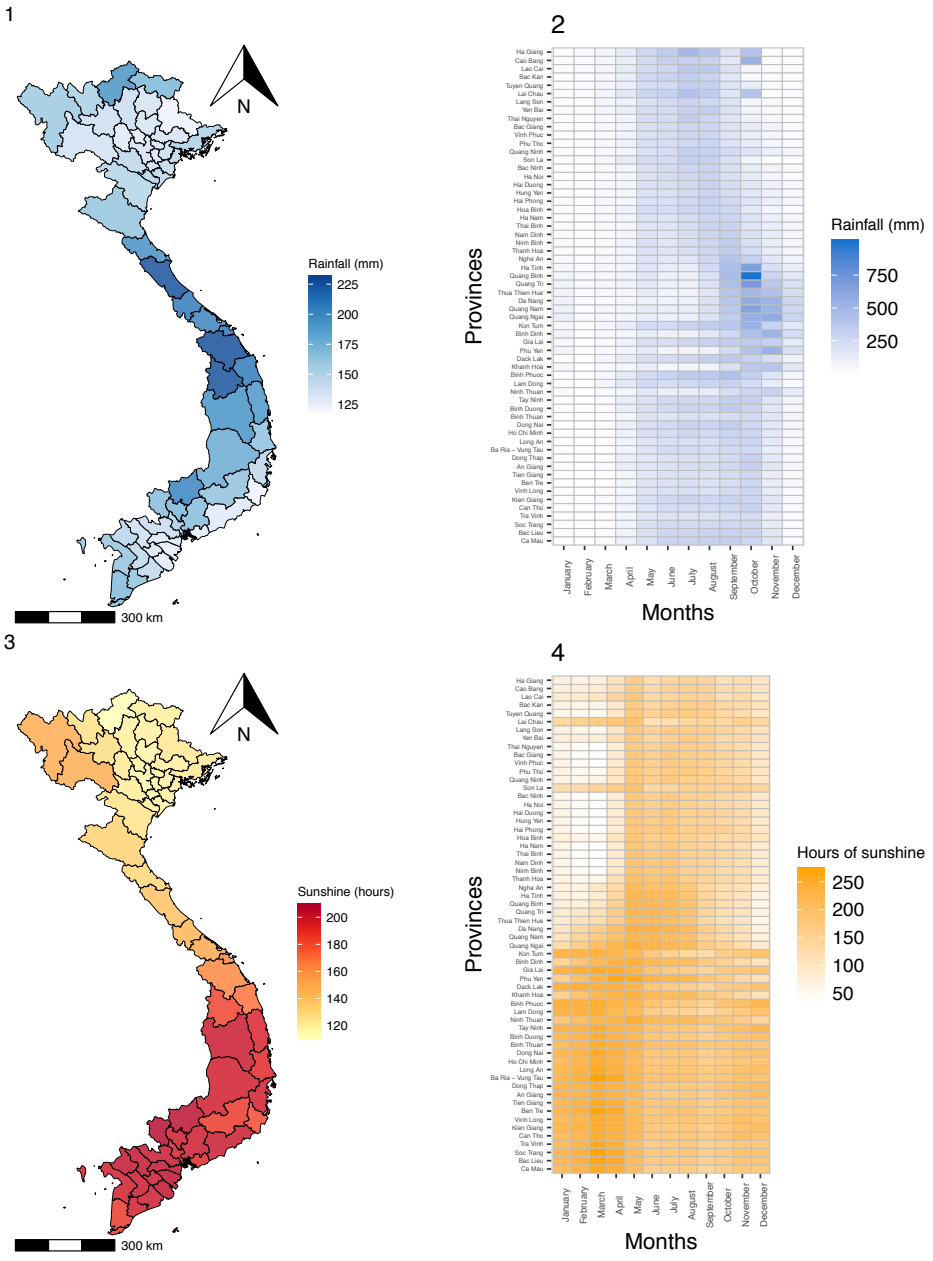
(1) The mean monthly maximum temperature by province
(2) The mean maximum temperature/month by province
(3) The mean monthly minimum temperature by province
(4) The mean minimum temperature/month by province

Figure 2.17 The mean monthly absolute and relative humidity by province from 1998-2016.



(1) The mean monthly relative humidity by province
(2) The mean maximum relative humidity/month by province
(3) The mean monthly absolute humidity by province
(4) The mean minimum absolute humidity by province

Figure 2.18 The mean monthly rainfall and hours of sunshine by province from 1998-2016.



(1) The mean monthly rainfall by province
(2) The mean maximum rainfall/month by province
(3) The mean monthly hours of sunshine by province
(4) The mean minimum hours of sunshine by province

The mean elevation was highest in the provinces in the Northern Midlands and Mountains and the Central Highlands with Lam Duong in the Central Highlands having the highest elevation weighted by population density (1046.16m). The mean elevation was lowest in Bac Lieu in the Mekong Delta (1.89m) (figure 2.19). The NDVI was highest in the Northern Midlands and Mountains and the Central Highlands and lowest in the coastal provinces of the Red River Delta, Da Nang on the Central Coast and the provinces in the Mekong Delta, southern Vietnam. The mean NDVI was highest in Binh Phuoc province in the Southeast (0.26, SD=0.16) and lowest in Ca Mau in the Mekong Delta (0.08, SD=0.06) (figure 2.21). Nationally, the NDVI was highest in May (mean=0.21, SD=0.15) and lowest in January (mean=0.13, SD=0.15).

The mean number of pigs per 100,000 human population was highest in northern Vietnam and lowest in the Mekong Delta and southern provinces of the Central Coast with the highest number in Bac Giang in the Northern Midlands and Mountains (64039.52, SD=10965.80) and lowest in Ho Chi Minh (3936.55, SD=340.65). The mean poverty rate was highest in Lai Chau (87.63%, SD=14.41%) and lowest in Binh Duong in the Southeast (5.07%, SD=5.14%). Nationally, there has been a decrease in the poverty rate from 30.11% in 1998 to 8.17% in 2016. The mean number of hospitals per 1000km^2 was highest in Ho Chi Minh (23.30, SD= 3.68) and lowest in Quang Binh on the Central Coast (0.87, SD=0.21) (figure 2.20).

In 1998, ten provinces in northern Vietnam received the JE vaccine with a mean coverage of three doses of 93.23%. In 2015, all provinces except Cao Bang in the Northern Midlands and Mountains received the vaccine with a mean coverage of 93.13%. Where vaccination was rolled-out annual coverage ranged from 4.74 in Ho Chi Minh in 2008 to 100% in a number of provinces (figure 2.21).

Figure 2.19 The mean elevation by province weighted by population density.

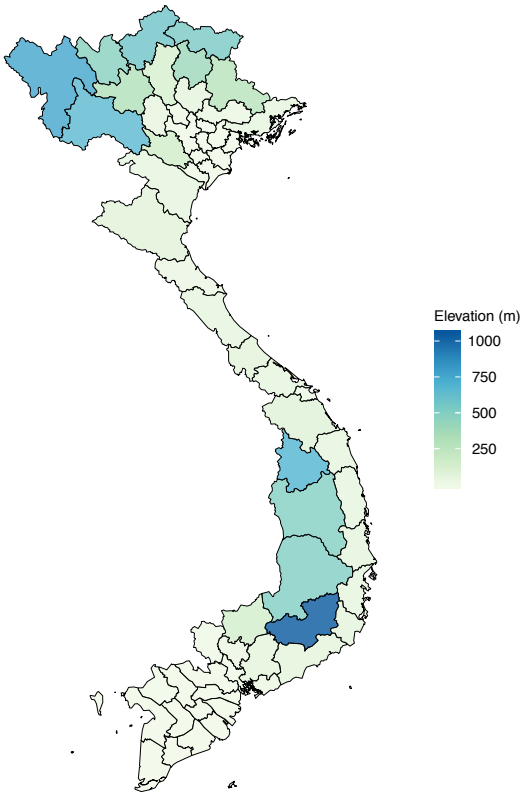


Figure 2.20 The mean NDVI, number of pigs per 100,000 population; poverty rate and number of hospitals per 1000 km^2 per province from 1998-2016.

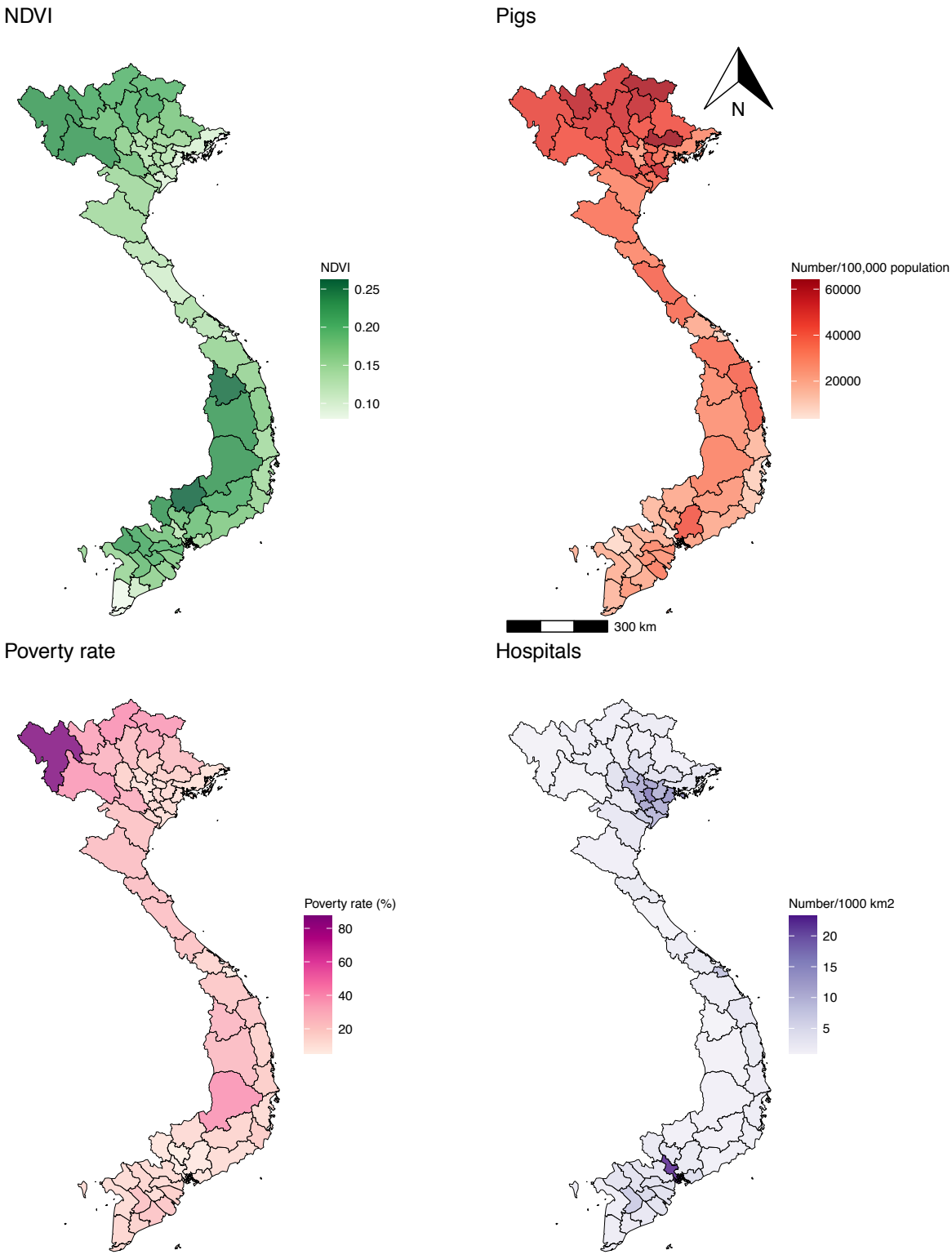
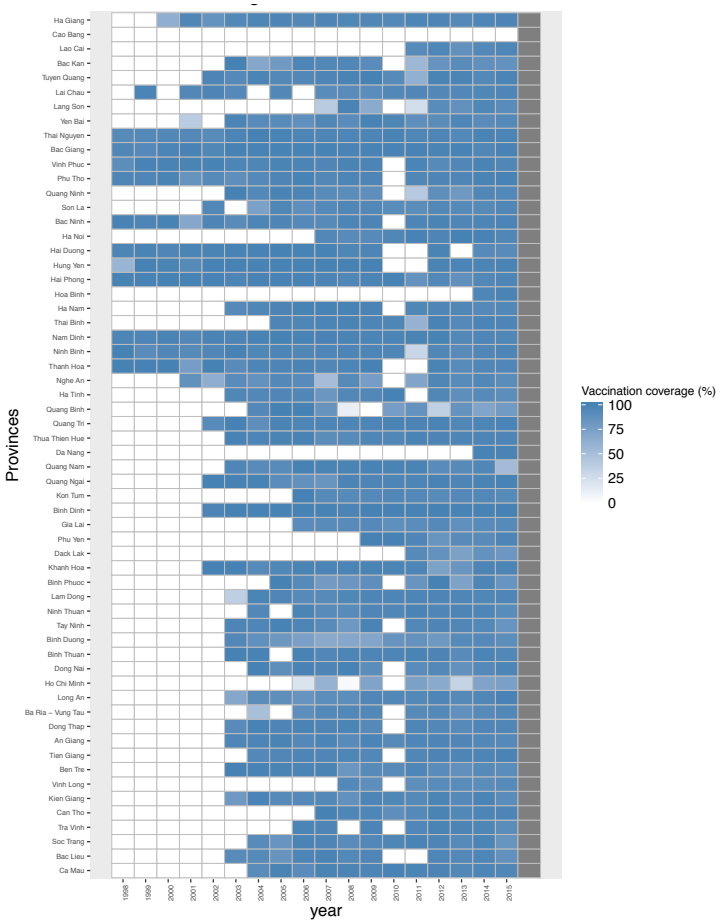


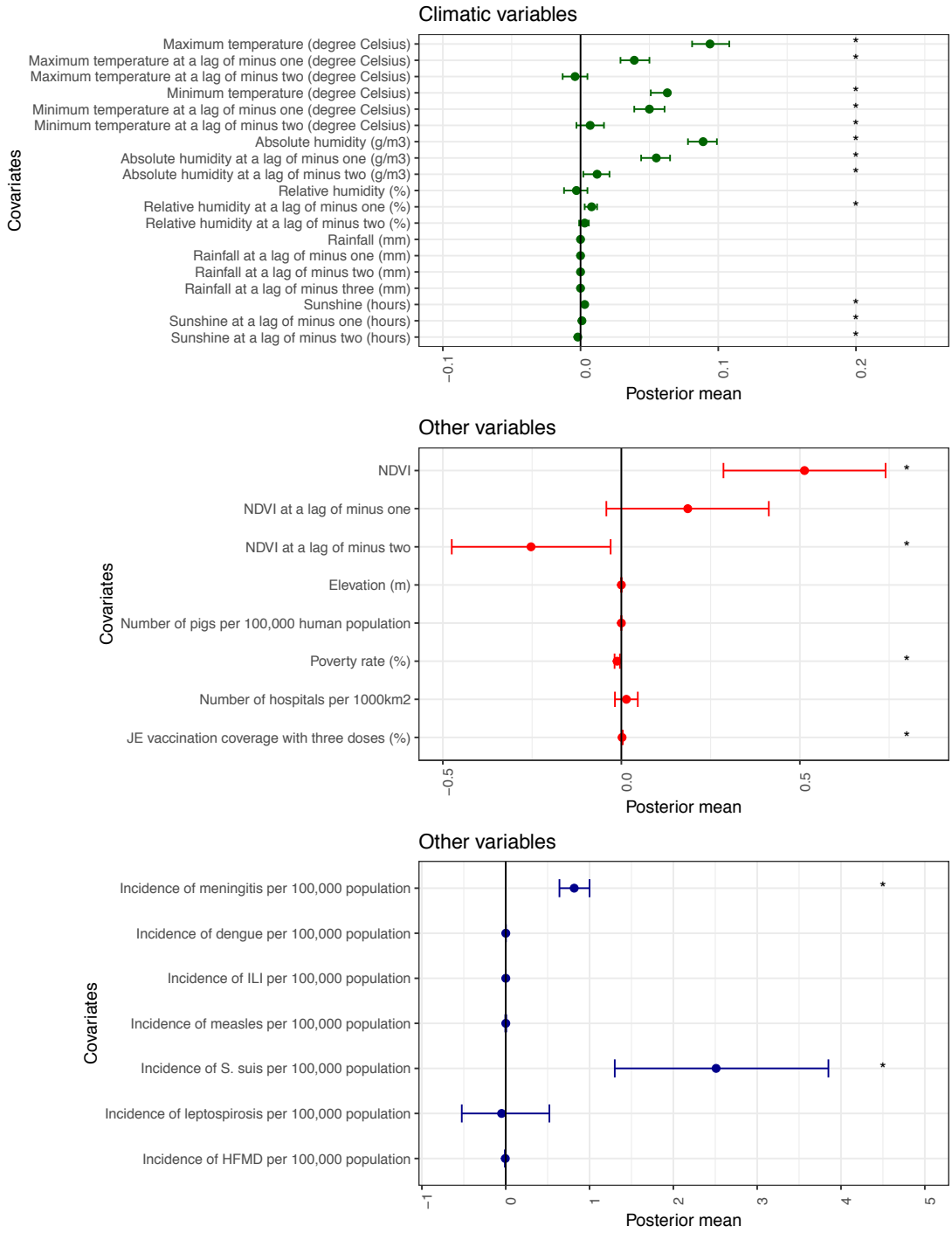
Figure 2.21 Three dose vaccination coverage against JEV by province and year.



UNIVARIATE ANALYSES: OUTCOME OF THE NUMBER OF CASES OF AES

In all negative binomial GAMs there was evidence of spatial autocorrelation of the residuals ($p<0.001$) (table A2.9, appendix 2.1). Univariate INLA models showed a positive association of maximum and minimum temperature with the number of cases of AES (posterior mean=0.094, 95% credible interval=0.081-0.108; and posterior mean=0.063, 95% credible interval=0.051-0.076, respectively) including at a lag of one month (posterior mean=0.039, 95% credible interval=0.029-0.050; and posterior mean=0.050, 95% credible interval=0.039-0.061); and absolute humidity including with a lag of one and two months (posterior mean=0.089, 95% credible interval=0.078-0.099, posterior mean=0.055, 95% credible interval=0.044-0.065). Relative humidity was positively associated at a lag of one month (posterior mean=0.008, 95% credible interval=0.003-0.012) and rainfall showed no association with the number of cases. Sunshine was positively associated with the number of cases (posterior mean=0.003, 95% credible interval=0.002-0.003) including at a lag of one month (posterior mean=0.001, 95% credible interval=0.001-0.002) but negatively associated at a lag of two months (posterior mean= -0.002, 95% credible interval= -0.002- (-0.001). NDVI was positively associated with the number of cases (posterior mean=0.513, 95% credible interval=0.286-0.740) but showed no association at a lag of one month and was negatively associated with a lag of two months (posterior mean = -0.253, 95% credible interval= -0.475 – (-0.030). Elevation, the number of pigs per 100,000 population and the number of hospitals per 1000km^2 showed no association with the number of cases but JE vaccination coverage showed a positive association. Incidence of meningitis and *S. suis* were the only syndromes/pathogens to show a positive association with the number of cases of AES with the others showing no association (figure 2.22).

Figure 2.22 The posterior means of the INLA univariate models by each covariate with an outcome of number of cases of AES. The dot represents the posterior mean value and bars represent the 95% credible intervals. The presence of an asterix (*) indicates 95% credible intervals which do not cross zero.



CORRELATION BETWEEN THE COVARIATES

The greatest correlation between climatic covariates was seen between the absolute humidity and maximum temperature (0.90); the absolute humidity and minimum temperature (0.88) and the maximum and minimum temperatures (0.85) (figure 2.23). There was no evidence of a strong correlation between any of the pathogens/syndromes however, he greatest correlation between pathogens/syndromes was seen between HFDM and ILI (0.17); AES and ILI (0.12) and dengue and ILI (-0.10) (figure 2.24).

Figure 2.23 Matrix of correlation coefficients between different climatic covariates using Pearson's correlation coefficients. The size of the dot corresponds to the magnitude of correlation with red being a negative correlation and blue being positive.

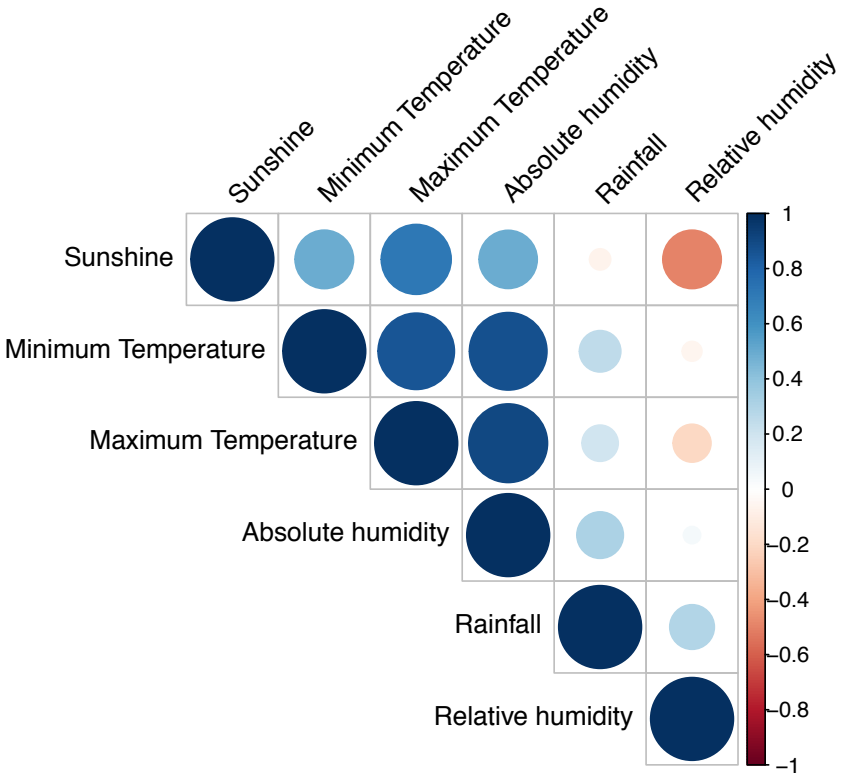
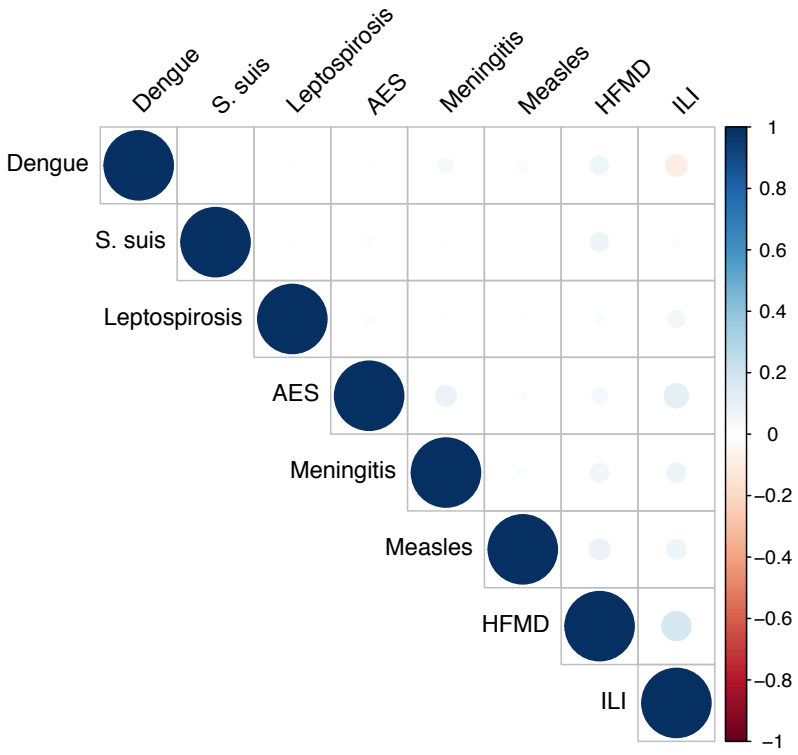


Figure 2.24 Matrix of correlation coefficients between different pathogens and syndromes using Pearson's correlation coefficients. The size of the dot corresponds to the magnitude of correlation with red being a negative correlation and blue being positive.

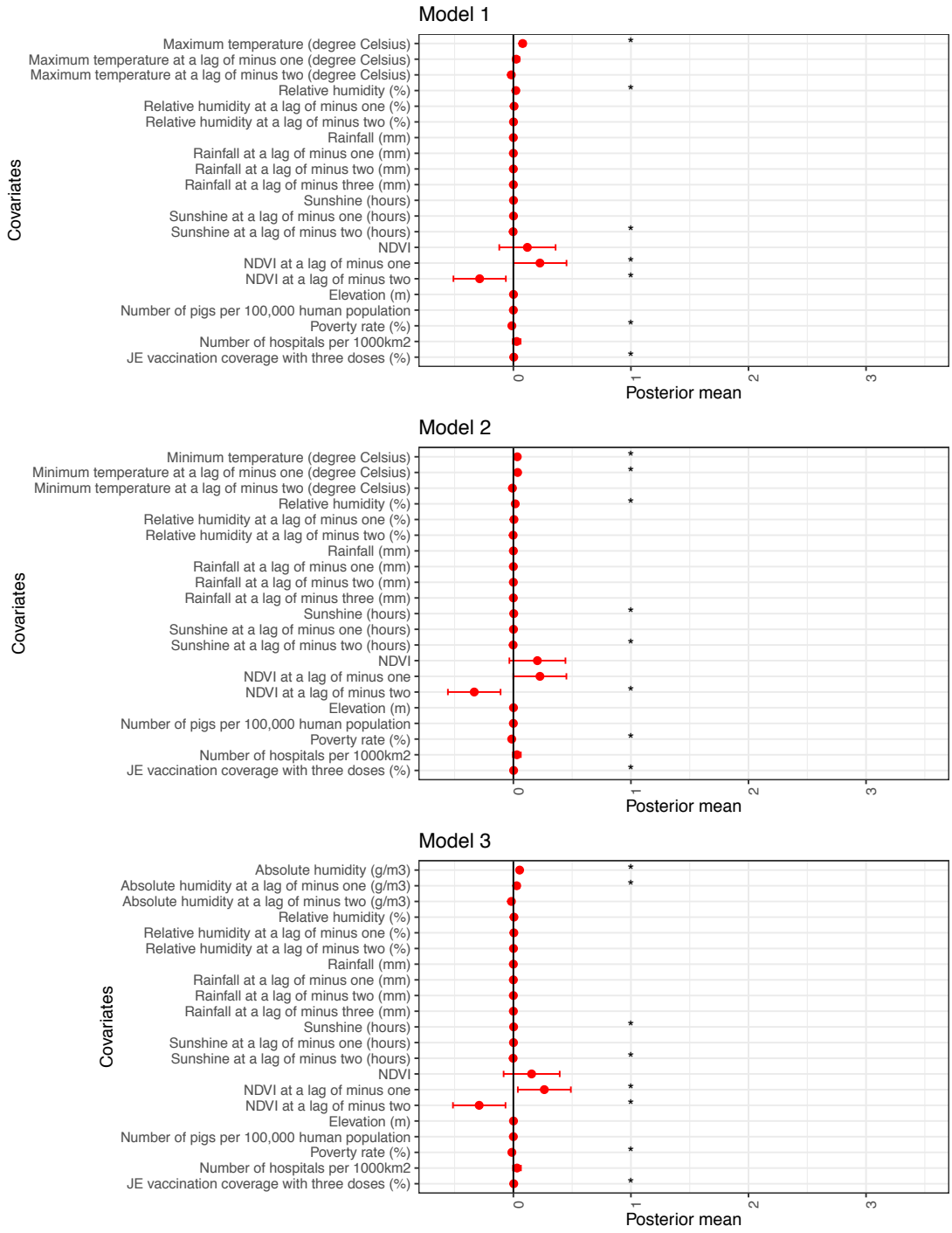


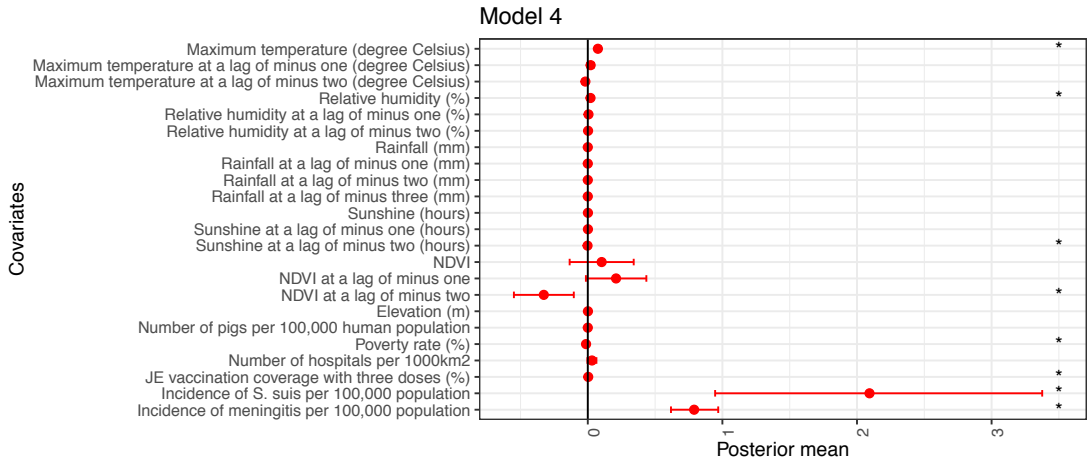
MULTIVARIATE ANALYSES: OUTCOME OF THE NUMBER OF CASES OF AES

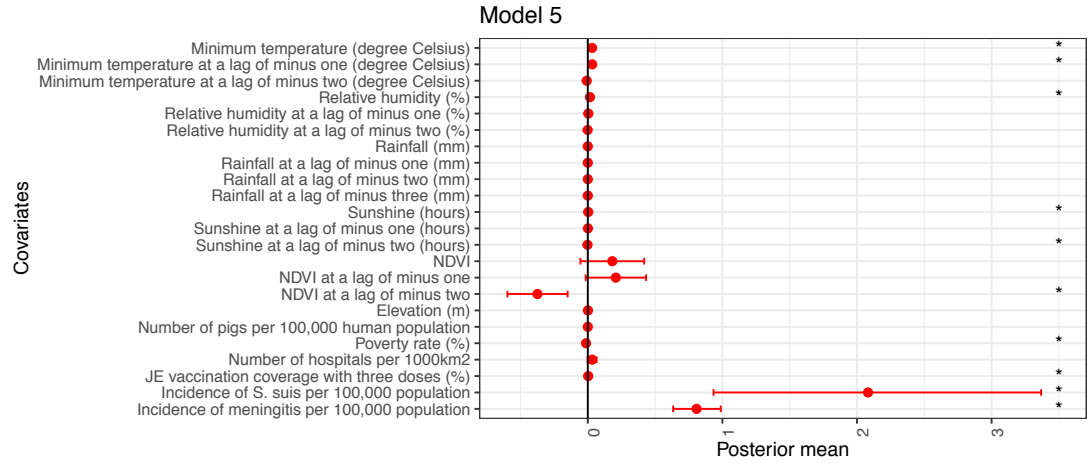
In the multivariate analysis, maximum temperature, minimum temperature and absolute humidity were positively associated with the number of cases of AES in all four models. Minimum temperature and absolute humidity were also positively associated at a lag of one month. Relative humidity was positively associated but only in models 1, 2 and 4 and there was no effect of rainfall on the number of cases. Sunshine was positively associated with the number of cases of AES in models 2, 3, 5 and 6 and positively associated at a lag of minus two months in all models (figure 2.25).

NDVI at a lag of one month was positively associated with the number of cases of AES in models 1, 3 and 6 and a lag of two months, negatively associated with the number of cases of AES in all models. Poverty rate was negatively associated and JE vaccination coverage positively associated with the number of cases of AES in all models but elevation, the number of pigs per 100,000 population and the number of hospitals per 1000km^2 showed no association in any models. The incidence of meningitis and *S. suis* were positively associated with the number of cases of AES in models 4, 5 and 6 (figure 2.25 and tables A2.11-A2.16, appendix 2.1). The model with the lowest DIC and WAIC was 4, (43917.46 and 44049.91, respectively) and highest, 2 (44047.71 and 44180.42, respectively) (table 2.4). In all models, the estimate of risk was highest in the provinces of Lai Chau in the Northern Midlands and Mountains (ranging from a posterior mean of 1.85 in model 3 to 1.97 in model 5) and Son La also in the Northern Midlands and Mountains (ranging from a posterior mean of 1.81 in model 5 to 1.94 in model 1), both of which are in northern Vietnam bordering Lao PDR. The estimate of risk was lowest in Phu Yen on the Central Coast, ranging from a posterior mean of -1.97 in model 4 to -2.17 in model 2 and Bac Kan in the Northern Midlands and Mountains ranging from a posterior mean of -1.38 in model 6 to -1.48 in model 2 (figure 2.26).

Figure 2.25 The posterior means of the INLA multivariate models by incidence of pathogens/syndromes with an outcome of number of cases of AES. The dot represents the posterior mean values and bars represent the 95% credible intervals.




Model 4
Covariates
Maximum temperature (degree Celsius)
Maximum temperature at a lag of minus one (degree Celsius)
Maximum temperature at a lag of minus two (degree Celsius)
Relative humidity (%)
Relative humidity at a lag of minus one (%)
Relative humidity at a lag of minus two (%)
Rainfall (mm)
Rainfall at a lag of minus one (mm)
Rainfall at a lag of minus two (mm)
Rainfall at a lag of minus three (mm)
Sunshine (hours)
Sunshine at a lag of minus one (hours)
Sunshine at a lag of minus two (hours)
NDVI
NDVI at a lag of minus one
NDVI at a lag of minus two
Elevation (m)
Number of pigs per 100,000 human population
Poverty rate (%)
Number of hospitals per 1000km2
JE vaccination coverage with three doses (%)
Incidence of S. suis per 100,000 population
Incidence of meningitis per 100,000 population
0
1
2
3
Posterior mean


Model 5
Covariates
Minimum temperature (degree Celsius)
Minimum temperature at a lag of minus one (degree Celsius)
Minimum temperature at a lag of minus two (degree Celsius)
Relative humidity (%)
Relative humidity at a lag of minus one (%)
Relative humidity at a lag of minus two (%)
Rainfall (mm)
Rainfall at a lag of minus one (mm)
Rainfall at a lag of minus two (mm)
Rainfall at a lag of minus three (mm)
Sunshine (hours)
Sunshine at a lag of minus one (hours)
Sunshine at a lag of minus two (hours)
NDVI
NDVI at a lag of minus one
NDVI at a lag of minus two
Elevation (m)
Number of pigs per 100,000 human population
Poverty rate (%)
Number of hospitals per 1000km2
JE vaccination coverage with three doses (%)
Incidence of S. suis per 100,000 population
Incidence of meningitis per 100,000 population
0
1
2
3
Posterior mean

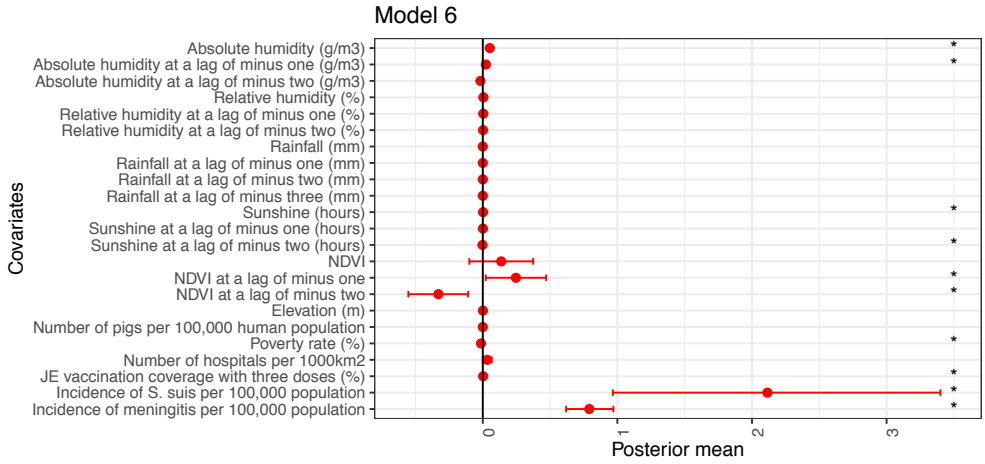
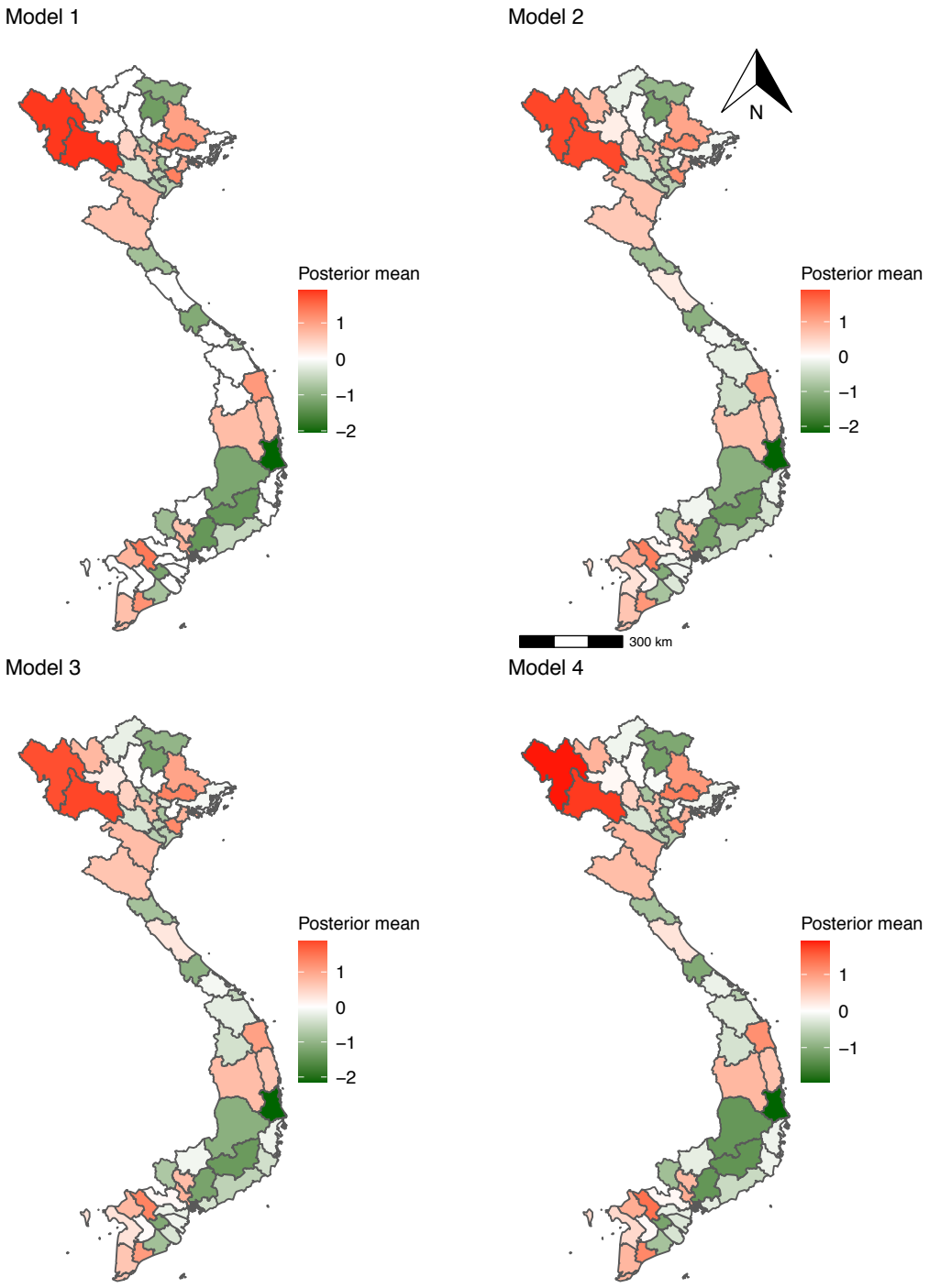
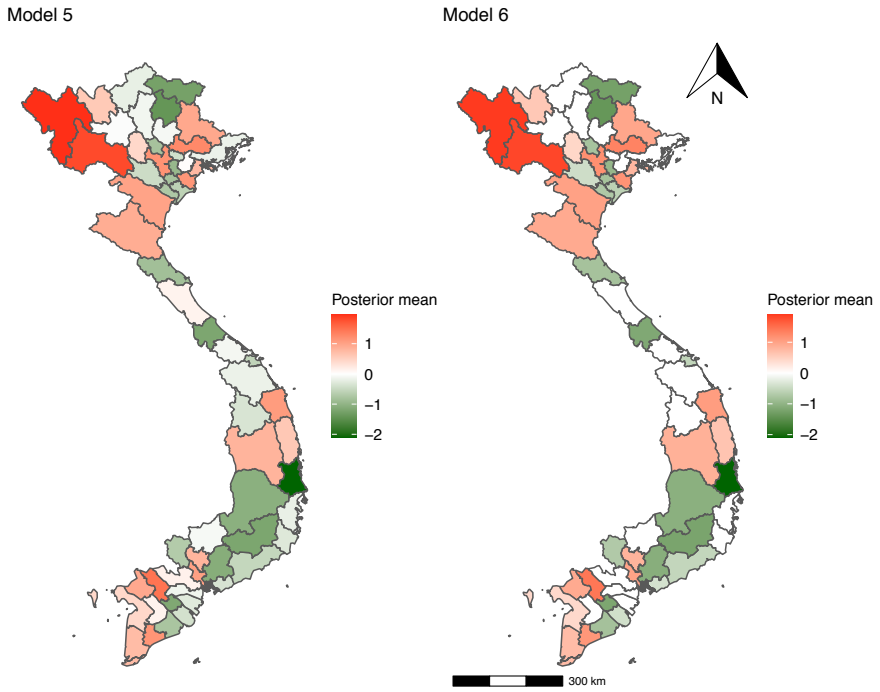

Model 6
Covariates
Absolute humidity (g/m3)
Absolute humidity at a lag of minus one (g/m3)
Absolute humidity at a lag of minus two (g/m3)
Relative humidity (%)
Relative humidity at a lag of minus one (%)
Relative humidity at a lag of minus two (%)
Rainfall (mm)
Rainfall at a lag of minus one (mm)
Rainfall at a lag of minus two (mm)
Rainfall at a lag of minus three (mm)
Sunshine (hours)
Sunshine at a lag of minus one (hours)
Sunshine at a lag of minus two (hours)
NDVI
NDVI at a lag of minus one
NDVI at a lag of minus two
Elevation (m)
Number of pigs per 100,000 human population
Poverty rate (%)
Number of hospitals per 1000km2
JE vaccination coverage with three doses (%)
Incidence of S. suis per 100,000 population
Incidence of meningitis per 100,000 population
0
1
2
3
Posterior mean

Table 2.4 The DICs and WAICs of the multivariate INLA models in figure 2.25.

Model	DIC	WAIC
1	44023.26	44155.75
2	44047.71	44180.42
3	44026.70	44158.98
4	43917.46	44049.91
5	43939.64	44072.36
6	43920.87	44053.27

Figure 2.26 The predicted posterior mean from each of the models by province. Only those provinces in which the 95% credible interval did not cross zero are included.



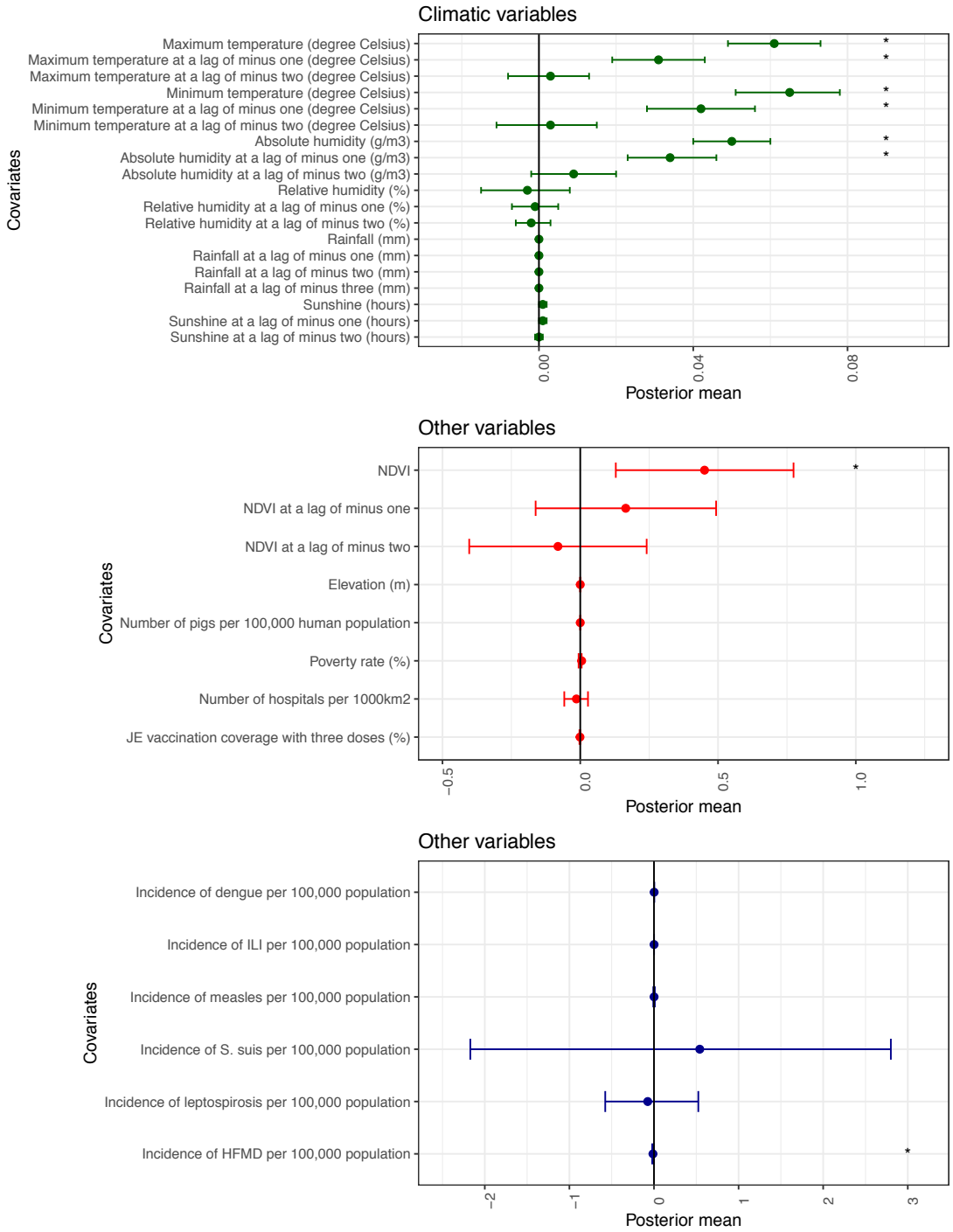


UNIVARIATE ANALYSES: OUTCOME OF THE NUMBER OF CASES OF MENINGITIS

In all GAM models, there was evidence of spatial autocorrelation of the residuals with a p<0.001 (table A2.11, appendix 2.1). Using INLA, there was evidence of a positive association of maximum and minimum temperature (posterior mean=0.061, 95% credible interval=0.049-0.073; and posterior mean= =0.065, 95% credible interval=0.051-0.078) with the number of cases of meningitis including at a lag of one month (posterior mean=0.031, 95% credible interval=0.019-0.043; and posterior mean=0.042, 95% credible interval=0.028-0.056, respectively); and absolute humidity (posterior mean=0.05, 95% credible interval=0.04-0.06) including at a lag of one month (posterior mean=0.034, credible interval=0.023-0.046). Other climatic variables did not show any correlation with the number of cases. NDVI was positively associated with the number of cases of meningitis (posterior mean=0.451, 95% credible interval=0.129-0.774) but showed no association at a lag of one or two months. Elevation, the number of pigs per 100,000 population, the number of hospitals per 1000km^2 and JE vaccination showed no association with the number of cases. HFMD was the only pathogen to show an association with the number of cases of meningitis which was negative (posterior mean= -0.012, 95% credible interval= -

0.012 – (-0.003) with the other syndromes/pathogens showing no association (figure 2.27).

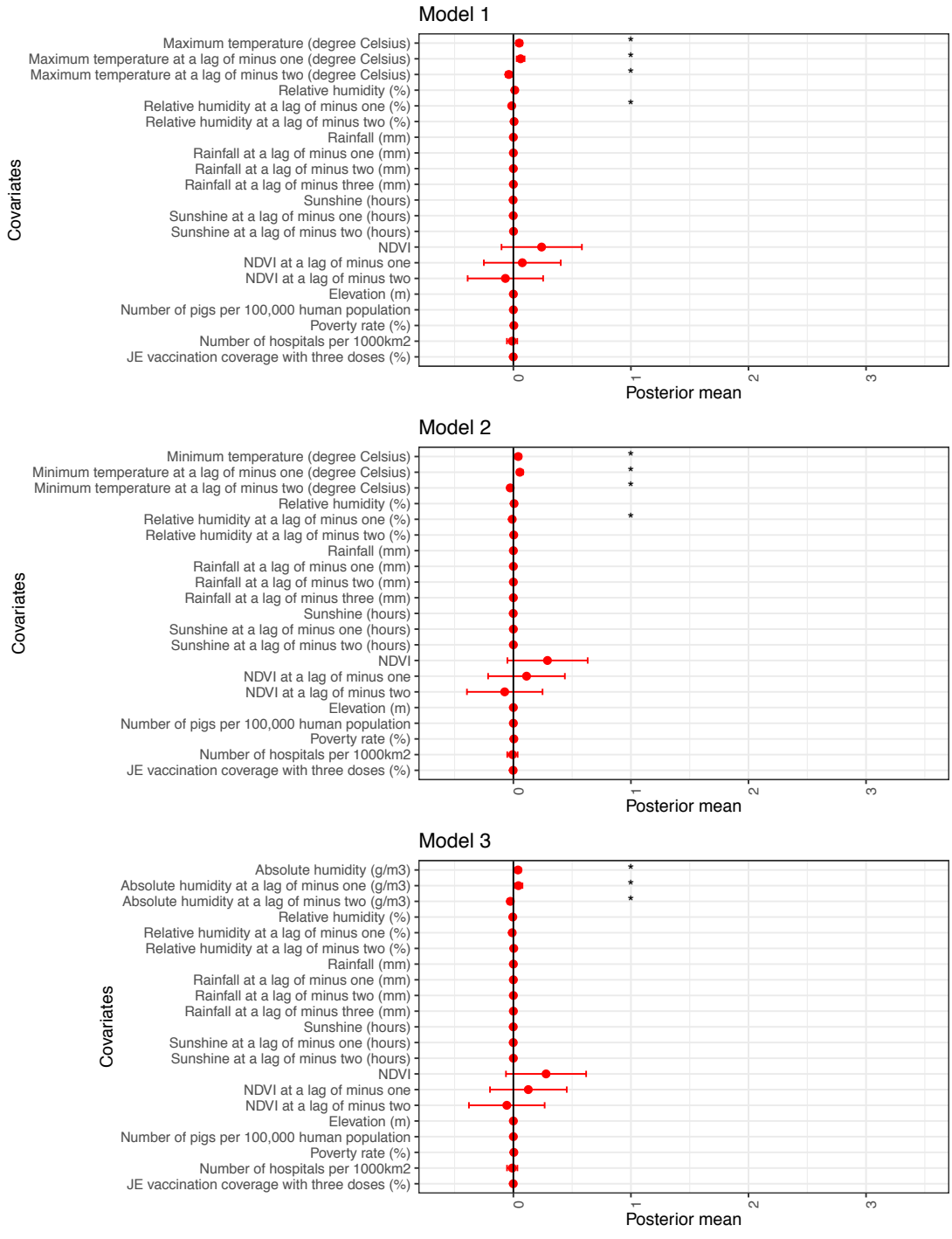
Figure 2.27 The posterior means of the INLA univariate models by each covariate with an outcome of number of cases of meningitis. The dot represents the posterior mean value and bars represent the 95% credible intervals. The presence of an asterix (*) indicates 95% credible intervals which do not cross zero.



MULTIVARIATE ANALYSES: OUTCOME OF THE NUMBER OF CASES OF MENINGITIS

In the multivariate analysis, maximum temperature and minimum temperatures and absolute humidity including at a lag of one month were positively associated with the number of cases of meningitis and at a lag of two months, negatively associated. Relative humidity was negatively associated at a lag of one month. Rainfall and sunshine showed no correlation with the number of cases and there was no association between other covariates and the cases of meningitis with the exception of the incidence of HFMD which was negatively associated (figure 2.28 and tables A2.17-A2.22, appendix 2.1). The DIC and WAIC ranged from 23733.78 and 23814.86, respectively in model 4 to 23745.28 and 23825.56 in model 2 (table 2.5). In all models, the estimate of risk was highest in the provinces of Son La, in the Northern Midlands and Mountains (ranging from a posterior mean of 2.24 in model 6 to 2.30 in model 1) and Thai Binh in the Red River Delta (ranging from a posterior mean of 1.99 in model 2 to 2.01 in model 4). The estimate of risk was lowest in Bac Kan in the Northern Midlands and Mountains ranging from a posterior mean of –2.23 in model 2 to -2.28 in model 4 and Thua Thien Hue on the Central Coast ranging from a posterior mean -2.16 in model 2 to -2.23 in model 4 (figure 2.29).

Figure 2.28 The mean posterior estimates of the INLA multivariate models by incidence of pathogens/syndromes with an outcome of the number of cases of meningitis.



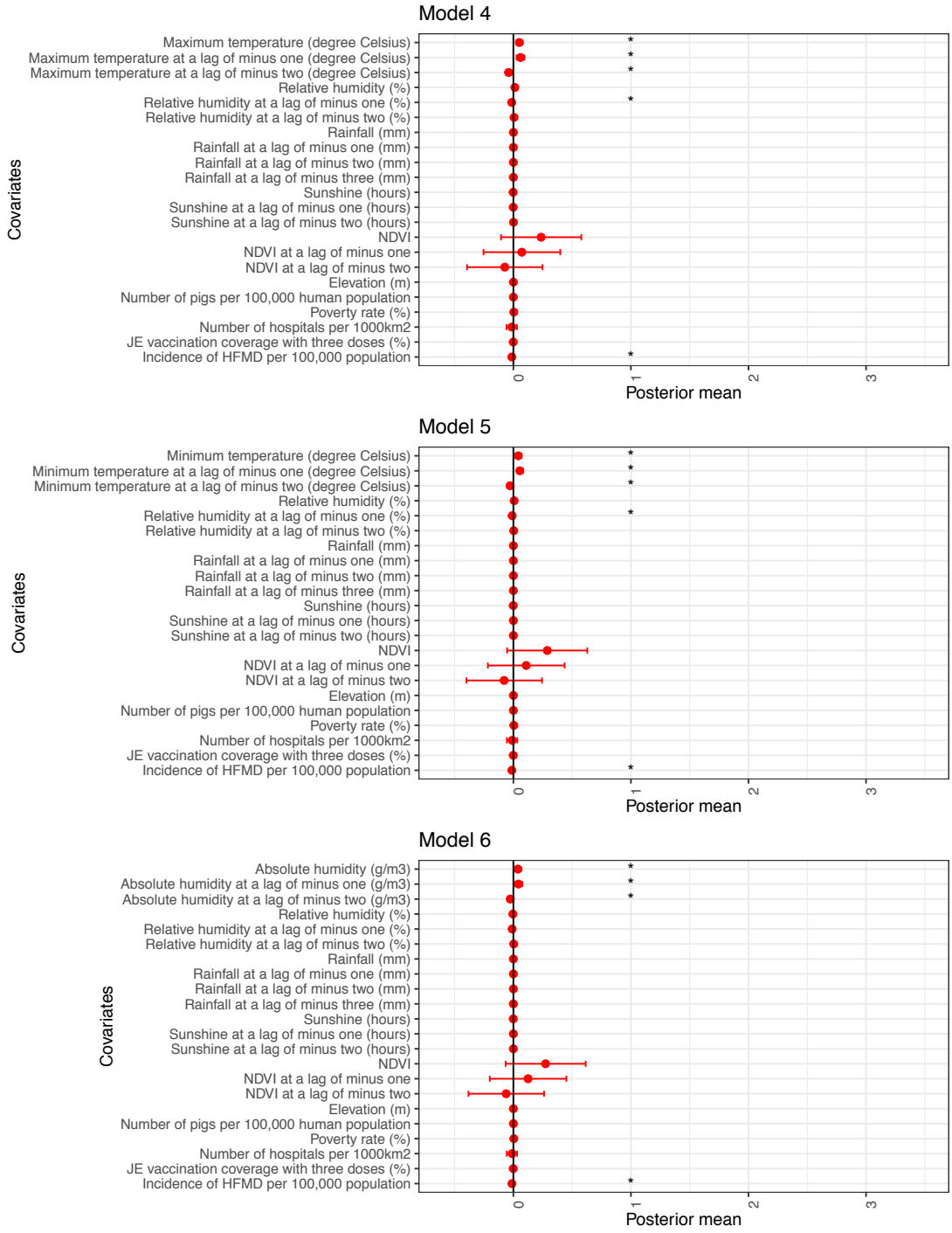
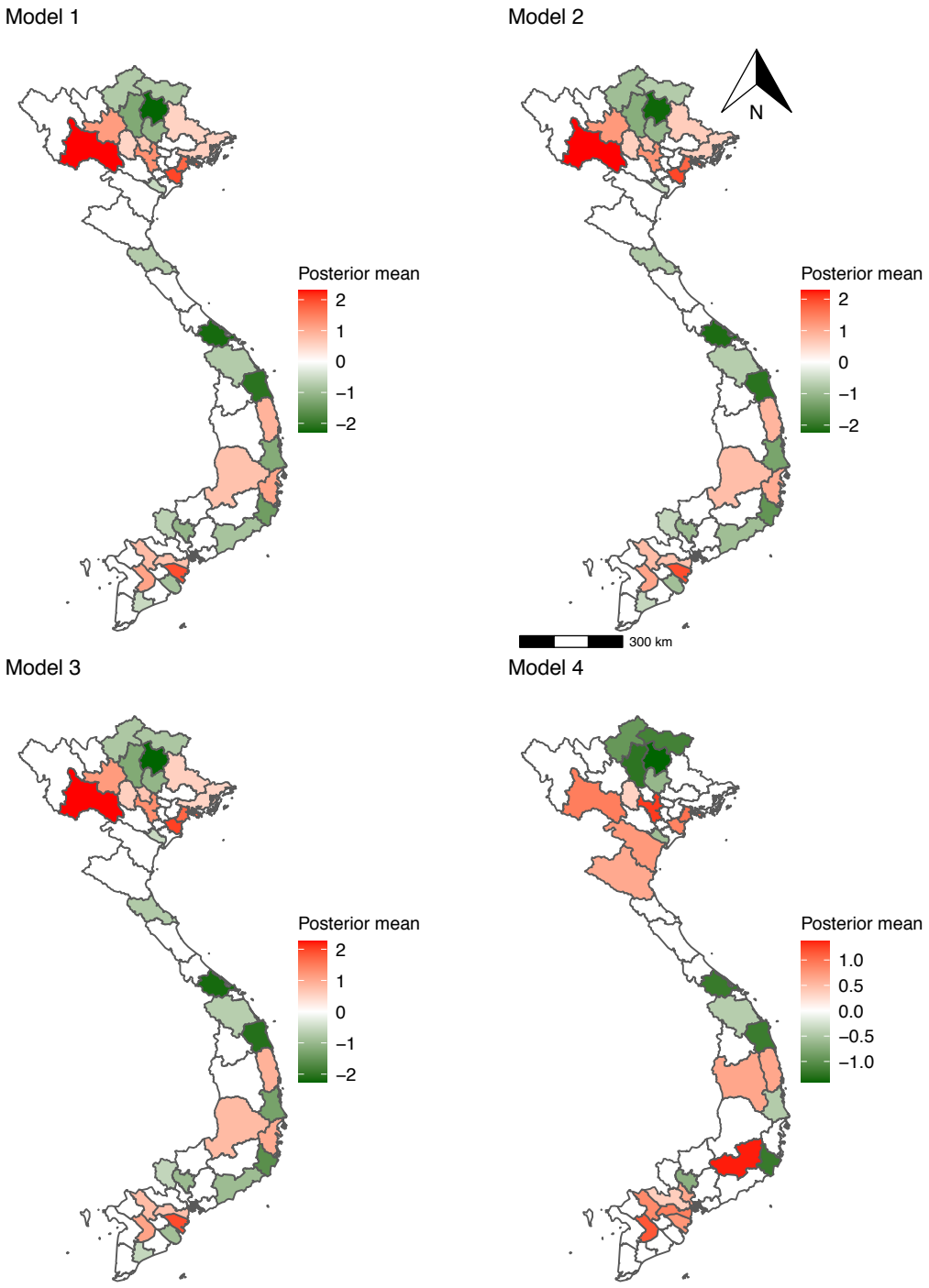
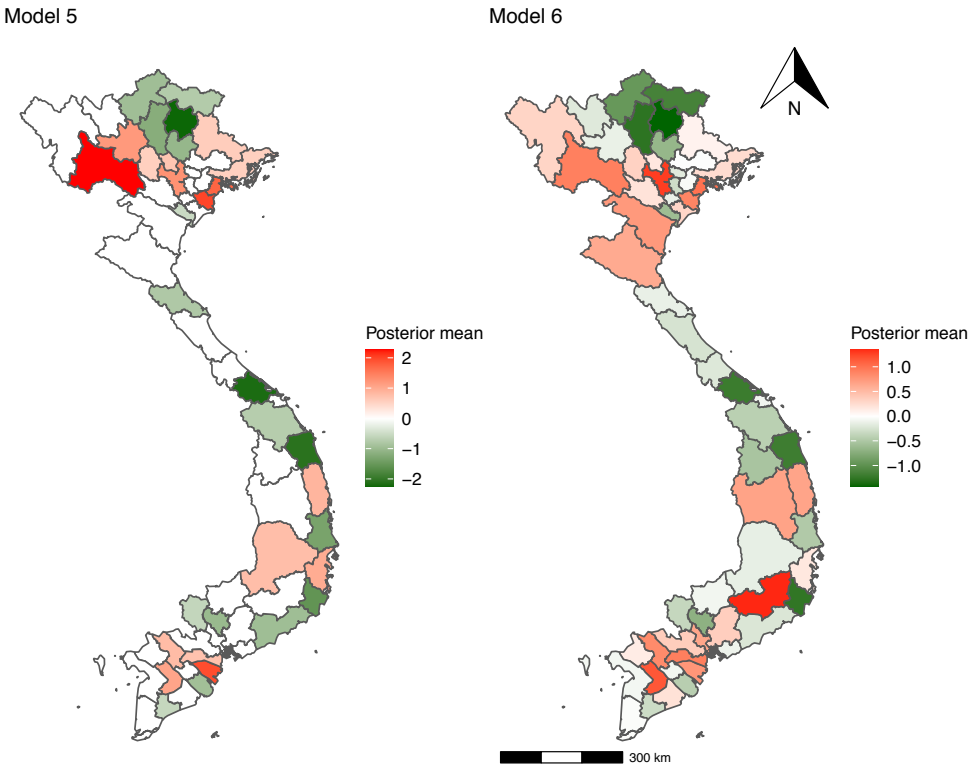

Model 4
Covariates
Maximum temperature (degree Celsius)
Maximum temperature at a lag of minus one (degree Celsius)
Maximum temperature at a lag of minus two (degree Celsius)
Relative humidity (%)
Relative humidity at a lag of minus one (%)
Relative humidity at a lag of minus two (%)
Rainfall (mm)
Rainfall at a lag of minus one (mm)
Rainfall at a lag of minus two (mm)
Rainfall at a lag of minus three (mm)
Sunshine (hours)
Sunshine at a lag of minus one (hours)
Sunshine at a lag of minus two (hours)
NDVI
NDVI at a lag of minus one
NDVI at a lag of minus two
Elevation (m)
Number of pigs per 100,000 human population
Poverty rate (%)
Number of hospitals per 1000km2
JE vaccination coverage with three doses (%)
Incidence of HFMD per 100,000 population
0
1
2
3
Posterior mean
Model 5
Covariates
Minimum temperature (degree Celsius)
Minimum temperature at a lag of minus one (degree Celsius)
Minimum temperature at a lag of minus two (degree Celsius)
Relative humidity (%)
Relative humidity at a lag of minus one (%)
Relative humidity at a lag of minus two (%)
Rainfall (mm)
Rainfall at a lag of minus one (mm)
Rainfall at a lag of minus two (mm)
Rainfall at a lag of minus three (mm)
Sunshine (hours)
Sunshine at a lag of minus one (hours)
Sunshine at a lag of minus two (hours)
NDVI
NDVI at a lag of minus one
NDVI at a lag of minus two
Elevation (m)
Number of pigs per 100,000 human population
Poverty rate (%)
Number of hospitals per 1000km2
JE vaccination coverage with three doses (%)
Incidence of HFMD per 100,000 population
0
1
2
3
Posterior mean
Model 6
Covariates
Absolute humidity (g/m3)
Absolute humidity at a lag of minus one (g/m3)
Absolute humidity at a lag of minus two (g/m3)
Relative humidity (%)
Relative humidity at a lag of minus one (%)
Relative humidity at a lag of minus two (%)
Rainfall (mm)
Rainfall at a lag of minus one (mm)
Rainfall at a lag of minus two (mm)
Rainfall at a lag of minus three (mm)
Sunshine (hours)
Sunshine at a lag of minus one (hours)
Sunshine at a lag of minus two (hours)
NDVI
NDVI at a lag of minus one
NDVI at a lag of minus two
Elevation (m)
Number of pigs per 100,000 human population
Poverty rate (%)
Number of hospitals per 1000km2
JE vaccination coverage with three doses (%)
Incidence of HFMD per 100,000 population
0
1
2
3
Posterior mean

Table 2.5 The DIC and WAIC of multivariate models in figure 2.28 with an outcome of the number of cases of meningitis.

Model	DIC	WAIC
1	23740.83	23821.04
2	23745.28	23825.56
3	23741.23	23822.74
4	23733.78	23814.86
5	23738.95	23820.30
6	23734.79	23817.52

Figure 2.29 Choropleth maps to show the predictions from the models in figure 2.29 as shown by the estimate (posterior mean).




Model 5
Posterior mean
2
1
0
−1
−2
Model 6
N
Posterior mean
1.0
0.5
0.0
−0.5
−1.0
300 km

2.4 DISCUSSION

A number of studies have been conducted in Vietnam, and regionally, exploring the aetiology, and on occasion, the spatio-temporal distribution of CNS infections. However, the majority of these have been cross-sectional studies conducted at a few sites. This is the first known study in Vietnam to explore the spatio-temporal distribution of both AES and meningitis at the national level using surveillance data over decades. Whilst other studies have utilised national surveillance data to explore the spatio-temporal distribution of pathogens causing CNS infections, none have explored the differences between these. This was therefore the first objective of this study.

OBJECTIVE 1: TO COMPARE AND CONTRAST THE SPATIO-TEMPORAL DISTRIBUTION OF AES AND MENINGITIS IN VIETNAM WITH OTHER SYNDROMES AND PATHOGENS

A decline in both the national incidence of AES and meningitis was seen over time. In the case of AES this becomes particularly apparent from 2005 onwards. However, increases in incidence of AES were seen in provinces in northern Vietnam which border Lao PDR and those in the central highlands with the mean incidence of AES and meningitis over the study period highest in Son La province. A clear effect of seasonality with peaks of incidence of AES in the summer months of June and July was seen in the northern but not southern provinces of Vietnam. A higher incidence of meningitis is seen in Son La and Yen Bai in northern Vietnam in the summer months but there was no clear difference in seasonality by latitude. If we compare the spatio-temporal distribution of AES and meningitis with other pathogens, it is difficult to find any clear similarities with the exception of JE and AES which both peak in the summer. The incidence of dengue was strongly seasonal yet the peaked occurred slightly later than AES in July and August and there were very few cases in northern Vietnam. Furthermore, unlike AES and meningitis there is not a decline in cases of dengue over time but instead, there was evidences of epidemics in 1998, 2010 and 2016. Although the spatial pattern of ILI showed similarities with AES and meningitis with the highest incidence in northern Vietnam and the central highlands, the peaks tended to be later than those of AES. The incidence of measles was also highest in the northern provinces bordering Lao PDR however, peaks in incidence were seen from March to May. Overall, the incidence of HFMD was is highest in southern Vietnam with peaks in the months of September to November. The incidence of

leptospirosis was higher in northern Vietnam with more cases in the summer months however, the number of cases was comparatively lower making it more difficult to draw comparisons. Similarly, due to the lower number of cases of *S. suis*, possibly due to under-reporting it was difficult to compare this pathogen (Huong *et al.*, 2019).

Given the evidence for spatial autocorrelation in the GAM models, Bayesian modelling was used in the form of INLA with neighbouring province added as a random effect. Results from these models showed that *S. suis* and meningitis were the only pathogens/syndromes positively associated with the number of cases of AES in the univariate analysis and remained so in the multivariate models. This is possibly due to the specificity of the case definition of AES which is discussed further in the next chapter (Venkatesan *et al.*, 2013) whereby clinicians are reporting some cases of meningitis as AES and vice versa. As the positive association between HFMD and meningitis present in the univariate analysis when adjusting for other covariates was reversed in the multivariate analysis, it can be concluded that there was no association between any of the pathogens/syndromes (with the exception of AES) and meningitis.

OBJECTIVE 2: TO ESTIMATE POPULATION LEVEL RISK FACTORS FOR AES AND MENINGITIS IN VIETNAM

The second objective of this research was to estimate the population level risk factors for AES and meningitis in Vietnam. In the multivariate analysis, there is a correlation between temperature and absolute humidity and with the exception of two models, relative humidity and the number of cases of AES, and between temperature and absolute humidity and the number of cases of meningitis. In the descriptive analysis, it is evident that the seasonal pattern of temperature and absolute humidity corresponds with the spatio-temporal distribution of incidence of AES.

Climatic factors affect the transmission dynamics of a number of pathogens (Metcalf *et al.*, 2017). As described in chapter one, of the potential pathogens causing AES, vector-borne diseases are particularly influenced by climate as the breeding and survival of mosquitoes and other vectors such as chiggers is dependent on temperature and humidity (Keiser *et al.*, 2005; Niaz and Reisen, 1981; Liu *et al.*, 2018;

Reinhold, Lazzari and Lahondere, 2018; Delatte *et al.*, 2009; Lv, Guo and Jin, 2018; Xu and Chen, 1960; Traub and Wisseman, 1974). As a result, there is evidence of seasonality of diseases such as JE, dengue and scrub typhus corresponding with the climatic factors (Solomon *et al.*, 2000a; Lowe *et al.*, 2011; Xu *et al.*, 2017). Other pathogens causing CNS infections also show seasonality such as enterovirus 71, causing HFMD, *S. suis* and influenza virus (Chang *et al.*, 2012; Wertheim *et al.*, 2009a; Wertheim *et al.*, 2009b; Thai *et al.*, 2015). Like the vector-borne diseases, influenza has been shown to be positively associated with absolute humidity (Thai *et al.*, 2015) and HFMD with temperature and relative humidity (Chang *et al.*, 2012). In Vietnam, the incidence of *S. suis* also corresponds spatio-temporally with that of AES (Wertheim *et al.*, 2009b; Nghia *et al.*, 2011). However, whether the climate has a direct impact on these pathogens or whether it is due to human behaviour remains to be determined. In some of the multivariate models there is evidence of a positive association between temperature and humidity at a lag of one month and the number of cases of AES or meningitis. In the case of vector-borne diseases, this is likely to be accounted for by the time taken for the vector to complete its life-cycle and subsequently transmit the pathogen to humans as was demonstrated in a study of JE in Nepal (Impoinvil *et al.*, 2011). Interestingly, in this study there was no evidence of an association between rainfall and the number of cases of AES and meningitis despite studies showing evidence of an association between this climatic factor and the survival of mosquitoes (Reisen, Aslamkhan and Basio, 1976; Longbottom *et al.*, 2017; Koenraadt and Harrington, 2008; Seidahmed and Eltahir, 2016) and in some situations the number of cases of leptospirosis (Chadsuthi *et al.*, 2012; Triampo *et al.*, 2007).

Of the non-climatic variables, NDVI was positively associated with the number of cases of AES with a lag of one or two months in selected multivariate models whereas no association was seen with the number of cases of meningitis. Other work has shown NDVI to be positively associated with cases of JE in China (Tian *et al.*, 2015) although this association is not necessarily linear and the risk may reverse over a particular threshold of greenness (Wang *et al.*, 2014). NDVI has also been shown to be positively associated with the incidence of scrub typhus in Taiwan (Kuo *et al.*, 2011) and WNV in the United States (Chuang and Wimberly, 2012). However, a negative association was seen between DENV and NDVI in Taiwan possibly due to the role of population density in transmission (Huang *et al.*, 2018). The lag of one or two months between NDVI and number of cases of AES in this study, might therefore

suggest that vector breeding and development may occur prior to transmission of the pathogen.

There was no association between the number of cases of AES or meningitis and elevation in any of the multivariate models. This is in keeping with the univariate analysis which also showed no association and the descriptive analysis which demonstrated that incidence of AES and meningitis was highest in provinces in the northwest of Vietnam and the Central Highlands which are at a higher altitude. Despite JE and *S. suis* being the most common pathogens causing CNS infections in children and adults respectively in Vietnam there was an absence of an association of pigs with cases of AES and meningitis in this study (Le *et al.*, 2010). However, although there is a potential risk of acquiring JEV from being in close proximity to pigs, this remains undetermined (Liu *et al.*, 2010; van den Hurk, Ritchie and Mackenzie, 2009). The greatest risk of *S. suis* is most likely to come from the consumption of or occupational exposure to raw pig blood or viscera (Huong *et al.*, 2014b; Nghia *et al.*, 2011). It is therefore possible that these activities are more frequent in provinces with higher numbers of pigs however, further work would be needed to evaluate this. There is a spatio-temporal association with PPRS and *S. suis* (Huong *et al.*, 2016) but it is unknown whether there is any relationship with the number of pigs per province at the national level. The poverty rate showed no association with either AES or meningitis despite highest incidence in provinces in northwest Vietnam and the Central Highlands which are poorer. It is possible that this is either not the driving force behind the cases or the difference in poverty rate for many provinces was quite similar, with Lai Chau being an outlier. Alternatively, there may be under-representation of those with CNS infections who are poorer as they are less likely to access healthcare. The inclusion of the number of hospitals per km^2 was to account for access to healthcare which may bias the results. Although we might have expected a negative association given the higher number of cases in rural areas, no association was seen in the multivariate analysis. This might suggest that cases are being under-reported in these areas or that this effect is not contributing to risk.

OBJECTIVE 3: TO DETERMINE THE POTENTIAL EFFECT OF THE JE VACCINE ON THE INCIDENCE OF AES AND MENINGITIS IN VIETNAM

The third objective of this work aimed to determine the potential effect of the JE vaccine on the incidence of AES and meningitis. In 1998 vaccination against JE was taking place in ten provinces. By 2015 this had expanded to all provinces except Cao Bang. In the multivariate models, vaccination coverage was positively associated with the number of cases of AES and showed no association with the number of cases of meningitis. Although a negative association might have been expected as has been shown in Nepal (Upreti et al., 2017), this result is possibly due to the presence of vaccination campaigns which tend to take place in response to outbreaks of JE.

OBJECTIVE 4: TO USE THE RESULTS FROM QUESTIONS 1, 2 AND 3 TO ESTIMATE THE AETIOLOGY OF AES AND MENINGITIS IN VIETNAM

In summary, this study suggests of the potential pathogens causing a CNS infections, the spatio-temporal distribution of JE most closely resembles that of AES. However, it is unknown whether all of the cases are in fact due to JEV without diagnostic information. It is therefore possible that other pathogens contribute towards the cases. Leptospirosis has a similar distribution to AES and meningitis however, as the numbers are relatively small further investigation would be recommended. The seasonality of ILI and measles do not correspond with that of AES but it is interesting that the incidence of these pathogens is also highest in the provinces bordering Lao PDR and the central highlands and it is possible that they may contribute to some of the cases. Additionally, is possible that *O. tsutsugamushi* which is a common cause of CNS infections in neighbouring countries (Dittrich *et al.*, 2015) and is also seasonal, contributes to some of these cases. Other pathogens known to cause CNS infections which are transmitted by mosquitoes and therefore influenced by the temperature and absolute humidity include WNV, ZIKV and CHIKV. Interestingly, there is no evidence that these are currently circulating widely in Vietnam despite suitable climatic conditions. The reasons for this are unknown however, it is possible that the circulation of both JEV and DENV may provide some immunity against other flaviviruses including WNV and ZIKV (Slon Campos, Mongkolsapaya and Screaton, 2018). Finally, we should continue to consider novel pathogens as a potential aetiology as was proposed by Vietnam Initiative on Zoonotic Infections (VIZIONS) (Rabaa *et al.*, 2015).

In the case of JE, we might wonder why incidence remains high despite good vaccination coverage. As JEV is zoonotic, it is not possible to obtain herd immunity through vaccination of the human population (World Health Organization, 2010). Therefore, 'pockets' of non-vaccinated individuals could continue to contribute to the relatively higher incidence of the disease in selected provinces. The reasons for a lower vaccination uptake amongst these populations could be wide ranging including vaccine hesitancy which has been shown to be associated with lower socioeconomic status (Larson *et al.*, 2014), poor access to locations where vaccination is taking place or inability to afford vaccines. Additionally, a porous border exists between Lao PDR and Vietnam (Okello *et al.*, 2015). Given that the JE vaccine was only introduced into the EPI in Lao PDR in 2015 (World Health Organization, 2015a) it would be worth exploring whether cases of JE in border provinces are occurring amongst Laotians.

LIMITATIONS AND FURTHER WORK

Ecological studies are observational studies where the unit of analysis is the population rather than the individual (Sedgwick, 2014). These types of study are often inexpensive and quick to undertake as data can often be obtained from surveys, registries or similar (Morgenstern, 1995). As data can be obtained from wide ranges in space and time ecological studies they are ideal for conducting spatio-temporal analysis (Meliker and Sloan, 2011) and may also be necessary if it is difficult to measure the exposure at the individual level, for example analyzing the effect of a population intervention (Morgenstern, 1995). However, as a result of aggregating the data, the studies are subject to ecological fallacy whereby the associations derived from the analysis are assumed to apply at the individual level (Sedgwick, 2014). Furthermore, adjustments for covariates at the population level may not provide a sufficient means of controlling for confounding (Morgenstern, 1995) particularly if the distribution of exposures and potential confounders within the groups studied is unknown (Guthrie, 2001). As a result of this, ecological studies may be better at generating rather than testing hypotheses (Morgenstern, 1995).

Given that this study is conducted at the provincial level it is subject to ecological fallacy (Piantadosi, Byar and Green, 1988). Therefore, we cannot make any assumptions about individual risk of CNS infections. Additionally, it should be acknowledged that surveillance data in particular is often subject to reporting biases

(Lee *et al.*, 2018) with potential differences in the interpretation of case definitions between medical staff and healthcare centres. Reporting rates may also have differed over time as the country grew economically, healthcare improved and the capacity for diagnostics increased. However, it is possible that a proportion of the population were missed either because they could not afford or did not have access to healthcare or, their symptoms were too mild to seek healthcare leading to selection biases.

In addition to the potential biases with the surveillance data, I did not have evidence about the accuracy of the covariates and whether this data was validated. For example, I did not know how the number of pigs per province were counted, how the measurements used to determine poverty rate were determined and the quality of the recordings obtained from the weather stations. As data did not exist for many covariates for each year, this was imputed. Although imputation can reduce biases associated with missing data, there are caveats, particularly if the data was not missing at random as was the case for this study. In such situations the imputation itself may lead to additional biases (Sterne *et al.*, 2009). For many of these covariates, it would be difficult to obtain data outside of the General Statistics Office for Vietnam. However, an alternative to using interpolated data from weather stations, climatic data can be obtained from satellite imagery such as land surface temperature (LST) (Li *et al.*, 2013b) but given the computational complexities with obtaining monthly provincial level data over this time period this was not utilized.

The accuracy of vaccination data can be particularly challenging as reported data often shows a higher coverage than is true (Murray *et al.*, 2003). It should also be noted that vaccination coverage acts only as a proxy for immunity to JEV. To better understand the effect of immunity we would need to take into account the age distribution of those vaccinated in each province, the vaccine efficacy and duration of protection, and the likelihood of natural immunity based on the force of infection.

Despite including data on the incidence of many pathogens, surveillance data was not available for a number of key pathogens causing CNS infections either in Vietnam or neighbouring countries including *O. tsutsugamushi* (Dittrich *et al.*, 2015), TBM (Thwaites and Tran, 2005) and Herpes Simplex encephalitis (Tan le *et al.*, 2014).

Based on the results and limitation of this the following recommendations would be made for future work:

1.To conduct a cross-sectional study to ascertain the aetiology in provinces with a high incidence of CNS infections such as Son La and Lai Chau. This would help guide public health measures such as vaccination against pathogens such JEV, measles virus or influenza virus; or the provision of education about the prevention of bites from vectors or the consumption and handling of raw pig blood and viscera as required.

2. To consider comparing models using:

- tasselled cap wetness and brightness with those using NDVI. These variables were used by Longbottom et al. 2017 for their predictions of the suitability of *Culex tritaeniorhynchus* and take into account aridity which can affect mosquito dessication.

- LST with those using temperature interpolated from weather station data.

3. To compare the results from the INLA models with other methods such as MCMC, potentially with the aim of improving the fit of the model.

4. To evaluate the reasons for the increase in incidence of AES in provinces in northwest Vietnam bordering Lao PDR. This might include looking at the migration across the border and JE vaccination status.

CONCLUSION

This work provides an insight into the risk factors associated with CNS infections in Vietnam based on surveillance data. The spatio-temporal patterns of AES correlated most strongly with JE however, other pathogens or syndromes showing similar patterns such as leptospirosis or ILI cannot be excluded. To understand this better, we would need to either expand diagnostic testing as part of surveillance or conduct a cross-sectional study in areas of high incidence.

CHAPTER 3 A PILOT CASE-CONTROL STUDY TO ASSESS THE RISK FACTORS FOR ACUTE ENCEPHALITIS SYNDROME IN PATIENTS ADMITTED TO THE NATIONAL HOSPITAL FOR TROPICAL DISEASES FROM THE RED RIVER DELTA REGION IN NORTHERN VIETNAM

3.1 INTRODUCTION

EPIDEMIOLOGICAL STUDIES

Epidemiological studies can be divided into descriptive and analytical. Analytical studies aim to infer causality by allowing associations between outcomes and exposures to be measured (Carneiro, 2017b). These studies are divided into ecological studies, cross-sectional studies, cohort studies and case-control studies. This thesis has so far, used ecological data to explore the epidemiology of CNS infections in Vietnam. Ecological and cross-sectional studies are advantageous in that they are cheap and quick to undertake. However, they are often subject to biases and confounding. Cohort studies, whereby a population who have been exposed are followed-up over time to determine if they develop the outcome have fewer problems with bias and confounding however, are expensive and can be take a long time to conduct. Despite case control studies being subject to selection bias which may lead to the association between the exposure and outcome differing in the study population compared to the target population (Geneletti, Richardson and Best, 2009) and information bias which may result from inaccurate reporting of the exposures, potentially due to recall bias (Shapiro, 2004) and possible confounding if this is not taken into account in the study design or analysis (Smith and Day, 1984). They offer a more efficient approach to ascertain association whereby cases with the outcome are recruited and exposures measured at the same time (Carneiro, 2017a).

CASE CONTROL STUDIES ADDRESSING RISK FACTORS FOR CNS INFECTIONS

A number of cross sectional studies have been conducted in Vietnam to determine the aetiology of CNS infections (Tan le *et al.*, 2014; Le *et al.*, 2010; Ho Dang Trung *et al.*, 2012; Taylor *et al.*, 2012) however, case control studies to understand the risk factors for infection have mainly focussed on single pathogens, in particular *S. suis*. A study conducted at the Hospital for Tropical Diseases, Ho Chi Minh City between 2006 and 2009 recruited 101 patients with *S. suis* meningitis, 303 unmatched hospital controls and 300 community controls matched on residency and age. Participants were interviewed by staff who completed a questionnaire to ascertain their risk factors for *S. suis* (Nghia *et al.*, 2011). In Hanoi, a case control study was conducted to understand the association between *S. suis* and porcine reproductive and respiratory

syndrome (PRRS) recruiting cases with confirmed *S. suis* and hospital controls with sepsis not due to *S. suis* from the National Hospital for Tropical Diseases. Cases and controls were compared with outbreaks of PRRS both in space and time (Huong *et al.*, 2016).

In 1998, case control studies were conducted in northern Vietnam comparing risk factors for adults with AES compared to adults without AES and children with JE compared to children without AES. Epidemiological information was collected about the home environment including mosquito prevention measures, proximity to animals and JE vaccination status however, it was unclear whether the study staff visited the home of the participants to obtain this (Lowry *et al.*, 1998). There is also no evidence that a similar study has been conducted since in Vietnam although a more recent study was undertaken in northern India in 2016 which recruited paediatric patients with AES and community controls. Field staff visited the homes of both cases and controls to obtain information on mosquito prevention measures, water and sanitation, proximity of vegetation to the house and presence of livestock. The data was collected electronically using tablets (Singh *et al.*, 2016).

Chapter two of this thesis showed that AES was seasonal in northern Vietnam and positive correlated with temperature, humidity and normalised difference vegetation index which may suggest that many cases were due to by vector-borne diseases. However, this analysis was subject to ecological fallacy with covariates provided at the level of province.

To better understand the risk factors for AES in northern Vietnam a multi-centre case-control study was therefore planned with the following objectives:

1. to describe the spatial and temporal distribution of cases hospitalised with AES who reside in the Red River Delta region, northern Vietnam
2. to compare the socio-demographic, environmental, entomological and zoonotic risk factors between AES cases and healthy controls.

Nested within this study two cross-sectional studies were to be performed to compare by aetiological agent:

3. the clinical features on admission with outcome at hospital discharge and community follow-up
4. the socio-demographic, environmental, entomological and zoonotic risk factors associated with AES.

A pilot study was designed to help ensure the methods proposed for the case-control study were achievable and acceptable in this setting with the following objectives:

1. to assess the recruitment rate of cases and controls
2. to determine the feasibility of visiting participants within four weeks of hospital discharge
3. to understand the practicality of undertaking participant questionnaires and the acceptability of photographing the local environment and housing during the community visits.

Unfortunately, for logistical reasons, it was not possible to proceed to the full case control study. Therefore, the results of the pilot study are presented here. In addition to the objectives for the pilot study, the results of the clinical features, outcome and risk factors within the home environment are presented and discussed to provide suggestions for the development of the CRF and questionnaire for the future case control study.

3.2 METHODS

The pilot study was conducted at the National Hospital for Tropical Diseases (NHTD) in Hanoi from 16th December 2016 to 15th June 2017. NHTD is a tertiary referral hospital for tropical and infectious diseases for the region with 634 in-patient beds (personal communication, OUCRU, Hanoi). The study flow chart for cases and controls are shown in figures 3.1 and 3.2 respectively.

Figure 3.1 The flow of the cases through the study.

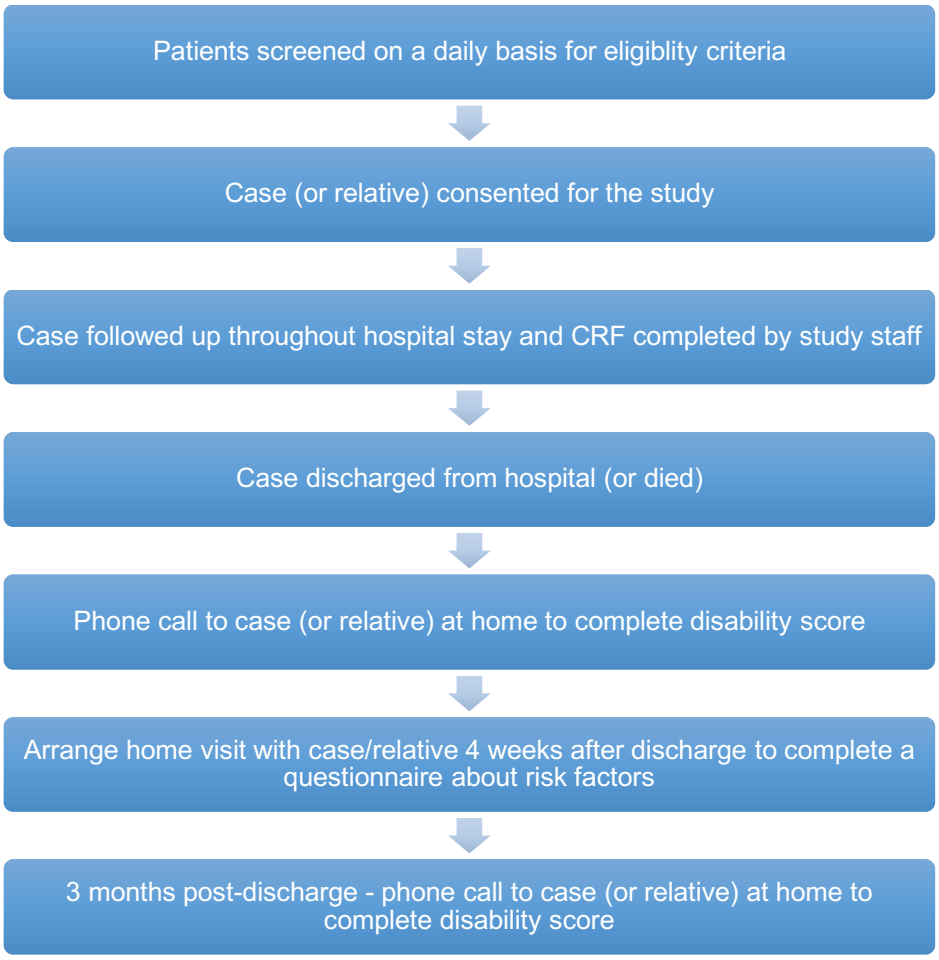
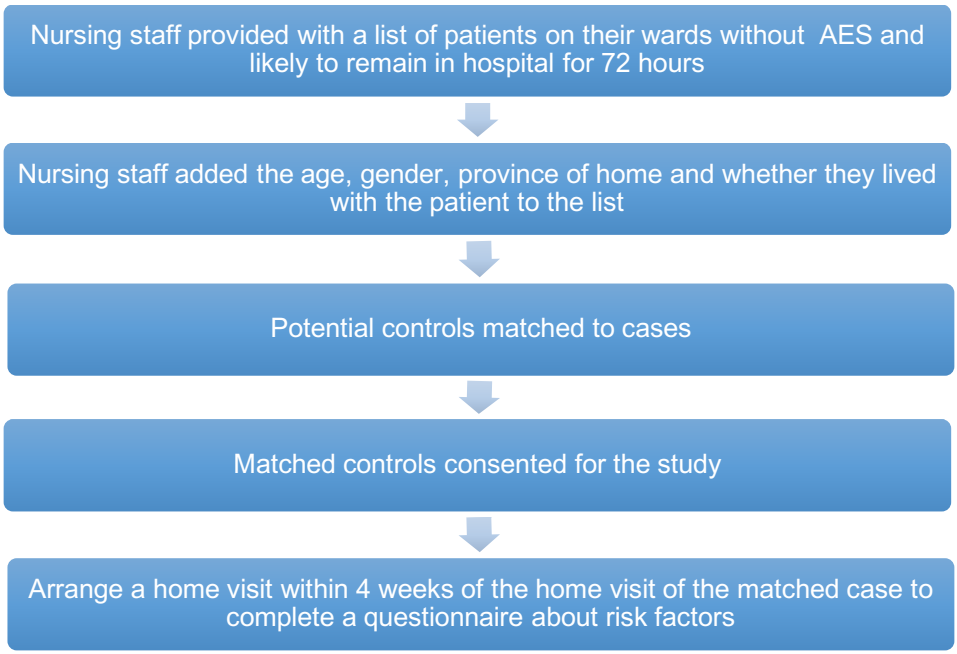


Figure 3.2 The flow of the controls through the study.



Cases and controls were screened and recruited to the study in parallel. Patients admitted to the emergency department, intensive care unit (ICU), paediatric ward or general medical ward with suspected AES were screened by the study doctors for eligibility. Those who consented to be in the study, were followed through their hospital admission until discharge or death with their clinical details completed using a case report form (CRF). At the time of discharge and three months following discharge, the participant or their relative was contacted by telephone to complete an outcome score. Additionally, the cases or their relatives were contacted to arrange a home visit within four weeks of discharge to complete a questionnaire about risk factors for AES (figure 3.1).

A list of potential controls was compiled by study nurses for their respective wards on a daily basis. These potential controls were matched to cases by the clinical trials team and consented by the study nurses. A home visit to complete a questionnaire about risk factors for AES was arranged within a four-week window period of undertaking a home visit to the matched case (figure 3.2).

PARTICIPANT RECRUITMENT

CASES

Cases were patients hospitalised with AES according to the WHO clinical criteria: “a person of any age, at any time of year with an acute onset of fever (before or after 10 days of admission to the initial hospital/clinic) and either a change in mental status (including symptoms such as confusion, disorientation, coma or inability to talk) or a new onset of seizures (excluding simple febrile seizures) or other early clinical findings including an increase in irritability (in paediatric cases), somnolence or abnormal behaviour as well as focal neurological signs (World Health Organization, 2016).”

Cases were also required to be living in one of the provinces in the Red River Delta region of northern Vietnam including Bac Ninh, Ha Nam, Hai Duong, Hung Yen, Nam Dinh, Ninh Binh, Thai Binh, Vinh Phuc or the municipalities of Hanoi and Haiphong and were required to provide signed informed consent with permission to visit the home or if they lacked capacity, informed consent could be provided by a relative or guardian.

Cases were excluded from the study if they had evidence of an encephalopathy due to non-infectious cause (e.g. cerebrovascular accident, brain tumour, electrolyte imbalance (where not a complication of an underlying encephalitis or meningo-encephalitis); encephalopathy due to an infectious cause other than a central nervous system infection e.g. sepsis secondary to pneumonia or a post-surgical meningitis/encephalitis. Cases could only be recruited once into the study.

Cases were recruited from emergency department, intensive care unit (ICU), paediatric ward and general infection ward. Written informed consent was obtained from the case whilst in hospital or if the case lacked capacity, their relative or if a child aged under sixteen years, their parent or guardian. If during the home visit, the case had re-gained capacity, they were re-consented. Children aged twelve to fifteen years provided assent (appendix 3.1).

CONTROLS

Controls were relatives and living in the same house of a patient with diagnosis of infection other than AES, and a likely length of hospital stay of 72 hours or more were to be recruited at a ratio of three controls to one case. Controls were matched to cases on province of home, gender, and age. Age was matched as follows: +/- one year in those aged under 3 years; +/- 2.5 years in the case of children aged 3-21 years; +/- 5 years in those aged 22-49 years and +/-10 years (in those aged over 50 years. Controls were required to provide written informed consent or in the the case of children. Where relatives of cases were required to answer questions as proxy respondents (for example, if the case was too unwell to do so), it was aimed for an equal number of proxy respondents for controls to reduce the effect of response bias.

Controls were excluded if they fulfilled the criteria for AES or had a past medical history of AES; lived in the house of someone with symptoms of AES or a past medical history of AES; had previously participated in the study. Controls could only be recruited once into the study.

Where more than one suitable control existed, staff were instructed to assign numbers to each, and select one randomly using a random number table or random number generator. If a control declined participation, a subsequent control was then approached where available.

Written informed consent was obtained from controls during their hospital visit. However, assent from children aged 12-15 years could be obtained at their home if they were not present at the hospital (appendix 3.2).

DATA COLLECTION

ADMISSIONS TO NHTD

A list of patient admissions over the study time period was provided by NHTD. Data on those with meningitis or meningo-encephalitis (Vietnamese: viêm não or viêm não màng não), the month and ward of admission of home province were extracted and to be used as baseline numbers of potential cases.

SCREENING TOOLS

Study doctors were asked to complete a screening log of all potential cases of AES who were not successfully recruited into the study and the reasons why. This was collected on a weekly basis (appendix 3.3). Nursing staff were originally asked to actively find controls who matched the cases however, it was noticed that there was very scanty recruitment of controls and therefore from 27th March onwards, additional support for recruitment was provided directly by OUCRU. The nursing staff were provided with lists of the patients on their wards and then asked to list the age, gender and province of their relatives and whether they lived with the patient. CTU staff then matched these with cases. The original feedback form is provided in appendix 3.4 however as this was not completed by nursing staff, staff from CTU used their own system as a screening log.

CASE REPORT FORMS (CRFS)

Paper CRFs were completed by the study doctors for all cases. These included clinical details, medication, laboratory results and diagnosis (appendix 3.5). The covariates included the screening criteria for eligibility to understand which signs and symptoms of AES were seen most commonly and provinces from which the participants were recruited; the demographics of the participants to determine if there was any difference between the age and gender by aetiology; the duration of symptoms prior to seeking medical care and the duration of time before transfer to NHTD to understand the process of medical care in this setting; the Glasgow Coma Score, admission to ICU and intubation status as a marker of severity of illness; information on the past medical history to understand whether there may be any predisposing factors associated with AES; and the medications taken prior to

admission to understand whether prior antibiotic usage may be the cause of partially-treated bacterial meningitis in cases with a negative CSF and blood cultures (Pandit *et al.*, 2005) and steroids in the case of afebrile AES (Ward, Levin and Phillips, 2001). The results of the CSF were used to determine whether those in whom a pathogen was not detected were more likely to have a bacterial or viral aetiology. Any missing information was added by the CTU staff using the patients' medical records.

The Liverpool Outcome Score (LOS) was completed for children (University of Liverpool, 2006) and the Extended Glasgow Outcome Scale (GOS) for adults (Medaille and Usinger, 2019) (appendix 3.5) at discharge and three months after discharge as feasible. This was undertaken by telephone interview with the case or if the case had died, lacked capacity or was a child, by the parent, guardian or relative. For the purpose of this study, the same LOS and Glasgow Outcome Scale were used at discharge and three months post-discharge.

FOCUS GROUP DISCUSSIONS (FGDS)

FGDs were undertaken to determine the understanding of vector-borne diseases and prevention measures, animal contact and acceptability of home visits. It was anticipated that these would include a total of twenty relatives (five men from urban areas; five women from urban areas; five men from rural areas and five women from rural areas) of those with AES or an infection for which the duration of hospital admission was likely to be 72 hours or more. The decision to define the home location as rural or urban was at the discretion of the study nurse and the communities could include those outside the Red River Delta. These discussions aimed to help identify environmental and behavioural risk factors of vector borne diseases reported by the local community with questions being ordered in terms of priority in case some participants may have wished to leave the FGD early (appendix 3.6). It was anticipated that the information obtained could be used to refine the questionnaire conducted at the home of the participants. Informed written consent was obtained from participants at the beginning of the FGD (appendix 3.7).

QUESTIONNAIRES AT THE HOME

Cases and controls were interviewed by field staff within their homes about their environmental, socio-demographic and zoonotic risk factors for AES during the month prior to becoming unwell for cases, and within the previous two months for controls (appendix 3.8). A verification form of the risk factors was completed by the field staff. This included recording observations around the home (including taking photos) and obtaining global positioning system (GPS) (appendix 3.8). The covariates were selected based on the risk factors for pathogens which were common causes of CNS infections in Vietnam or the neighbouring countries as outlined in chapter one.

If the case had died, following agreement with family members, the home was visited and a proxy respondent was interviewed. Similarly, if a case lacked capacity to answer questions or was a child aged under sixteen years, the questionnaire was completed by a relative, parent or guardian of their behalf. The questionnaire was conducted using the application 'ODK Collect' which is part of the electronic data capture software Open Data Kit (ODK) (Open Data Kit, 2020) using Samsung Galaxy Android smartphones. At the time of the home visit, the study participants also received an education leaflet about potential causes of AES (appendix 3.9).

DIAGNOSTICS

A lumbar puncture was undertaken in patients with AES at the discretion of the treating doctor as part of routine medical care and where no contraindications existed. At NHTD, the routine tests for AES include a CSF cell count and biochemistry; CSF and blood culture; Ziehl Nielsen staining, PCR and GeneXpert for *M. tuberculosis* (MTB); PCR for *S. suis*; ELISA (IgM) for Japanese encephalitis virus; NS1 and serology (IgM) for dengue virus (DENV) and PCR for herpes simplex virus (HSV). Other tests which are available include a Cryptococcal antigen, malaria film and antigen, PCR for enterovirus, PCR and serology (IgG) for rickettsia, serology for toxoplasma and serology for certain helminth infections. All tests are performed at the discretion of the treating doctor or medical team. The results of tests performed during hospital admission were documented in the CRF (appendix 3.5).

All CSF samples underwent repeated tested by OUCRU Hanoi for a panel of pathogens with the exception of those which already had a confirmed diagnosis of *S. suis*. In this case, the pathogen was not tested for again. The choice of pathogens

was decided based on previous literature of the causes of CNS infections in Vietnam and neighbouring countries (Le *et al.*, 2010; Taylor *et al.*, 2012; Ho Dang Trung *et al.*, 2012; Dittrich *et al.*, 2015; Turner *et al.*, 2017). The pathogens and testing methods are outlined in table 3.1. Fast Track Diagnostics (FTD) multiplex PCR kits for viral meningitis, bacterial meningitis and tropical fever core have been used for other aetiology studies of encephalitis in similar settings (Turner *et al.*, 2017; Goel *et al.*, 2017) and less expensive than using in-house methods. The multiplex PCRs were performed in accordance with the manufacturers' guidelines (appendix 3.10). However, for pathogens including *S. suis*, *O. tsutsugamushi*, measles virus and rubella virus which were not included in the FTD multiplex PCR, an in-house PCR was used (appendix 3.11). JEV and DENV were tested in accordance with the standard operating procedure from the Lao-Oxford-Mahosot Hospital Wellcome Trust Research Unit (LOMWRU) (appendix 3.12 and appendix 3.13) using the InBios JE Detect IgM Antibody Capture ELISA and Panbio® Dengue IgM Capture, in addition to the PCR for DENV. The pathogens and the methods by which they were tested are given in table 3.1.

Table 3.1 The pathogens tested for and methods of diagnosis.

Pathogens	Diagnostic test
HSV 1 and 2	FTD (FTlyo viral meningitis) multiplex PCR
Enterovirus	FTD (FTlyo viral meningitis) multiplex PCR
VZV	FTD (FTlyo viral meningitis) multiplex PCR
Mumps virus	FTD (FTlyo viral meningitis) multiplex PCR
N. meningitidis	FTD (FTlyo bacterial meningitis) multiplex PCR
S. pneumoniae	FTD (FTlyo bacterial meningitis) multiplex PCR
H. influenzae	FTD (FTlyo bacterial meningitis) PCR multiplex
DENV	FTD tropical fever core multiplex PCR
***Rickettsia* species**	FTD tropical fever core PCR multiplex PCR
***Salmonella* species**	FTD tropical fever core multiplex PCR
WNV	FTD tropical fever core multiplex PCR
***Leptospira* species**	FTD tropical fever core multiplex PCR
CHIKV	FTD tropical fever core multiplex PCR
S. suis	In-house PCR
Measles virus	In-house PCR
Rubella virus	In-house PCR
O. tsutsugamushi	In-house PCR
JEV	InBios JE Detect IgM Antibody Capture ELISA
DENV	PanBio Dengue IgM Capture ELISA

STUDY CONDUCT ASSESSMENTS

In addition to the screening tools for cases and controls group discussions took place at three and five months after the start of recruitment with hospital staff to obtain their views on any problems with the study in terms of recruitment and performing the questionnaire at the homes of the participants (appendix 3.14). Field staff were asked to provide feedback for each home visit including the time taken to reach the home and any problems with data collection (appendix 3.15). Group discussions also took place with the field staff at three and five months to identify any further issues (appendix 3.16).

DATA MANAGEMENT

Data from the paper CRFs and feedback forms was entered into the OUCRU electronic database 'CliRes' and downloaded as Microsoft Excel files for analysis. Data entered into the ODK application on the tablets was sent to an ODK Aggregate server hosted at the University of Liverpool. The data was downloaded as .csv files and .jpeg files for images for analysis. The data was cleaned using R statistical software with any queries referred back to the CTU for clarification. Any results which were still outside the range were removed from the analysis. A recruitment log of the participants was kept as a Microsoft Excel file by the CTU to record the participant details, their discharge date, follow-up and matched controls. Laboratory results were entered directly into Microsoft Excel.

The discussions with the study staff as part of the study conduct assessment were recorded using dictaphones. The recordings were transcribed into Vietnamese and then translated into English.

SAMPLE SIZE

As this was a pilot study, no formal sample size estimation was undertaken. Within six months we estimated that fifty cases and one-hundred and fifty controls could be recruited based on known admissions and the resources available.

ANALYSIS

QUANTITATIVE

The number of admissions to NHTD with meningitis or meningo-encephalitis were totalled by month, ward and province and where available, the number of patients screened for eligibility as either a case or control. Using the screening logs for controls, the ratio of availability of potential controls by province, gender, age and whether they were living with the patient (relative) were calculated to ascertain where difficulties in recruitment may lie as a result of the matching.

The final aetiology for cases of AES was based on a combination of the diagnosis made NHTD and the laboratory results from OUCRU. In the absence of laboratory confirmation, a final diagnosis of *S. suis* or MTB was provided, based on clinical judgement alone. Two cases with laboratory confirmed JEV by NHTD were given a final diagnosis of JEV despite no pathogen being detected by OUCRU. This is due to the possibility of sample degradation during storage (Khalakdina *et al.*, 2010).

R statistical software was used for all quantitative analysis. Given the small numbers with individual pathogens, clinical data was summarised for all cases rather than individual aetiologies. The number and percentage were given for categorical covariates, and median and range for continuous covariates. The completion of the Glasgow Outcome Score did not appear consistent with the guidelines in many cases. Therefore, a judgement was made as to the final score which best fitted the guidelines.

Conditional logistic regression was attempted to compare the risk factors ascertained from the questionnaires conducted at the home between cases and controls. However, due to the small number of matched controls to cases, the model was unable to converge. As a result of this, an unconditional logistic regression was used however, to account for the small sample size, Firth's bias reduction method was applied using the R package 'logistf' (Ploner, 2018). If the number of events in a database is too low for example, lower than the number of risk factors, overfitting can occur whereby the predictions made by the models may be inaccurate and the model can fail to converge. A number of methods can be used to overcome this. Compared to other methods, Firth's penalized method can introduce bias however, it is a less challenging approach (Rahman and Sultana, 2017).

QUALITATIVE

For each of the pre-defined questions in the FGDs, a list of words and frequency with which they were appeared in the transcript was constructed using the R package 'tidytext' to produce a codebook (appendix 3.17). Words which appeared twice or more by either group were used to summarise the findings, referring back to the transcript to confirm the context in which they were discussed. If a word appeared once but the context in which it was provided was considered to be a useful finding this was also included in the summary. As unique identifiers were not provided and

consent was not obtained from participants to show individual quotes, the comments in the summaries may refer to one or more of the participants. Data was only available for the rural men and women.

Study conduct assessments took place at three and five months with hospital and field study staff to obtain their views about any problems with study in terms of recruitment and performing the questionnaire. The study conduct assessments were semi-structured, using a set of pre-defined questions with the facilitator using prompts where required to explore topics in more depth. The assessments were recorded using dictaphones, transcribed into Vietnamese and translated into English.

For each of the groups: field staff, nursing staff and doctors, the assessments at three and five months were combined. Key themes were constructed which aimed to address the study objectives with summaries of the text provided for each.

ETHICS

The study was approved by the Oxford Tropical Research Ethics Committee (OxTREC) (reference 42-15), the University of Liverpool ethics committee (RETH001070), the University of Liverpool Sponsorship (UoL001172) and the National Hospital for Tropical Diseases ethics committee.

3.3 RESULTS

OBJECTIVE 1: TO ASSESS THE RECRUITMENT RATE OF CASES AND CONTROLS

CASES

Screening

During the study recruitment period, three hundred and fifty-five patients were admitted to NHTD with a diagnosis of meningitis or meningo-encephalitis (viêm não or viêm não màng não). Tables 3.3-3.5 show the distribution of these admissions by month and ward of admission and province in which they live. The number of patients admitted was higher during the spring compared to winter months, with the June predicted to be the month with highest number of admissions (n=45 until 15th). Most patients were admitted to the emergency department and the general infection ward (86%) and were from the Red River Delta (69.6%).

Table 3.3 Month of admission of patients admitted to NHTD over the study recruitment period with an initial diagnosis of AES or meningo-encephalitis according to hospital data.

Month	Number of patients
December (16-31st)	18 (5.1%)
January	39 (11.0%)
February	52 (14.7%)
March	66 (18.6%)
April	66 (18.6%)
May	69 (19.4%)
June (1-15th)	45 (12.7%)

Table 3.4 Ward of admission of patients admitted to NHTD over the study recruitment period with an initial diagnosis of AES or meningo-encephalitis according to hospital data.

Ward	Number of patients (%)
Emergency	111 (31.3%)
Paediatrics	25 (7.0%)
ICU	2 (0.6%)
General Infection	194 (54.7%)
Virology and parasitology	23 (6.5%)

Table 3.5 Province of patients admitted to NHTD over the study recruitment period with an initial diagnosis of AES of meningo-encephalitis according to hospital data.

Province	Number of patients
Red River Delta (n=247, 69.6%)	
Bac Ninh	14 (3.9%)
Ha Nam	18 (5.1%)
Ha Noi	85 (23.9%)
Hai Duong	13 (3.7%)
Hai Phong	19 (5.4%)
Hung Yen	7 (2.0%)
Nam Dinh	27 (7.6%)
Ninh Binh	32 (9.0%)
Thai Binh	19 (5.4%)
Vinh Phuc	13 (3.7%)
Other (n=108, 30.4%)	
Bac Giang	8 (2.3%)
Cao Bang	4 (1.1%)
Dak Lak	1 (0.3%)
Dien Bien	1 (0.3%)
Dong Nai	1 (0.3%)
Ha Giang	4 (1.1%)
Ha Tinh	12 (3.4%)
Hoa Binh	5 (1.4%)
Lang Son	5 (1.4%)
Nghe An	6 (1.7%)
Phu Tho	17 (4.8%)
Quang Ninh	8 (2.3%)
Son La	6 (1.7%)
Thai Nguyen	6 (1.7%)
Thanh Hoa	18 (5.1%)
Tuyen Quang	6 (1.7%)

Study conduct assessments

Recruitment of cases took place from 16th December 2016 until 1st June 2017. During this time, fifty cases were consented for the study, however, twelve were later identified to be ineligible for the study, leaving thirty-eight for analysis.

Feedback was provided by doctors for twenty-eight patients who were not successfully recruited into the study for either one or more reasons. Thirteen patients lived outside of the Red River Delta, eight did not have impaired consciousness or seizures, seven had a post-operative meningitis or encephalitis and one did not have a fever.

Themes from the discussion groups

Four key themes emerged from the discussion groups with the doctors including 1) the study recruitment numbers and catchment area; 2) the process of informed consent; 3) the inclusion and exclusion criteria and 4) the transfer of cases to Dong Anh hospital and other departments.

1. Study recruitment numbers and catchment area

Despite there being many adult patients with AES, the doctors felt that the study catchment area was too small as many patients who fulfilled the inclusion for AES lived in provinces outside of the Red River Delta including Nghe An, Thanh Hoa, Bac Giang, Lang Son and Son La. Furthermore, they thought it more likely that patients with *S. suis* and MTB would be recruited if the catchment area was limited to the Red River Delta. They therefore suggested that the area be increased to include these areas. The lack of paediatric patients was thought to be due to a seasonal effect as most are normally admitted during the summer months.

2. Informed consent

Some doctors felt that the length of the ICF, including the need to verify consent by ticking boxes, was inconvenient. They commented that given the study collaboration with the UK, some participants may have believed that their homes would visited by foreign investigators, potentially causing anxiety.

3. Inclusion and exclusion criteria

Some study doctors found the inclusion and exclusion criteria difficult to understand, particularly when cases were enrolled on the basis of the clinical criteria but were later found to have a normal CSF result. This was a particular problem in those with a reduction in conscious level of unknown cause.

4. Transfer of cases to Dong Anh hospital and other departments

Due to cases being from NHTD to Dong Anh hospital (a second tropical diseases hospital which opened in 2016) and to other departments, study doctors found it difficult to know whether to recruit cases with an anticipated transfer and if so, how to manage their follow-up in the study.

Clinical results of cases recruited

The final aetiologies of the cases of AES were as follows: *S. suis* (n=7 (18.4%); TBM (n=7 (18.4%)); JEV (n=2 (5.3%)); *S. pneumoniae* (n=1 (0.3%)); mixed pathogens (n=4 (1.1%)) and no pathogen detected (n=17 (4.8%)). The mixed pathogens included one case with *S. suis* and *S. pneumoniae*, one case with *S. suis* and DENV, one case with MTB and VZV and one case with HSV and *S. pneumoniae*.

With regard to the inclusion criteria, cases were recruited from all provinces in the Red River Delta with the exception of Van Phuc. The highest number of cases were from Hanoi (n=10, 26.3%) (table A3.1, appendix 3.18). Following a change in conscious level, the most common neurological presentation was seizures (n=10, 26.3%) with very few or no cases having other signs or symptoms (table A3.2, appendix 3.18). The majority of cases were men (n=27, 71.1%), the median age was 60 years with a range of 9-85 years) (table A3.3, appendix 3.18). 23.7% (n=9) of patients were admitted to ICU and the median GCS on admission to the initial hospital was 11 (tables A3.4 and A3.5, appendix 3.18). The most common medications given prior to admission were the antibiotics, ceftriaxone (26.3%, n=10) followed by ampicillin (13.2%, n=5) (tables A3.6, appendix 3.18) with over half of the cases having a lumbar puncture prior to admission to NHTD (51.4%, n=19) (table A3.7, appendix 3.18). The median CSF white cell count was 310 cells/μl with a range of 3 – 15634

cells/μl with a median percentage of neutrophils of 70% (range 2-97%) and lymphoctyes 10% (1-96%) (table A3.8, appendix 3.18). The median length of hospital stay was 21 days, with 52.6% (n=20) discharged home and 7.9% (n=3) discharged to die (table A3.9, appendix 3.18). The Glasgow Outcome Score was completed for twelve cases at a median time of 27 days after discharge from NHTD with the majority of cases having an upper or lower severe disability (75%, n=9) (table A3.10, appendix 3.18). The score was completed again for thirteen cases at a median time of 108 days post discharge where the percentage of those with an upper or lower severe disability had reduced to 53.9% (n=7) (table A3.11, appendix 3.18). The Liverpool Outcome Score was completed for one child. This was a score of two ("Severe sequelae, impairing function sufficient to make patient dependent") at discharge and three ("Moderate sequelae mildly affecting function, probably compatible with independent living") three months after discharge.

Screening

Data was available from for a total of 1311 potential controls whose relatives were from the Red River Delta. Of these:

- nine individuals who met the matching criteria refused participation in the study and four were discharged before consent.
- 102 patients had no relative visiting so matching could not take place.
- 182 patients had already been discharged and 14 transferred to Dong Anh hospital so could not be matched

The first control was recruited on 24th January 2017. By 15th June 2017, twenty-one controls were recruited, matching with twelve cases. Eight controls were inappropriately recruited as three did not match the case on location and five did not fulfil the inclusion criteria (or there was missing data with regard to eligibility). One control was recruited despite not fully completing the consent form. Table A3.12, appendix 3.18 shows the feasibility of recruiting potential controls if the matching criteria had been altered based on the province of the home. Matching only on gender, sufficient numbers of females would have been recruited however, the number of men would have been insufficient for many provinces. However, once age and living with the patient were included as matching criteria, insufficient numbers would have been recruited from every province except Hanoi.

Study conduct assessments

Themes from the discussion groups

Two key themes were found from the discussion groups with the nursing staff: 1) the recruitment of controls and 2) the follow-up of patients after discharge to complete the disability scores.

1. Recruitment of controls

Nurses experienced difficultly finding appropriately matched controls. The recruitment of male controls was particularly difficult as most of the visiting relatives were female. Additionally, it was challenging to find matches of an older age as relatives tended to be young. The nurses emphasised that they believed the age bands for matching were too narrow. In situations where the nursing staff found a control who matched on age, gender and province, they may not have lived in the same house as the patient as sometimes family members visit patients because they work in Hanoi but do not live with them. Furthermore, the relatives sometimes hire a helper who is of no relation to the patient and therefore ineligible for the study. In addition to the difficulty with age-matching, it was challenging to find relatives who came from outside of Hanoi.

Although the nurses often carried out work in shifts, they did not consider this to affect the recruitment controls as they felt they could work more closely with relatives outside of the working hours, particularly with those visiting patients in ICU.

When consenting controls, the nurses felt that as the relatives were healthy, there was some reluctance to participate in the study. Additionally, some participants appeared uncomfortable with the prospect of people visiting their homes to take photos. There were different opinions regarding reimbursement for the participants. Some nurses believed that the controls should receive a higher payment for participation. However, other nurses didn't think that reimbursement influenced a control's decision to participate and if they wished to be in the study they would.

2. Follow-up of patients after discharge to complete the disability scores

The nurses claimed that there was some difficulty following up patients after discharge, especially when transferred to another hospital. The nurses emphasised that it could be challenging to contact patients at certain times of the day. They also found the disability scoring system quite long and the order of the questions could be confusing.

During FGDs at five months after the start of recruitment, the nurses again explained the difficulty with recruiting controls who were reluctant to agree to the study. According to the nursing staff the relatives of patients were afraid of having 'troubles' and 'did not like anything related to the patients and hospital'.

OBJECTIVE 2: TO DETERMINE THE FEASIBILITY OF VISITING PARTICIPANTS WITHIN FOUR WEEKS OF HOSPITAL DISCHARGE

Thirty-seven cases received a home visit. Of these, data was available for twenty-seven after excluding those who were ineligible. Fifteen controls received a home visit with data available for six after excluding those recruited in error or who did not fully complete the consent form. The median time between discharge and home visit was 39 days with a range of 15 to 98 days using data available from 25 cases. The median time between the visits to the homes of cases and controls was 20 days with a range of -5 days to 71 days.

Feedback from field staff was not fully completed. Therefore, accurate information on the date the distances travelled and time taken to reach the house is missing. How key themes from the discussion group at three months since the start of the study are shown below under objective 3.

OBJECTIVE 3: TO UNDERSTAND THE PRACTICALITY OF UNDERTAKING PARTICIPANT QUESTIONNAIRES AND THE ACCEPTABILITY OF PHOTOGRAPHING THE LOCAL ENVIRONMENT AND HOUSING DURING THE COMMUNITY VISITS

THE DISCUSSION GROUPS WITH THE FIELD STAFF

Two main themes emerged from the discussion groups with the field staff: 1) the acceptability of the questionnaire and 2) using the ODK Collect application.

1. Acceptability of the questionnaire

Generally, cases were happy to complete the questionnaire and taking photos of the home environment was acceptable. However, field staff occasionally experienced some reluctance from other family members in the home. There were also some difficulties finding a suitable proxy respondent, particularly as this participant was not informed of the study beforehand. On one occasion, the participant's neighbours were suspicious of the field staff but after showing identification and explaining the study, the issue was resolved. Most questions were easy for staff to follow however, some participants would be inconsistent with their answers, for example, declaring the use of treated water and then changing it to rain water.

2. Using the ODK Collect application

In general, there were few problems with the Android or the ODK application. However, the battery on the Android could run out quickly. During the group discussion at five months following the start of recruitment, field staff advised that the maximum travel time was a return trip totalling six hours. There were some difficulties if the participants' homes were in remote areas where public transport was unavailable and could not be located using GPS. Additional challenges occurred when the incorrect phone number for the participant was provided. Despite the staff being flexible with regard to arranging home, visits, they found it difficult to enquire persistently about discharge dates which may have led to some withdrawing from the study. Additionally, only one out of four relatives of patients who died accepted a home visit.

FGDS WITH RURAL PARTICIPANTS

The responses to the questions for the FGD which are outlined in appendix 3.6 are shown below.

1. In the area where you live are there places where you think you would be bitten by mosquitoes? What time of day?

Both women and men talked about the presence of vegetation such as plants and trees around the house which attract mosquitoes. Both rural women and men also

identified water storage as a place where there are mosquitoes with rural women discussing rubber tyres and rural men discussing water storage tanks. The rural groups discussed natural water sources as sites for mosquitoes including rivers and ponds with the men also discussing rice fields. Rural women discussed their keeping of animals, with a reference to the abundance of excreta and mosquitoes whereas some of the urban women believed that the presence or absence of mosquitoes around their homes was dependent on the presence of livestock kept by neighbours with some urban women claiming not to see any mosquitoes around their homes.

Rural women considered the afternoon, particularly the late afternoon to be the time at risk for bites. Some also discussed a risk of bites in the morning. Rural men also considered the late afternoon and early evening to be a risky time and urban women, the evening and night.

2. What are the ways in which you try to prevent mosquito bites when you are inside your house? What else could you do, even though you currently don't? Why don't you do these things?

Both men and women discussed the use of bednets for the prevention of mosquito bites. The rural participants claimed that their communes were sprayed two to three times per year whereas some of the urban women sprayed their homes themselves. Neither rural men nor women used a 'cream' to prevent mosquito bites with some women stating that they either had no time to apply it or were concerned about side effects however, some of the urban women used this on their children or grandchildren. Rural women discussed closing doors and windows to prevent mosquito entry to the house with all groups mentioning that door nets were used rarely, if at all by either themselves or their communities. Urban women discussed the use of rackets and machines to kill mosquitoes.

3. Why do you try to avoid mosquito bites?

The avoidance of mosquito bites to prevent disease was mentioned by all groups however, there was some discrepancy amongst the rural women with some believing that mosquitoes could only cause fever and not transmit disease.

4. Do you know of any diseases caused by mosquito bites? Tell me about these diseases and how do you get these? (If not discussed, then ask 'Tell me what you know about Japanese Encephalitis and Dengue Fever")

Without prompting, those from all groups recognised dengue fever as being transmitted by mosquitoes. However, the rural men considered malaria to be the main infectious disease transmitted by mosquito bites, stating that there were many cases in their area. JEV was mentioned only within the FGD with rural women.

5. Would you spend time in rice fields e.g. what time of year?

Both men and women worked in the field or had neighbours who did so. Both men and women work in the field during the two farming seasons which the rural participants stating that occurs between October and January and the summer months. Urban women also discussed growing vegetables all year round.

6. Which animals are there in your community?

6a. Do you have any type of contact with each of these animals? If Present, which animals do you have contact with and what is the type of contact?

- e.g.do they live in the house, do you look after any animals

Both rural women and men discussed keeping dogs, cats, chickens and pigs. Dogs and cats were kept by rural participants to protect the house with cats being used to catch mice. The presence of rodents around the home environment was raised as an issue by all groups. Rural men discussed raising chickens for food. Rural men also stated that they kept goats and cattle. In terms of contact, feeding animals was common amongst both groups and showering pigs was discussed by rural women. Men also discussed slaughtering animals.

7. Which animals do you eat? What do other people in your community eat? How often (use counters)? Which of these are eaten raw?

Both men and women discussed eat pork, chicken, beef and fish. Rural groups also ate mice. The rural women stated that they sometimes prepare and eat mice and the blood of hedgehogs and ducks, whereas urban women discussed the consumption of pork blood puddings by family members. The rural men claimed that although family

dogs and cats were not slaughtered for food, the raw meat would be purchased from the market on special occasions.

8. How would you feel if we came to your house to perform a questionnaire?

9. How would you feel if we took photographs around your house?

Generally, the men had no problem with the idea of a home visit however, there were mixed opinions from the women. Some women were concerned that they lived far away and other were embarrassed due to their house being 'poor' or 'ugly'. The women stated that during a house visit they would like some advice on how to prevent mosquitoes and improve hygiene.

THE RESULTS OF THE QUESTIONNAIRE CONDUCTED DURING THE HOME VISIT

The key results from the questionnaire are shown in tables A3.13 to A3.17, appendix 3.18.

29.6% (n=8) of cases and 33.3% (n=2) of controls worked as farmers. 81.5% (n=22) of cases and all of the controls had received some form of education. The majority of cases and controls had a monthly household income of up to 10 million Vietnamese Dong (VND) (81.5% (n=22) and 66.6%, n=4, respectively). The majority of cases and controls shared their bedroom with one other person or no other people (85.2%, n=23 and 83.3%, n=5, respectively) and 11.1% of cases (n=3) and 16.7% (n=1) of controls used a water source which included an unprotected well, river, stream, pond or lake (table A3.13, appendix 3.18).

All cases and controls used a simple bed net for mosquito prevention but only one case used an insecticide treated bed net. All of the controls and 74.1% (n=20) of cases slept under a bed net in the two months prior to conducting the questionnaire or the month prior to becoming unwell, respectively. 29.6% of cases compared to

100% of controls claimed that indoor residual spraying (IRS) of the house had been previously carried out (table A3.14, appendix 3.18).

Five of the cases (18.5%) and none of the controls had contact with a pig in the month prior to becoming unwell or the last two months, respectively. 74.1% (n=20) of cases and 83.3% (n=5) of controls had seen rats around the house or place of work in the month prior to becoming unwell or the last two months, respectively and 66.7% (n=18) of cases and 50% (n=3) of controls ate or prepared raw meat, viscera or blood in the month before becoming unwell or the last two months (table A3.15, appendix 3.18).

29.6% (n=8) of cases and 50% (n=3) worked in rice fields and 48.1% (n=13) of cases and 33.3% (n=2) of controls had walked through grassland in the month before becoming becoming unwell or the last two months respectively. 22.2% (n=6) of cases and 33.3% (n=2) of controls had travelled outside the province in the month before becoming unwell or the last two months respectively (table A3.16, appendix 3.18). Only two cases could recall having been vaccinated against JEV and none against *N. meningitidis* or *H. influenza*. Three cases and one control recalled being vaccinated against measles (table A3.17, appendix 3.18).

There was no evidence for a difference any of the potential risk factors between cases and controls with the exception of IRS (p=0.002) (table A3.14, appendix 3.18).

3.4 DISCUSSION

This pilot case control study conducted at the National Hospital for Tropical Diseases in Hanoi aimed to assess the feasibility and acceptability of using a number of methods both in terms of study design and data collection to understand the risk factors for AES. The results of these were to be used to develop a future, larger case control study. The use of electronic data capture at the homes of cases and controls worked well. However, despite adequate recruitment of cases, most had a bacterial meningitis rather than a viral encephalitis and recruitment of controls was unsuccessful. The main outcomes are discussed below for each of the objectives.

OBJECTIVE 1: TO ASSESS THE RECRUITMENT RATE OF CASES AND CONTROLS

CASES

Use of the WHO case definition for AES

The target of fifty cases was met without difficulty. This was to be expected given that two hundred and forty-seven of patients with AES or meningo-encephalitis from the Red River Delta region were admitted to NHTD over the same time period. However, the majority of cases had a bacterial meningitis due to *S. suis* or TBM with recruitment of patients with a viral encephalitis posing a challenge. In retrospective epidemiological studies, a case definition is imperative to ensure that there is reliability in determining the presence of disease and also selecting controls who are appropriate (Sharma, 2011). As detailed in chapter one, there are many case definitions for encephalitis. However, a number of these are reliant on diagnostic tools which may not be available in resource-poor settings. Many countries use the WHO definition of AES for surveillance of JE (Heffelfinger *et al.*, 2017). The WHO definition is stated as "a person of any age, at any time of year with an acute onset of fever (within 10 days of admission to initial hospital/clinic) and either a change in mental status (including symptoms such as confusion, disorientation, coma or inability to talk) or a new onset of seizures (excluding simple febrile seizures) or other early clinical findings including an increase in irritability (in paediatric cases), somnolence or abnormal behaviour as well as focal neurological signs" (World Health Organization, 2016). Despite its use, the sensitivity and specificity in determining laboratory confirmed JE was shown to be 65% and 39%, respectively. Adding in features such as acute flaccid paralysis and meningism improved this the sensitivity to over 85% but reduced the specificity to 26% with acute flaccid paralysis (AFP) and 23% with meningism (Solomon *et al.*, 2008). It is therefore possible that this case definition was not specific enough for this study. However, had recruitment occurred during the peak season for AES, or in a paediatric hospital, it is possible that its specificity would have been sufficient.

The procedural aspects of recruitment

The doctors did not appear to struggle with consenting patients however, unfortunately, twelve of the fifty cases did not fulfil the inclusion criteria despite being recruited into the study. It is unknown whether these were due to errors with data entry. However, the doctors did struggle in determining whether patients with a reduced level of consciousness might be eligible and the difficulty in differentiating between encephalopathy and encephalitis is already known (Quist-Paulsen *et al.*, 2019). Unfortunately, the doctors did not routinely complete the feedback forms, thereby making it difficult to understand how many patients were screened.

The case report form

Generally, there little missing data from the CRFs. However, it was evident from the case report form that many participants had received treatment and had diagnostics undertaken prior to admission to NHTD. Although it was not the purpose of this pilot study to determine clinical risk factors, it would be imperative to collect this data from the hospital from which the cases were transferred as the CSF results from NHTD could indicate a partially treated bacterial meningitis (McGill *et al.*, 2016).

The follow-up after discharge

Unfortunately, follow-up after discharge was not completed for all participants as the nursing staff often found it difficult to make contact. Therefore, this loss to follow-up would need to be accounted for in the design of the larger case control study. Additionally, it was apparent that the nursing staff struggled with the completion of extended GOS. Therefore, either further training or a change to the scoring system would be needed.

CONTROLS

The procedural aspects of recruitment

The recruitment of controls using the planned methodology was inadequate. Despite support from the staff from OURCU half-way through the recruitment process, it remained too difficult to match controls on all criteria required.

The study base principle assumes that case and controls are representative of the same underlying population, including in time (Wacholder *et al.*, 1992a). When the cases and controls are a random sample of those with and without disease within the study base, this principle has been met (Wacholder *et al.*, 1992a). As confounding can lead to biased results, controls can be matched to cases to help reduce this. For example, socioeconomic confounders may be reduced by matching the home of the control to the neighbourhood of the case. However, we need to be careful not to overmatch, whereby the population are too similar in terms of the exposures being studied. This can reduce the precision of the effect size (Wacholder *et al.*, 1992a). With regard to the comparable accuracy principle, the degree of measurement errors between the cases and controls should be equal (non-differential) (Wacholder *et al.*, 1992a).

In this study, it was noted that there was a gender imbalance in the relatives caring for patients with more females and males whilst cases were predominantly male. Additionally, matching on the same province and relatively narrow age ranges as the case added to this difficulty. As a result of this, the number of controls recruited was far below the number required (twenty-one compared to one-hundred and fifty).

In addition to the methods of selection of controls, the types of controls also need to be considered. For example, controls may include population controls, neighbourhood controls, hospital controls or relative controls each with their own advantages and disadvantages (Wacholder *et al.*, 1992b; Grimes and Schulz, 2005). It was not feasible to recruit population controls for this study given the number of local permissions required in advance. Therefore, in order to recruit sufficient number of controls for the future study, either the matching criteria would need to be changed for the relative controls or consider the use of hospital controls. Hospital controls are patients admitted to the same hospital as the cases and therefore are considered to be from the same secondary base. However, care must be taken if patients live in different catchment areas depending on the severity of the disease (Wacholder *et al.*, 1992b). Additionally, the exposure may be distorted as there may be selective inclusion of patients with related diseases (Wacholder, 1995). Despite this, hospital controls may be more convenient and provide information which is of comparable quality to the cases (Wacholder *et al.*, 1992b).

OBJECTIVE 2: TO DETERMINE THE FEASIBILITY OF VISITING PARTICIPANTS WITHIN FOUR WEEKS OF HOSPITAL DISCHARGE

The median time between discharge from NHTD and home visit was 39 days indicating that many were seen outside of the four-week period. However, some cases were transferred to other hospitals for which the date of discharge was not necessarily known and the field staff found it challenging to repeatedly contact the relatives of the cases to arrange a time to conduct a home visit and therefore, this process may need to be reconsidered in a future study.

OBJECTIVE 3: TO UNDERSTAND THE PRACTICALITY OF UNDERTAKING PARTICIPANT QUESTIONNAIRES AND THE ACCEPTABILITY OF PHOTOGRAPHING THE LOCAL ENVIRONMENT AND HOUSING DURING THE COMMUNITY VISITS

Field staff found that the questionnaire was generally acceptable to the cases and families and during the FGDs, with the exception of some concerns about the how the staff may perceive their home participants implied that they would be happy to be visited. However, the use of a proxy respondent had to be abandoned that this was found not to be feasible. As shown in the results of the data, there was little missing data, indicated as ‘unknown’. The ODK Collect application also worked well for the majority of the time with the exception of a period during which the forms could not be sent over Wi-Fi following the installation of a new Secure Sockets Layer (SSL) certificate for the server hosted at the University of Liverpool.

A number of electronic data capture (EDC) tools now exist for collecting data during such studies (King *et al.*, 2014). EDC tools offer a number of advantages over paper data collection both in terms of data quality as in-built validation methods reduce the need for mistake to be made during data entry and transcription (Maduka, Akpan and Maleghemi, 2017; Meyer *et al.*, 2013). Additionally, the data can be sent to the end user in real-time (Zhang *et al.*, 2017b) and finally, the data is more secure as a result of the absence of the need to print and transport paper copies of questionnaires (King *et al.*, 2013). However, challenges of EDCs including the ability to charge the battery of the mobile device and the availability of internet access to send the data are acknowledged (Style *et al.*, 2017). The use of electronic data collection in this study

allowed for the rapid acquisition of information but also minimised the need for data cleaning improving the efficiency of the data management and analysis. The provision of GPS coordinates and photos around the home allowed myself, as the lead investigator who did not conduct home visits to be able to remotely monitor the visits and visualise the living environment.

RECOMMENDATIONS FOR THE FUTURE LARGER CASE-CONTROL STUDY

Following this pilot study, a number of recommendations are made below to progress to a future larger case control study to understand risk factors for AES.

CASE RECRUITMENT

1. To include cases who have ≥5 white cells/μl or more in the CSF. This may help to improve the specificity of recruiting those with a CNS infection and also to help doctors to decide whether to recruit patients when they are unsure about the diagnosis. However, the exclusion of those in whom a lumbar puncture is contraindicated may lead to a recruitment bias.

2. To consider excluding patients with TBM or bacterial meningitis based on hospital diagnostics. However, this would only be possible at sites where these diagnostics are available, potentially excluding more rural hospitals where many cases of AES may be seen. Additionally, cases who die before their results are available may lead to recruitment bias.

3. To consider recruiting patients only during the summer months e.g. May to September. This is the peak season for viral encephalitis and will avoid recruiting an excess of patients with bacterial meningitis or TBM during the winter months. This will help to improve the cost and efficiency of the study.

4. To ensure a greater proportion of cases are children, a paediatric hospital site should be included.

5. To expand the study catchment area to include the whole of northern Vietnam and possibly the north-central provinces. The data from chapter two shows that many cases of AES come from the border provinces of Son La and Lai Chau. However, if house visits are still to be performed, travel to these provinces would have to be taken into consideration. This may require the employment of additional field staff thereby increasing study costs. Alternatively, the questionnaire could be performed in the hospital with GPS coordinates of the home of the case obtained using the full address of the participants. Home visits could be reserved where spatial clusters of disease are found which require further investigation. Prior to undertaking this method, it would be useful to compare if there are any discrepancies in results between home and hospital conducted questionnaires before moving to this method.

6. Where possible, to employ a study team consisting of one or two study doctors or nurses who can recruit across different wards.

CONTROL RECRUITMENT

1. To amend the matching process for the controls. The age bands for matching could be wider; for example, matching those aged 15 years and below and above 15 years would be satisfactory to account for differences in aetiologies by age. Additionally, instead of matching on province, it may be more efficient to match on urban/rural living. The parameters for urban/rural would need to be set, most likely depending on population density.

2. To recruit patients without CNS infection rather than relatives as controls. Although it is acknowledged that there may be some biases, this primarily might reduce the problem of matching on gender and age. These patients may also feel more comfortable about participating in a study compared to the healthy population.

3. To recruit controls directly from the community using a random selection of communes within the study catchment area instead of hospital controls. This is a more efficient process as field staff can go from door to door to find an appropriate match. This would also avoid the need to follow-up controls until

discharge and then find a suitable time frame in which to visit the home. If either a blanket permission for the study catchment area and time-frame could be provided or permission obtained on the day this may be feasible. However, this approach needs to be further explored.

4. To remove the need for a proxy control (used when the questionnaire is answered by the relative of a matched case).

STUDY DATA COLLECTION

1. To recommend that field staff explain the study and agreement of the participant carefully to all family members when visiting the home where applicable to avoid any misunderstanding. If there is still disagreement arrange the visit at a different time or give the option to the participant to withdraw if they feel if would make relatives unhappy.

2. To advise field staff to fully charge the Android after every use and take a battery pack with them to the homes.

3. To review the questionnaire and amend the wording of any questions which caused confusion or add additional skip patterns where required.

4. To have a centrally managed log system for monitoring follow-up whereby one member of staff is responsible for contacting the participants to gain information about discharge and arrange home visits.

5. To use a single data collection method and database. As this study used a combination of methods, multiple databases with different data dictionaries were produced which reduced the efficiency of analysis.

6. To consider conducting home visits within four weeks of cases developing symptoms rather than discharge from hospital to account for the possibility of long hospital admissions and increased risk of recall bias. It is not necessary

for the participant to be at the home during the visit and providing the participant has capacity, sections of the question could be conducted whilst they remain an in-patient.

CONCLUSION

Despite its difficulties, this pilot study provided useful information to inform a larger case-control study to understand the risk factors for AES in northern Vietnam. Primarily, the either the matching criteria of the controls should be amended or the use of community controls should be considered; the study catchment area should include more rural areas in northern Vietnam; the cases should be recruited after the CSF results are available; and a paediatric hospital should be included. Furthermore, it may be more efficient to recruit only during the peak season for encephalitis. The use of the ODK application worked well and would be recommended for future studies.

CHAPTER 4 EVALUATION OF THE DAILY ACTIVITIES AND CONTACT PATTERNS OF HEALTHY ADULTS AND CHILDREN IN RURAL AND URBAN SETTINGS IN HA NAM PROVINCE, VIETNAM TO EXPLORE POTENTIAL RISK FACTORS FOR ACUTE ENCEPHALITIS SYNDROME

4.1 INTRODUCTION

THE ROLE OF HUMAN MOBILITY IN PREDICTING THE RISK FACTORS FOR AND TRANSMISSION OF PATHOGENS

The movement of humans can influence both the acquisition and transmission of a number of pathogens. Understanding the exposure to environmental pathogens through the utilisation of movement data can be beneficial for epidemiological studies (Owers *et al.*, 2018) and can help guide strategies for the control of infectious diseases (Pindolia *et al.*, 2012). The capture of socio-demographic data can help determine whether there are differences between groups of individuals with regard to their exposure and hence, potential risk of infection.

A study conducted in Uganda showed that the time mothers and young children spent at the shore of Lake Albert was positively associated with the prevalence of schistosomiasis, a water-borne parasite which is transmitted via snails (OR=2.1, 95%CI 1.2-3.7). Amongst young children alone, this association was even higher (OR=4.4, 95%CI 1.4-14) (Seto *et al.*, 2012). A study conducted in an urban informal settlement in Salvador, Brazil, aimed to identify exposure to environmental sources of *Leptospira*, a spirochete which is transmitted via the urine of an infected mammal. It was found that men visited a larger area compared to women over a 24-hour period. Moreover, there was significantly higher exposure to locations where there was evidence of rodent activity or waste amongst those infected with leptospirosis (Owers *et al.*, 2018). In south central Vietnam, a large-scale survey found that undertaking regular forest work and sleeping overnight in the forest was associated with an increased risk of infection with malaria (OR=1.76, 95%CI 1.05-2.94; and OR=2.86, 95%CI 1.62-5.07) (Erhart *et al.*, 2005).

As described in previous chapters, cases of AES in Vietnam can be due to a number of pathogens. Risk of infections from vector-borne diseases depends on the time at which the vectors bite, the number of infected vectors and the time a person spends at a location (Stoddard *et al.*, 2009). In children, the most common pathogen causing AES in Vietnam is JEV (Tan le *et al.*, 2014). A study conducted in Bali, Indonesia found that proximity to rice fields (<100m from the home) was a risk factor for

serologically confirmed JE (81% of cases compared to 59% of controls, p=0.0004), as well as playing outdoors (55% of cases compared to 41% controls, p=0.028) (Liu *et al.*, 2010). DENV is an another cause of AES in Vietnam (Tan le *et al.*, 2014; Phu Ly *et al.*, 2015) and a study conducted in Hanoi found that those who lived near an open sewer were more likely to have dengue fever or dengue haemorrhagic fever (p<0.01) (Toan *et al.*, 2015). Although not a known cause of AES in Vietnam, CNS manifestations of scrub typhus have been documented in neighbouring Lao PDR (Dittrich *et al.*, 2015). A case control study conducted in South Korea found that cases of scrub typhus were more likely to undertake dry field farming (p=0.013) (Kim *et al.*, 2018), and a case control study conducted in Beijing, China, found that the most common risk factors for scrub typhus infections included working in vegetable fields and hilly areas (OR=3.7, 95%CI 1.1-11.9 and OR=8.2, 95%CI 1.4-49.5) (Lyu *et al.*, 2013). Environmental risk factors may also play a part in the acquisition of non-infectious causes of AES. A case control study conducted in northern India found that those who had a litchi orchard within the vicinity of their home were more likely to have AES (OR=3.25, 95%CI 2.10-5.00) (Singh *et al.*, 2016). A separate study showed that the consumption of litchis was associated with AES (Shrivastava *et al.*, 2017).

Increasing travel networks increase the speed and magnitude of the transmission of pathogens (Tatem, Rogers and Hay, 2006). This is particularly true of pathogens spread by aerosol or droplet as demonstrated by the recent global spread of SARS-CoV-2 (Wells *et al.*, 2020). Air travel also plays an important role in the international spread of influenza A as demonstrated during the 2009 H1N1 pandemic (Kenah *et al.*, 2011; Mesle *et al.*, 2019). However, it is thought that short-distance commutes for work greatly influence the spread at a regional level, particularly from more to less populated areas (Charu *et al.*, 2017; Viboud *et al.*, 2006). For example, the seasonality of measles and rubella viruses has been shown to be dependent on human mobility at the local level, with epidemics propagating from larger to smaller cities (Marguta and Parisi, 2015; Wesolowski *et al.*, 2015a).

In addition to pathogens transmitted by droplet or aerosol, human mobility has also been implicated in the spread of pathogens transmitted via alternative routes. Ebola virus is primarily contracted following contact with infected bodily fluids (Bausch *et al.*, 2007). It is thought that the rapid progression of the 2014-2016 West African epidemic of Ebola virus disease was in part due to the effect of the movement of infected people from rural to urban areas and also across porous international borders between

Guinea, Sierra Leone and Liberia (Coltart *et al.*, 2017). Cholera is a diarrhoeal disease caused by the bacterium *Vibrio cholerae* which transmitted between people via the faecal-oral route (Zuckerman, Rombo and Fisch, 2007) and epidemics of cholera may be caused by the introduction of the pathogen into previously uninfected areas, potentially via asymptomatic shedding by those infected (Mari *et al.*, 2012).

In addition to pathogens which are spread between humans without an intermediate host, human mobility is also thought to be responsible for the spread of vector-borne diseases (Adams and Kapan, 2009). The *Ae. aegypti* mosquito generally does not travel far (Harrington *et al.*, 2005) and it is thought that the spread of DENV is the result of the movement of infected humans between mosquito communities (Adams and Kapan, 2009). At a fine spatial scale, it is thought that the movements of humans between houses plays an important role in the transmission of the virus (Stoddard *et al.*, 2013). At a larger scale, it is thought that the human mobility in combination with changes in climate which provide sites conducive to mosquito breeding, can result in the spread and introduction of the virus to new locations (Wesolowski *et al.*, 2015b; Thapa and Sapkota, 2017; Teurlai *et al.*, 2012).

THE COLLECTION OF MOBILITY DATA: GLOBAL-POSITIONING DEVICES (GPS)

Human mobility data can be collected by a variety of methods. At the population level, flight data, censuses, household and displacement surveys, mobile phone usage data and social media can be used to track movements. However, these have limitations in terms of confidentiality agreements and accessibility (Tatem, 2014). Mobile phone data may be subject to biases as not all of the population have access to phones or the internet (Tatem, 2014; Wesolowski *et al.*, 2012). Additionally, the data are anonymous and do not allow for differentiation of demographics.

At the individual level, the use of personal devices such as Global Positioning Systems (GPS) may be used to provide information. In Uganda, the association between water contact and schistosomiasis was determined by GPS data. Participants wore GPS devices for a period of 3 days to evaluate water contact and interpersonal interactions (Seto *et al.*, 2012). In Iquitos, Peru, the use of GPS devices to assess human movement patterns in relation to the transmission of DENV was shown to be feasible (Vazquez-Prokopec *et al.*, 2009). In Salvador, Brazil, GPS

devices were used to track the movements of those living in slum areas and their exposure to environments associated with leptospirosis (Owers *et al.*, 2018); and in Zambia to ascertain time spent away from the household and in areas at high-risk for malaria (Searle *et al.*, 2017).

GPS tracking as a means to understand human mobility may however, raise concerns by some populations. In Peru, focus group discussions took place prior to piloting the use of the GPS trackers during which participants worried whether their conversations would be recorded and also how to look after the unit correctly. During the pilot study itself, some participants admitted that they did not use the device for periods of time, either because they forgot to wear it, thought the device was not working or that the device was due to be exchanged (Paz-Soldan *et al.*, 2010). A similar study was performed by Katherine Anders, in southern Vietnam (unpublished). GPS devices were attached to 155 paediatric patients who had had clinically suspected dengue fever to evaluate their daily activities. Reasons for declining recruitment into the study included: a) concern about children damaging the device, b) the need to discuss participation with a family member first c) inconvenience e.g. the child lived with grandparents or parents didn't come home until late in the evening or d) the participant did not consider themselves to be at risk of mosquito bites.

HUMAN CONTACT PATTERNS

Human contact patterns differ between locations and ages and play a role in the acquisition and transmission of pathogens (Prem, Cook and Jit, 2017). Contact patterns are particularly important in the spread of pathogens by aerosol or droplets which require the close proximity of people for transmission (Leung *et al.*, 2017; Gastanaduy *et al.*, 2019). Transmission may be influenced by the age of the contacts and underlying immunity with studies looking at both serological and contact data to estimate the ability to maintain measles elimination (Funk *et al.*, 2019; Chun *et al.*, 2020) and the transmission of VZV (Ogunjimi *et al.*, 2009). The age of the contacts and impact of school holidays may also influence the seasonality of pathogens such as pertussis, measles, VZV and influenza (Eames *et al.*, 2012; Cauchemez *et al.*, 2008; Rohani, Zhong and King, 2010; Metcalf *et al.*, 2009; Jackson *et al.*, 2014). The type and frequency of contact may also be influenced by socio-cultural norms, demographics and the living and working environment (Horby *et al.*, 2011) with

pathogens such as enterovirus 71 being transmitted within households (Chang *et al.*, 2002; Hoang *et al.*, 2019a). In addition to pathogens spread by aerosol or droplets, contact patterns can also influence the transmission of pathogens spread by body secretions such as sexually transmitted infections (Datta, Mercer and Keeling, 2018) and Ebola virus disease (Cleaton *et al.*, 2016).

Contact surveys are used globally to understand the demographics of the respondents, their types of contact, and location, duration and frequency of each contact (Hoang *et al.*, 2019b). Contact diaries have been used to help understand these patterns in the context of the spread of infectious diseases in northern Vietnam. In Thanh Liem District, Ha Nam province, Vietnam details of physical and non-physical contact by 865 participants from 264 households in a semi-rural community were recorded. A mean of 7.7 different contacts per respondent per day were recorded with 91% of physical contacts occurring in the home (Horby *et al.*, 2011). A household-based survey amongst urban and rural populations of Guangdong, China (n=1821) was performed to ascertain both travel and contact patterns with those aged under 20 years having the longest duration of contact (Read *et al.*, 2014). A questionnaire administered to participants in a longitudinal cohort study of influenza in Hong Kong found that there was an association between the median number of contacts or locations and being infected (Kwok *et al.*, 2014).

JUSTIFICATION FOR THE RESEARCH AND QUESTIONS TO BE ADDRESSED

The aetiology of AES in Vietnam includes a number of pathogens for which the risk of acquisition and spread can be influenced by human movements and contact patterns. These include vector-borne diseases such as JE and dengue; and those caused by pathogens spread by aerosols or droplets such as VZV, influenza virus, measles virus and enterovirus 71. Additionally, diseases found to cause CNS infections in neighbouring Lao PDR such as scrub typhus and leptospirosis for which the risk of acquisition may also be dependent on human movements could potentially attribute to the aetiology in Vietnam.

The study of daily activities performed in southern Vietnam showed that GPS tracking of individuals was feasible. However, there was no evidence to suggest a similar study had been conducted in northern Vietnam and whether it would be feasible. Additionally, there was scope for building on the contact pattern work conducted by

Horby et al. 2011 in Ha Nam province, by analysing the differences between those from urban and rural areas, as well as investigating types of food consumed to elucidate the potential risk of zoonotic infections such as *S. suis* (Huong *et al.*, 2014b).

The aims of this study were therefore as follows:

1. To assess the acceptability and feasibility of using GPS devices and contact pattern diaries to understand the mobility amongst healthy participants living in Ha Nam province, northern Vietnam.

2. To compare the mobility patterns of different groups of participants including adults and children from rural and urban areas;

- at different times of the years
- during different days of the week

3. To evaluate the time spent in areas deemed to be at risk of mosquito bites

4. To compare the contact and behavioural patterns of adults and children living in rural and urban areas at different times of the year and different days of the week.

4.2 METHODS

STUDY LOCATIONS

This study was conducted in collaboration with the NIHE and the PMC in Ha Nam province (figure 4.1). Ha Nam province is located just south of Hanoi (figure 5.1) and in 2016 had a computed population of 803,700 (General Statistics Office of Viet Nam, 2018), a mean elevation weighted by population density of 7.38m and a normalised difference vegetation index (NDVI) of 0.2 (General Statistics Office of Viet Nam, 2018; NOAA: National Centers for Environmental Information, n.d.) The NDVI ranges from -1 to +1 where the higher the value, the more biomass of photosynthetically active vegetation exists. Negative values refer to non-biomass e.g. clouds, water or snow (Helbich, 2019). The NDVI of rice paddy has been shown be a positively related to both mosquito density and the number of Japanese encephalitis cases in China (Tian *et al.*, 2015) and also positively associated with scrub typhus in Taiwan (Kuo *et al.*, 2011). However, a negative associated was seen in Taiwan with dengue fever (Huang et al., 2018).

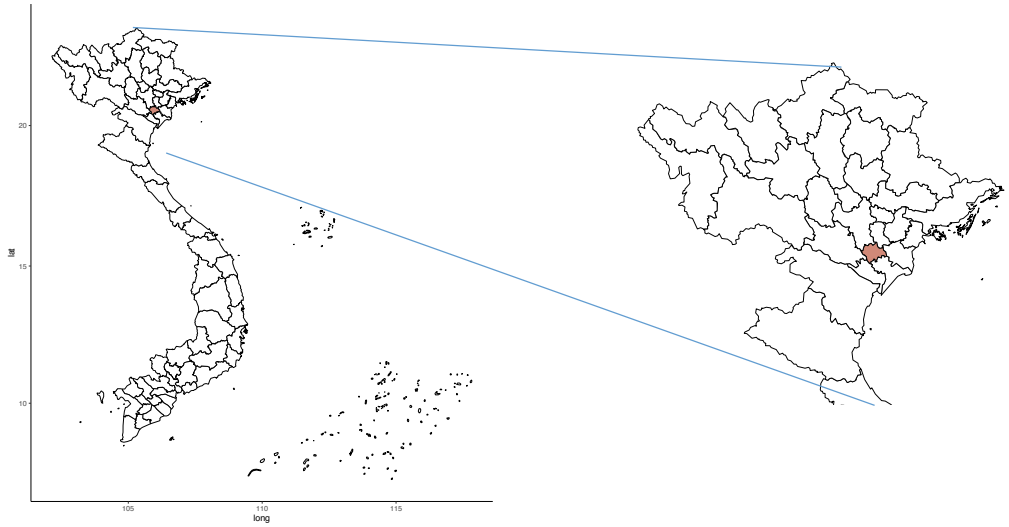
In 2016 there were two cases of AES giving an annual incidence of 0.25 cases per 100,000 population compared to the mean provincial incidence of 1.23 cases per 100,000 with a range of 0 to 137 cases per province (Ministry of Health Vietnam General Department of Preventive Medicine, 2019). However, sentinel site data recorded eight cases of JE in Ha Nam in 2016 making it the province (in addition to Hai Phong) with the second highest number of cases of JE after Son La (N=10).

Despite the lower incidence of AES compared to other provinces, Ha Nam had ongoing collaborations with OUCRU enabling permissions to be obtained to work in this area. Obtaining permissions to conduct the study in the border provinces which see a higher incidence of AES (chapter 2) was not feasible at this time.

The study was performed in an urban district (Phu Ly) and a rural district (Thanh Liem). In Phu Ly, two communes were selected to be used for the study which are Tran Hung Dao and Hai Ba Trung and in Thanh Liem, the communes are Thanh Ha

and Thanh Binh. The choice of districts and communes were decided in collaboration with the Ha Nam PMC.

Figure 4.1 Map of the location of Ha Nam province (coloured in red).



FOCUS GROUP DISCUSSIONS

To gauge the acceptability of conducting this study in the communities in Ha Nam province, FGDs took place to explore the attitudes and concerns towards wearing the GPS device and being asked questions about their daily activities (appendix 4.1). Four FGDs were held in both the urban and rural communes in December 2016, prior to the start of the study comprising: a) an urban FGD with eight men b) an urban FGD with eight women c) a rural FGD with eight men d) a rural FGD with eight women. Participants were selected using a purposive approach with the support of PMC staff, and provided informed written consent prior to the start of the FGD (appendix 4.2).

THE SELECTION OF PARTICIPANTS AND CONSENT

Six adults and six children were randomly selected from each of the four communes by study staff members from Ha Nam Preventive Medicine Center (PMC) giving a total of forty-eight participants. Participants were excluded if they were unable to

provide informed written consent; and in the case of children, had a parent or guardian who could provide this or if aged 12-15 years could provide assent. Initially informed consent was obtained at every visit, but this was later changed to provide consent for all future visits. The informed consent /assent form is provided in appendix 4.3 (most recent version only).

GPS DEVICES

During each study period of 72 hours, participants were provided with a GPS device (i-gotU GT-120) weighing 20g with dimensions of 44.5x28.5x13mm which they were advised to wear on their arm (Stothard *et al.*, 2011). This device has been used in previous studies (Seto *et al.*, 2012; Stothard *et al.*, 2011) and as part of the 13DXA study performed in southern Vietnam (K Anders, personal communication).

To validate the recordings from the i-gotU devices, GPS coordinates obtained from the devices were compared to Garmin etrex 20x devices at four different locations with five different devices tested. The mean latitudinal difference between the Garmin device and the iGoTU devices of 4.76×10^{-5} ºN and longitudinal difference of 4.27×10^{-5} ºE. Using the R statistical software 'distHaversine' function from the package 'geosphere' (Hijmans, 2019a), the mean distance between the Garmin GPS coordinates and i-gotU devices was 8.99m which was deemed acceptable for this study as a difference of 9m would be unlikely to change the outcome regarding whether the participant was within an area deemed at risk for mosquito bites.

The devices were also tested by study staff from NIHE and OUCRU to check the functionality and settings. Following testing by study staff, it was decided to set the device record a latitude and longitude every 2 minutes or every 3 seconds if the participant travelled at a speed of 10km/hour or over. This provided a balance between the collection of enough GPS locations to sufficiently make inferences about the data whilst maintaining sufficient battery power before the device could be charged overnight by the participants. To commence recording, the devices are required to obtain an initial signal and coordinate before locking the device to prevent participants disengaging the system. This procedure was performed at the PMC in Phu Ly by staff from NIHE.

A 72-hour feasibility study was conducted during weekdays in February 2017 to ensure the devices functioned correctly. Following this, the participants wore the GPS devices again for seven, 72-hour periods. These included weekdays and weekends for each of the months of April 2017, July 2017, September 2017; and weekends only in December 2017.

CONTACT PATTERN DIARIES

Over the same time periods during which the participants wore the GPS devices, the study team visited the homes of participants on a daily basis to complete a paper contact pattern diary which asked questions about contact with other people, their ages, who they were, the type of contact and where the contact happened in addition to questions about where they travelled to, activities undertaken at these locations and food consumed. These questions were devised to cover multiple risk factors for AES of different cause and were in keeping with previous studies (Read *et al.*, 2012). Additionally, participants were asked about any problems they had with the device, whether they forgot to wear or removed it purposefully (appendix 4.4).

SAMPLE SIZE

Due to the descriptive nature of this study, a power calculation was not performed. A sample size of forty-eight participants was considered to be logistically and financially feasible.

DATA MANAGEMENT AND ANALYSIS

QUALITATIVE DATA

The qualitative data from the FGDs was analysed for each question. Common themes were compared between each of the groups including urban men and women and rural men and women. No individual quotes were used, and the data was not analysed quantitatively.

QUANTITATIVE DATA: MOVEMENT DATA

The igotU software (@trip) was used to download data from each of the GPS trackers as CSV files. The longitude and latitude coordinates obtained en route from the PMC to the participants' homes were removed and the device number and participant identification number were added as covariates. Data from the CSV outputs utilised for analysis included the date and time of each GPS recording, the longitude and latitude coordinates reported to six decimal places and the distance travelled between each coordinate (in metres).

Analyses were performed using R statistical software. Datasets from each of the participants were merged, cleaned and managed using the package 'tidyverse' (Wickham, 2017) and Base R (RDocumentation, n.d.-c).

Information about the participants' age and gender were merged from data from the contact diary. Participants aged 18 years and over were considered to be adults; those from Hai Ba Trung and Tran Hung Dao were coded as urban participants and those from Thanh Ha and Thanh Binh, rural participants. The months of February and December were classified as winter; April as spring; July as summer and September and October as autumn.

Boxplots were constructed to show the distances covered per participant over the study period between urban and rural participants, adults and children, males and females, during weekdays and weekends and each of the seasons. The Kruskal Wallis test was used to determine the differences between the distances.

The Kruskal Wallis test was used to determine differences in the time spent at each coordinate. Maps were constructed of varying time periods however, it was decided to show those where participants spent (2 minutes or longer and 6 hours or longer) at each coordinate. Two minutes was the standard time between logging GPS coordinates when the participant travelling at less than 10km/hr. Spending time at locations for less than two minutes was unlikely to be a risk factor for mosquito bites. The time spent in locations for six hours or longer might represent a working

environment, place of education or home. Maps were produced for each of the groups (urban and rural participants, adults and children, male and female participants, weekdays and weekends, and each of the seasons). Maps were also produced to show the number of participants at each of the coordinates for urban and rural adults and urban and rural children. In the latter case, the coordinates were given to four decimal places to better differentiate the number of participants at each location.

The distance between the centre of Phu Ly (105.9164, 20.5405) and Thanh Liem (105.9390, 20.4368) and each of the coordinates was calculated using the distHaversine() function from the package 'geosphere'. The distHaversine() function calculates the distance between two sets of coordinates as the crow flies. The Kruskal Wallis test was used to determine the difference between these distances.

QUANTITATIVE DATA: THE NORMALISED DIFFERENCE VEGETATION INDEX (NDVI)

NDVI raster files were obtained from the Copernicus Global Land Service at a resolution of 1km and to the nearest two weeks where possible (version 2.2) (Copernicus Global Land Service, 2018) to the nearest two weeks where available. Buffer zones of 100m were extracted around each coordinate using the R package 'raster' and the function extract() which took the mean of the NDVI. The difference between the NDVI values within each buffer zone were compared between each group of participants (adults and children, rural and urban participants, males and females and at weekdays and weekends) using the Kruskal-Wallis test. The NDVI values were then categorised into low (0-0.25), low-medium (0.26-0.50), medium (0.51-0.75) and high (>0.75) and the median and range of total time spent over the study period was compared between urban and rural participants, adults and children, males and females and between different times of the day and the seasons.

QUANTITATIVE DATA: CONTACT PATTERN DATA

Data from the paper contact pattern diaries was entered into the CliRes database. Data was then extracted and read into R as CSV files for analysis.

The same participants undertaking the GPS study also participated in a contact pattern diary at the same times of the year. For the purpose of the diary mornings were classified as 05:00 hours to 11:59 hours; afternoons as 12:00 hours to 17:59 hours; evenings as 18:00 hours to 20:59 hours. Monday-Friday were classified as weekdays and Saturday and Sunday, weekends. 'Contacts' were not given unique identifiers.

Data were cleaned for three diaries where the date had been entered incorrectly with one excluded as the correct date could not be confirmed. One diary was missing for a participant during the summer. Tables showing the total number of non-physical contacts defined as face to face and physical contact, defined as skin to skin or touch, for each group of participants and the median and ranges of the number of contacts per participant over the study period and per day were provided. Statistical differences between the numbers of contacts per participant per day were calculated using a negative binomial GLM using the R package 'MASS' with the identification of the participant fitted as a random effect (Venables and Ripley, 2002). As the date of birth was incomplete for a number of participants the age in years of the participant was calculated as 2017 minus the year of birth.

The median number of contacts per participant by age category were plotted for each of the groups including urban and rural participants, males and females and during weekdays and weekends, different seasons and times of the day. Negative binomial GAM to estimate the number of contacts according to the age of the participant and corresponding contact were fitted for each model with the exception of the contact per participant per day by time of day which required a Poisson model to allow the model to fit. The ages were fitted as smoothed continuous values using the default thin plate regression splines. The identification of the participant was fitted as a random effect for models comparing the number of contacts per participant, and the identification of the participant and the date of the interview as random effects for models comparing the number of contacts per participant per day. Plots of models were constructed using the function vis.gam() from the package 'mgcv' with the corresponding outputs including the estimated degrees of freedom, the chi-squared test and the p value. vis.gam allows for visualisation of the output of the GAM model using a perspective or contour plot (RDocumentation, n.d.-g).

Boxplots to show the difference in the numbers of contacts per participant according to whether the contact was a family member, friend or other were constructed for urban and rural participants, adults and children, males and female and during weekdays and weekends. Similarly, boxplots to show the difference in numbers of and types of contacts made at home or school/work were produced for adults and children. Statistical differences were calculated using negative binomial GAM with the participant identification as a random effect.

ETHICS

The study was approved by OxTREC (reference 42-15), the University of Liverpool ethics committee (RETH001072), the University of Liverpool Sponsorship (UoL001172a) and the NIHE ethics board (IRB-VN01057 -27/2016).

4.3 RESULTS

THE ACCEPTABILITY, FEASIBILITY AND QUALITY OF THE DATA COLLECTION

THE FEASIBILITY STUDY

During the initial feasibility study conducted during 72 weekday periods in February 2017 45,825 coordinates were obtained during the mornings, 43,933 during the afternoon, 22,751 during the evening and 28,772 during the night. The median time between signal recordings was 2.02 minutes. The results showed that for the majority of time, the GPS devices were working and recording signals appropriately. Additionally, it was suggested that most people were taking the GPS with them during their daily activities as the number of recordings was higher during the morning and afternoon when people were more likely to be visiting more places.

Knowledge of GPS devices

Knowledge of GPS devices differed between the groups. Both urban and rural men showed an understanding of the concepts of GPS devices explaining that these existed within smartphones and could be used to locate positions including of people but also to show the road and direction of travel and to track buses. Women showed less understanding with some being unaware of the existence of GPS devices and others unaware of its utility.

Attitudes towards wearing GPS devices and concerns

All groups were concerned that the GPS devices may adversely affect their health particularly if they were wearing it all day with potential effects on the brain or fertility being mentioned. However, some female participants asked whether the device would be able to detect diseases or whether it could be of benefit if they were unwell.

Privacy was a particular worry for some participants who required reassurance that the devices were unable to record sound. Furthermore, expressed anxieties that the devices may be used to 'spy' on them and asked about where these were manufactured. However, other participants appeared less concerned about privacy, stating that they had no worries about the study team knowing their movements which were often from the home to the market, school or work.

Many participants thought that the device would be uncomfortable to wear or be unsightly and that other people may wonder what it was. Many participants also believed that the children would struggle with the device as they would think it was a toy. Female participants were concerned that they may break the device, lose it or forget to put it on after a shower. Some also mentioned that it would be difficult for sellers to wear due to their contact with water. It was suggested that a 'GPS watch' may be a better option.

Attitudes towards participating in the contact pattern diary

All groups were concerned that staff would not find suitable times to visit their homes to complete the diary and some suggested an alternative may be to conduct the interview by mobile phone or to leave the diary to complete themselves. They also stated that the diary should not be too long. Some urban men suggested that it would be more suitable for women participate in the study as they were easier to visit and due to their jobs, travelled more.

Despite concerns raised, the groups were happy to take part in the study if it was for the benefit of the community.

FEEDBACK FROM THE PARTICIPANTS

Feedback was obtained on 1,163 occasions with eighty-one (7.0%) reports of problems with the GPS device over the study period. There were thirty-seven (3.2%) reports of participants forgetting to wear the device. Of these, twenty-one (56.8%) reports were from adults and sixteen were from children. There were six (16.2%) reports of participants forgetting to wear it for less than one hour, twenty-six (70.3%) between one and six hours and fifteen (40.5%) between seven and twenty-four hours. There were 1,105 (95%) reports of deliberately removing the device, with ten (0.9%) people removing it for less than one hour, 267 (24.2%) removing it from between one and six hours; and 809 (73.2%) between seven and twenty-four hours. The main reasons for removal of the GPS included charging the device (n=969 (87.7%)), bathing or washing (n=763 (69.1%)) or sleeping (n=219 (19.8%)). There were forty-seven (4.0%) comments that the device was big or heavy and in four (0.3%) occasions it was mentioned that friends borrowed the device.

QUALITY OF THE DATA COLLECTION

After data cleaning, a total of 671,202 longitude and latitude coordinates were obtained. Data from two participants during two seasons were removed as the devices were lent to friends; data was missing for one participant in July and two in September/October; data from an individual season* was removed on eighteen occasions where there were spurious coordinates for example, where it was not feasible for a participant to travel between the coordinates in the time provided e.g.

was travelling at a speed of 130km/hr or over; and maps were excluded for one participant who did not consent to this.

* December and February were counted as separate seasons given that the two 72 hour period could be separated.

MOBILITY PATTERNS

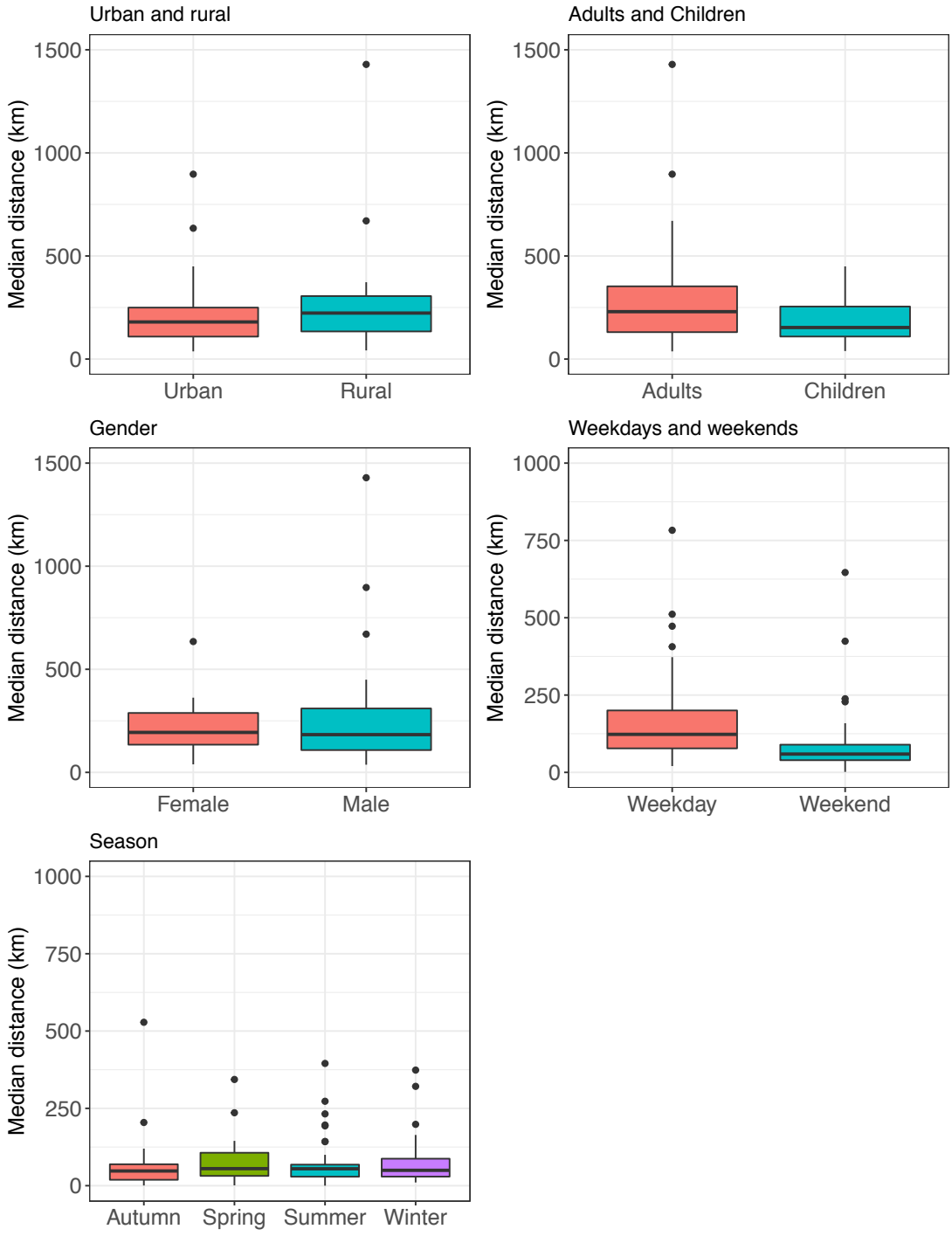
The participants included in the study included twelve female adults, twelve male adults, thirteen female children and eleven male children.

DISTANCES COVERED

The total distance covered over the study period amongst all participants was 11977.07km and the median distance covered per participant over the total study period was 189.21km (range = 37.26-1429.15km). The median total distance covered per participant from an urban area was 179.97 km (range 37.26-896.70km) and from a rural area, 223.16 km (range 41.99-1429.15km) with no evidence for a difference between the distances (p=0.275). Adult participants travelled a median of 229.91km (range 37.27-1429.15km) and children, 153km (range 38.92-449.50km) with no strong evidence for a difference between the two (p=0.095). Female participants, travelled a median of 193.81km (range 38.92-6345.08km) and male participants 183.09km (range (37.26-1429.15km) with no evidence for a difference between the two (p=0.992).

The median distance covered during weekdays was 122.95km (range 20.50-782.81km) compared to weekends (59.22km (range 1.5 – 64.34km) with strong evidence for a difference between the two (p<0.001) The median total distance covered per participant during the spring was 55.06km (range 9.39–343.43km); summer, 54.67km (range 0-395.26km)); autumn, 47.53km (range 5.48–528.35km) and winter 49.73km (range 10.41-373.76km) with no evidence for a difference between the seasons (p=0.339) (figure 4.2).

Figure 4.2 The distances covered per participant over the study period by group of participants and between weekdays and weekends and seasons. The upper hinge of each box represents to the 75th percentile and the lower hinge, the 25th percentile. The bold line located within the box represents the median value. The upper whisker is equal to 1.5*the inter-quartile range (IQR) and the lower whisker, 1.5*IQR. The individual dots represent 'outlying' points.



Rural adults travelled the most with a median distance covered per participant of 274.96 km (range 67.03-1429.15km) followed by urban adults (212.83km (range 37.26-897.70km)); rural children (170.87km (range 41.99-351.77km) and urban children (127.36km (range 38.92-449.50km)) but with no evidence for a difference between these (p=0.252). On an individual basis, those from all groups covered greater distances during the weekdays compared to the weekends. Evidence for a difference existed only amongst the children (p=0.003). Urban children travelled a median distance of 85.41km (range 38.92-372.64km) per participant during the weekdays compared to the weekends (median of 47.76km and range of 1.5-158.32km) and rural children, 119.52km (range 21.75-206.49km) during weekdays compared to weekends (median of 53.41km (range 19.28-145.28km).

THE TIME SPENT AT LOCATIONS

390,663 unique coordinates were obtained with a median of 7126 unique coordinates per participant (range 1641-30689). Unique coordinates are those which have a different longitude and latitude as the same coordinate can appear more than once in the database. The median time spent at the same coordinate per participant was 2.05 minutes with a range of 3 seconds to 93 hours.

There was no evidence for a difference in the median time spent at each coordinate between urban and rural participants (p=0.342), male and female participants (p=0.597) nor during each of the seasons (p=0.05). There was also no difference in median time spent at each coordinate at weekdays compared to weekends (p=0.929). including when stratified by age (adults, p=0.809 and children, p=0.560).

All groups of participants travelled outside of Ha Nam province during the study period. Using locations participants remained at for 2 minutes or longer, seven provinces (Hung Yen, Hai Duong, Bac Ninh, Ha Noi, Ninh Binh, Nam Dinh and Thai Binh) outside of Ha Nam were travelled to by adults or those from urban areas compared to five by children (Ha Noi, Hoa Binh, Nam Dinh and Thai Binh) and four by those from rural areas (Ha Noi, Bac Ninh, Hoa Binh and Thai Binh) (figures 4.3 and 4.4). Four provinces were travelled to on weekdays, compared to eight provinces at weekends (figure 4.5). The participants travelled to four provinces outside of Ha

Nam during the spring compared to three in the summer, four in the autumn and three in the winter (figure 4.6). One rural child travelled to Quang Nam, during a weekday in the winter. However, locations in which participants stayed six hours or longer were only within Ha Nam province (figure 4.7).

Figure 4.3 Maps to show median time spent per participant at the same coordinate for adults and children. The maps include the neighbouring provinces of Ha Nam with Hanoi city and Phu Ly marked. The maps are subset to show those where the median time was 2 minutes or 6 hours or longer. The size of the circle and colour indicates the time in hours.

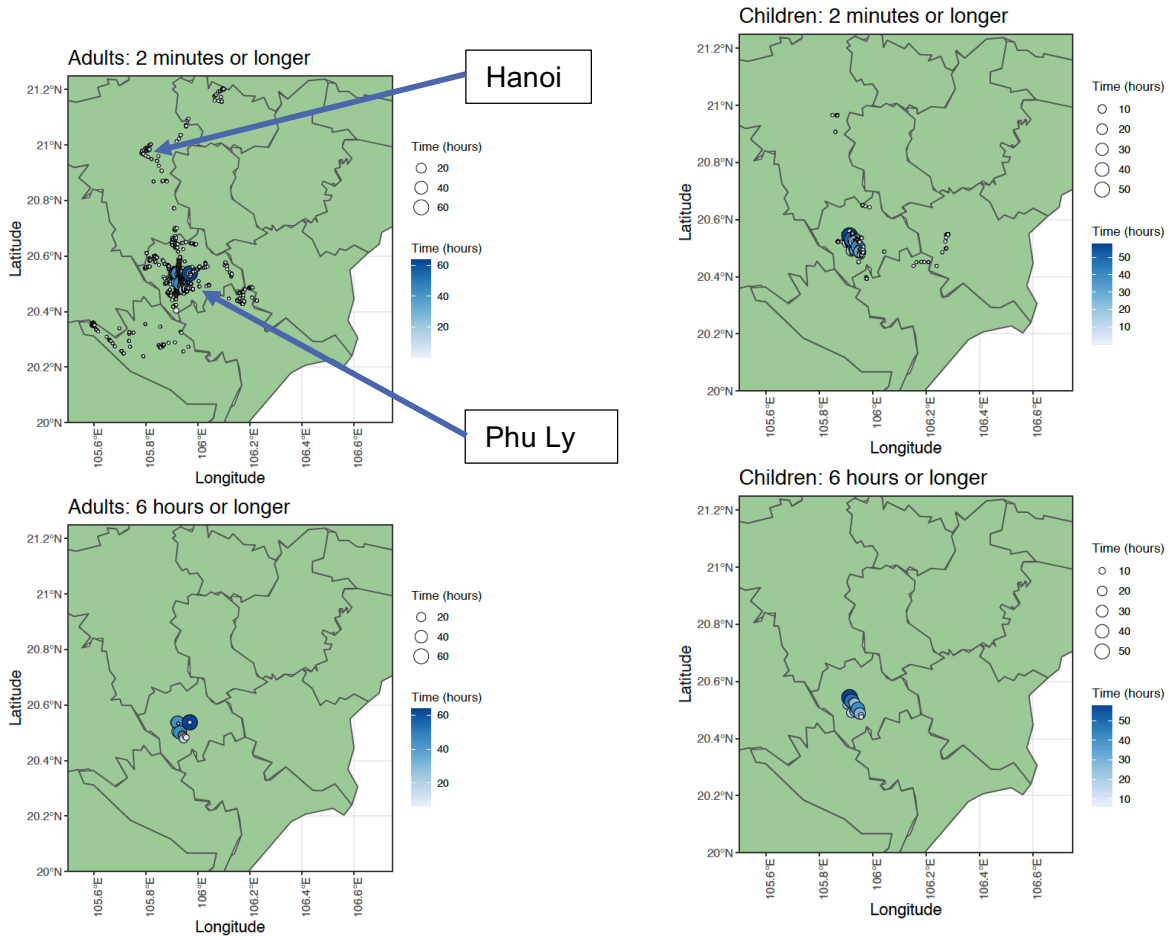


Figure 4.4 Maps to the median time spent at the same coordinate for rural and urban participants. The maps include the neighbouring provinces of Ha Nam with Hanoi city and Phu Ly marked. The maps are subset to show those where the median time was 2 minutes or 6 hours or longer. The size of the circle and colour indicates the time in hours.

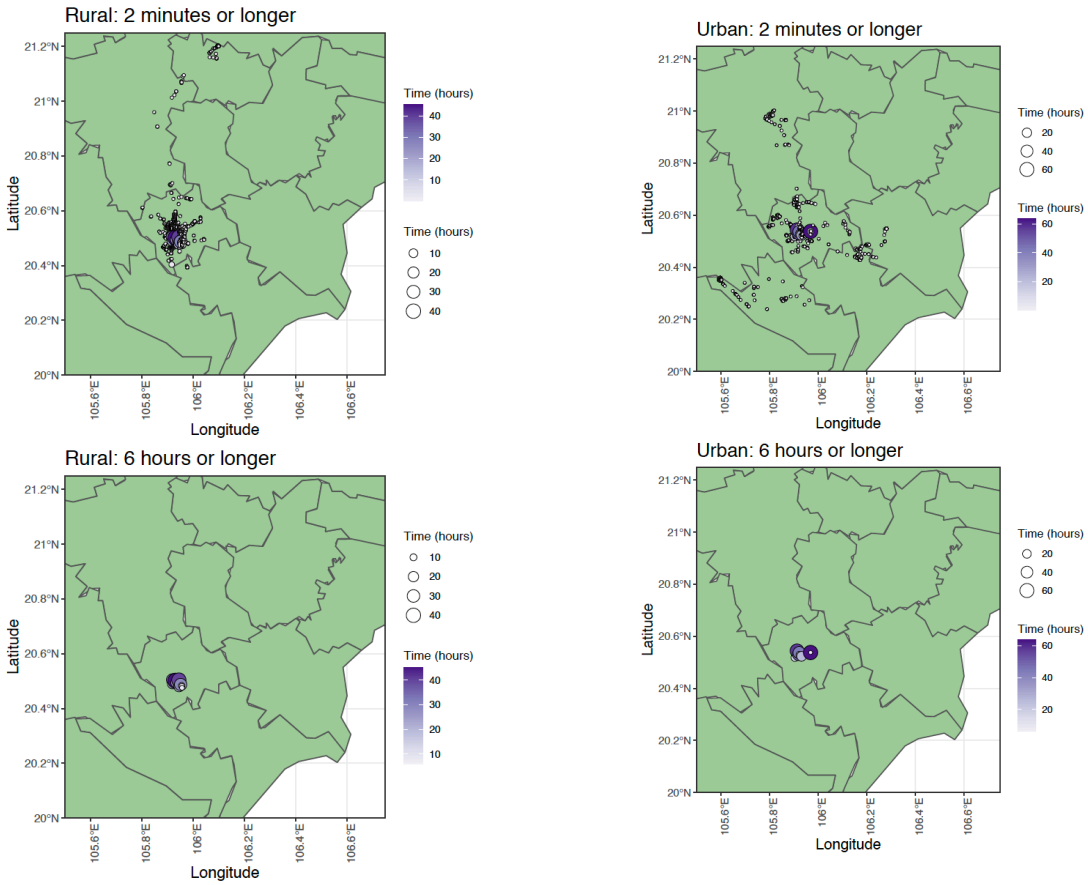


Figure 4.5 Maps to show the median time spent at the same coordinate per participant for weekdays and weekends. The maps include the neighbouring provinces of Ha Nam with Hanoi city and Phu Ly marked. The maps are subset to show those where the median time was 2 minutes or 6 hours or longer. The size of the circle and colour indicates the time in hours.

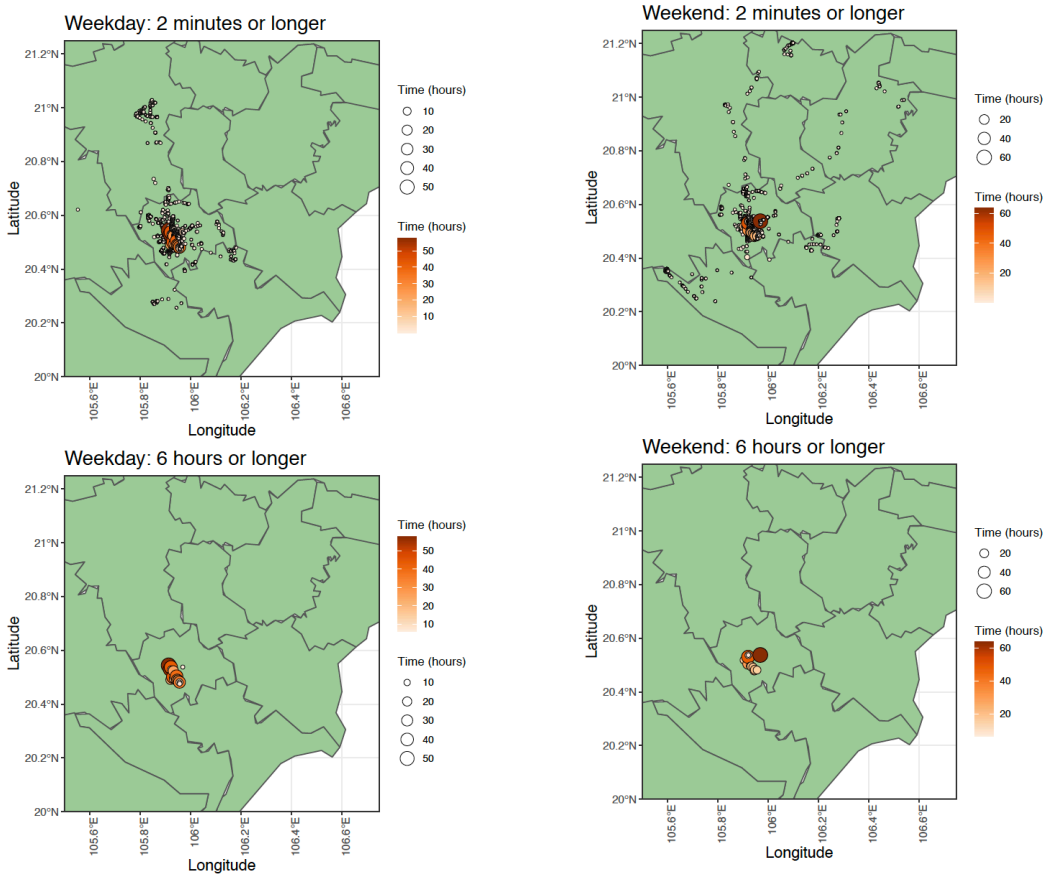
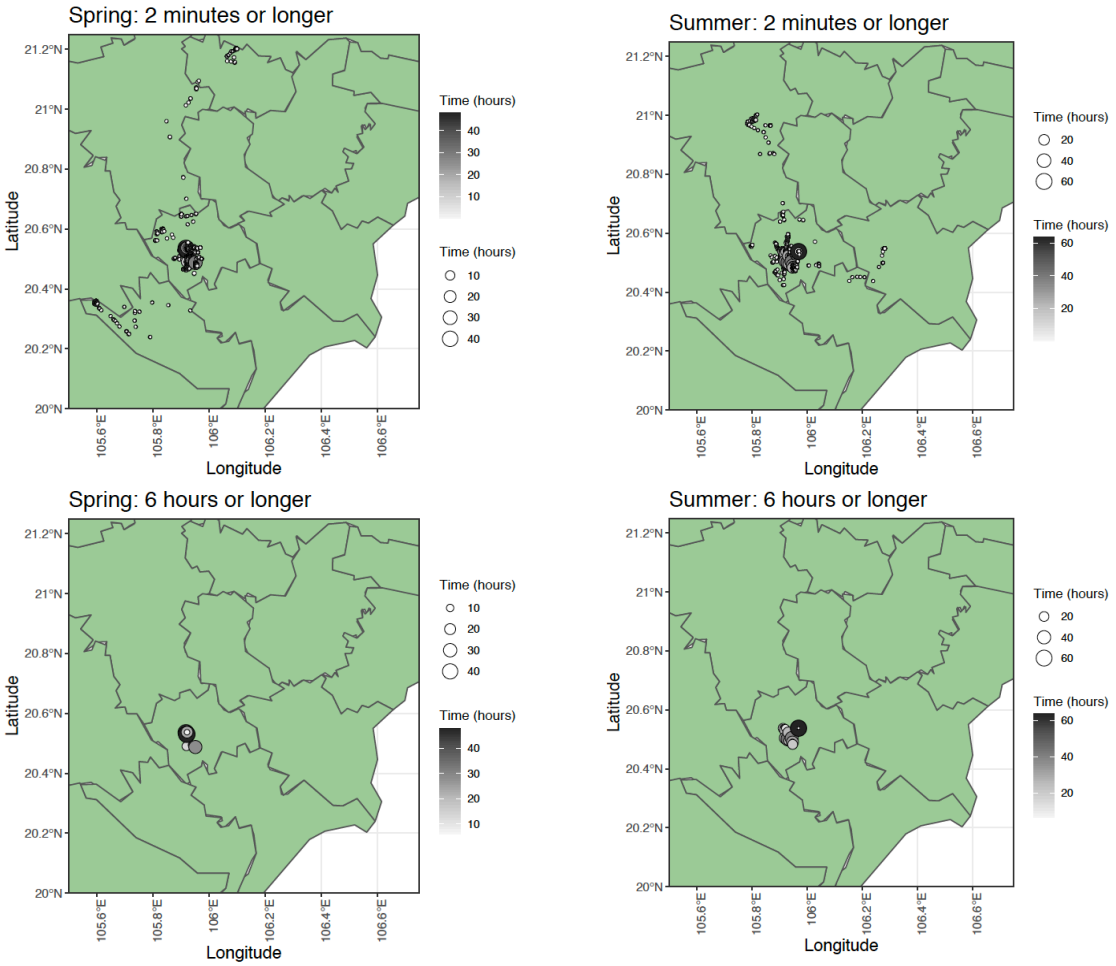
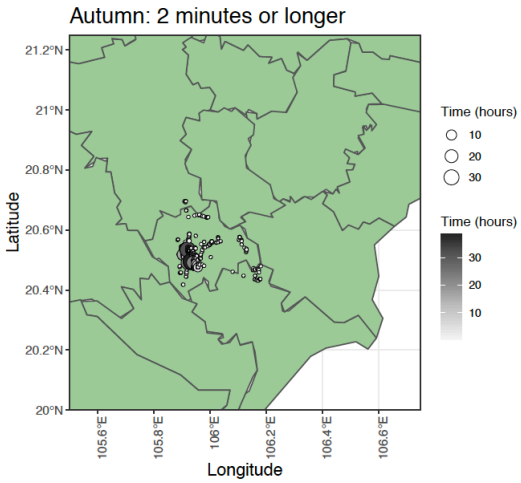
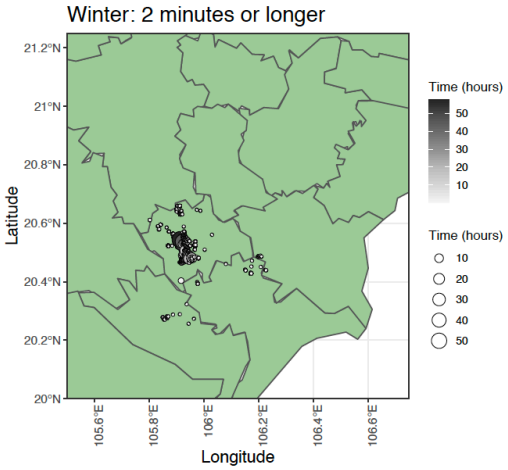
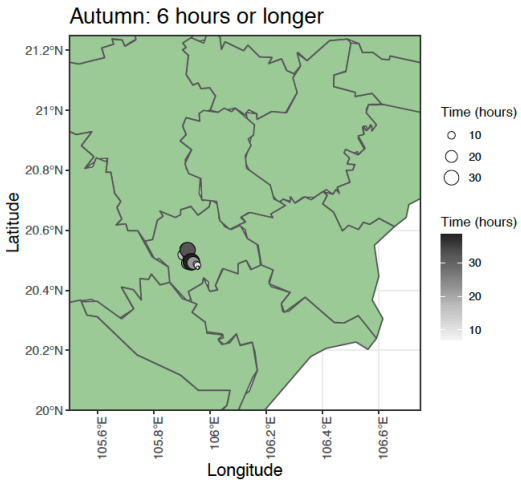


Figure 4.6 Maps to show the median time spent at the same coordinate per participant for each season. The maps include the neighbouring provinces of Ha Nam with Hanoi city and Phu Ly marked. The maps are subset to show those where the median time was 2 minutes or 6 hours or longer. The size of the circle and colour indicates the time in hours.




Autumn: 2 minutes or longer
Latitude
21.2°N
21°N
20.8°N
20.6°N
20.4°N
20.2°N
20°N
105.6°E
105.8°E
106°E
106.2°E
106.4°E
106.6°E
Longitude
Time (hours)
10
20
30
Time (hours)
30
20
10


Winter: 2 minutes or longer
Latitude
21.2°N
21°N
20.8°N
20.6°N
20.4°N
20.2°N
20°N
105.6°E
105.8°E
106°E
106.2°E
106.4°E
106.6°E
Longitude
Time (hours)
50
40
30
20
10
Time (hours)
10
20
30
40
50


Autumn: 6 hours or longer
Latitude
21.2°N
21°N
20.8°N
20.6°N
20.4°N
20.2°N
20°N
105.6°E
105.8°E
106°E
106.2°E
106.4°E
106.6°E
Longitude
Time (hours)
10
20
30
Time (hours)
30
20
10

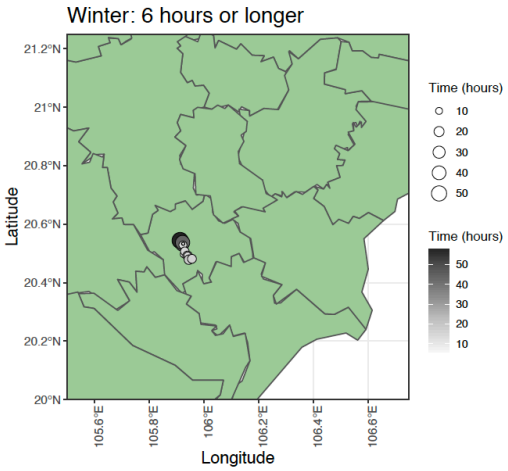
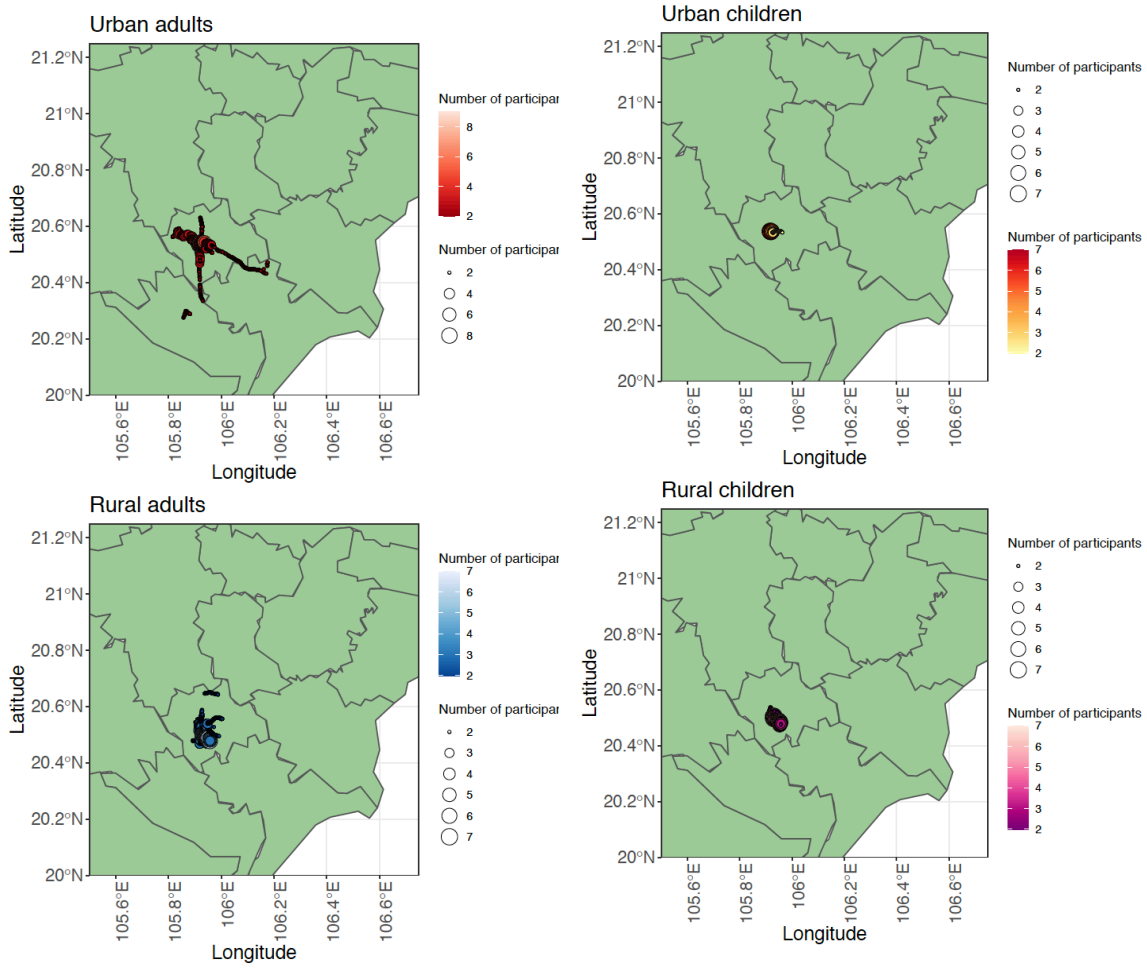

Winter: 6 hours or longer
Latitude
21.2°N
21°N
20.8°N
20.6°N
20.4°N
20.2°N
20°N
105.6°E
105.8°E
106°E
106.2°E
106.4°E
106.6°E
Longitude
Time (hours)
10
20
30
40
50
Time (hours)
50
40
30
20
10

Figure 4.7 The places travelled to by more than one participant over the study time period, subset by urban and rural adults and urban and rural children. The size and colour of the circles indicate the numbers of participants.



Per individual, urban participants travelled a median distance of 765.97m from the centre of Phu Ly with a range of 11.29m-52.43km compared to rural participants who travelled a median distance of 6.42km from the centre of Thanh Liem with a range of 1.68-86.86km with strong evidence for a difference between the two ($p<0.001$). Per participants, adults travelled a median distance of 3.53km from the centre of Phu Ly or Thanh Liem with a range of 7.15m–86.86km compared to children who travelled a median distance of 3.88km from the centre of Phu Ly or Thanh Liem with a range of 25.08m-47.88km with no evidence for a difference between the two ($p=0.483$). Per participants, females travelled a median of 2.35km (range 7.15m-52.43km) compared to males, 4.73km (range 25.08m-86.86km) with no evidence for a difference between the two ($p=0.177$).

NDVI

A measurement of the NDVI was available for 359,556 unique coordinates. Per participant, the median NDVI within a 100m radius of each recorded coordinate was 0.93 with a range of 0.12-0.93. Amongst urban participants the median NDVI per participant was 0.93 with a range of 0.12 to 0.93, compared to rural participants (median=0.69, range = 0.14-0.93) with evidence for a strong difference between the two ($p<0.001$). There was no difference in the NDVI per participant between adults and children with a median of 0.93 and a range of 0.12-0.93. The median NDVI amongst male participants was 0.93 with a range of 0.14-0.93) and female participants 0.93 with a range of 0.12-0.93) with no evidence of a difference between the two ($p=0.879$). The median NDVI at locations during the weekdays was 0.93 with a range of 0.12-0.93 and weekends, 0.93 with a range of 0.14-0.93 with no evidence of a difference between the two ($p=0.018$). The median NDVI at locations visited by the participants during the morning, afternoon or evening was the same (0.93) with a range of 0.12-0.93).

Across all demographics (urban and rural, male and female and adults and children) the most time was spent in areas of NDVI within the highest quartile (>0.75) and in general, the shortest amount of time in the lowest NDVI (0-0.25). The exception to this is the urban participants who spent the shortest time in the ‘lowest-medium’ NDVI (0.26-0.50). In the summer, autumn and winter the most time was spent in the highest

NDVI and the spring, the medium-highest (0.51-0.75). Similarly, throughout the day the most time was spent in the highest NDVI (tables A4.1-A4.5, appendix 4.5).

CONTACT PATTERNS

Over the study time period, there were a total of 12,804 non-physical (face to face) contacts and 2,911 physical (skin to skin touch) contacts. There was no evidence for a difference between the total number of non-physical or physical contacts per participant by age category. However, there was strong evidence for a higher number of non-physical contacts per participant per day amongst all age groups except those aged 13-17 years and 61 years compared to the youngest age group of 0-5 years. Those aged 0-5 years were more likely to have a higher number of physical contacts per participant per day compared to all age categories except those aged 6-12 years and those aged 18-35 years (tables A4.6 and A4.7, appendix 4.5).

There was strong evidence that men were more likely to have a higher number of non-physical contacts per participant per day compared to women but a lower number of physical contacts (tables A4.8 and A4.9, appendix 4.5). Similarly, there was strong evidence that urban participants were more likely to have a higher number of non-physical contacts compared to rural participants but fewer physical contacts (tables A4.10 and A4.11, appendix 4.5).

There was strong evidence for fewer non-physical contacts during weekends. This relationship also existed for physical contact but only with respect to the number of contacts per participant over the study period and not per participant per day (tables A4.12 and A4.13, appendix 4.5). There was strong evidence for a higher number of non-physical contacts per participant per day in the spring compared to the summer and winter but fewer physical contacts compared to all other seasons (tables A4.14 and A4.15, appendix 4.5). There was strong evidence for a higher number of non-physical contacts in the morning compared to the evening however, physical contact was greater in the evenings but only with respect to the total number of contacts per participant (table A4.16 and A4.17, appendix 4.5).

THE NUMBER OF CONTACTS BY AGE OF THE PARTICIPANT AND AGE OF THEIR CONTACT (PHYSICAL AND NON-PHYSICAL)

Data prior to modelling showed that amongst male participants, the most contact was seen between children and adults aged 36-60 years. This differed to female participants where, with the exception of participants aged 6-12 years, more contact was seen amongst adults. In both urban and rural participants, children have the most contact with adults aged 36-60 years however, urban participants aged 61 years and over also have higher numbers of contacts with those of the same age group. There was little difference between ages of participants and contacts at weekdays compared to weekends with most contact occurring between participants aged 0-5 years and contacts aged 36-60 years and adults with those aged 61 years and older (figure 4.8).

Negative binomial generalised additive models showed that, amongst urban participants, contact was estimated to be highest amongst participants aged 40-50 years and contacts aged 80 years and older, and participants aged 5 years and under and contacts aged around 40 years. Amongst rural participants, contact was estimated to be greatest amongst child participants and older contacts e.g. those aged over 70 years. Amongst male participants, contact was estimated to be highest amongst adult participants and elderly contacts and child participants and contacts aged 40-50 years. Amongst female participants, younger adult participants were estimated to have more contact with the elderly and child participants with adults aged 30-40 years. A similar pattern was estimated during weekends and weekdays with the highest contact between younger adult participants and elderly contacts and younger children and adult contacts aged 30-40 years (figure 4.9). Overall, there was strong evidence for differences in the number of contacts between age categories within each of the groups with the exception of the male participants which showed slightly weaker evidence (table 4.1).

Figure 4.8 The median number of contacts per participant over the study period by age category shown for urban and rural participants, males and females and during weekdays and weekends.

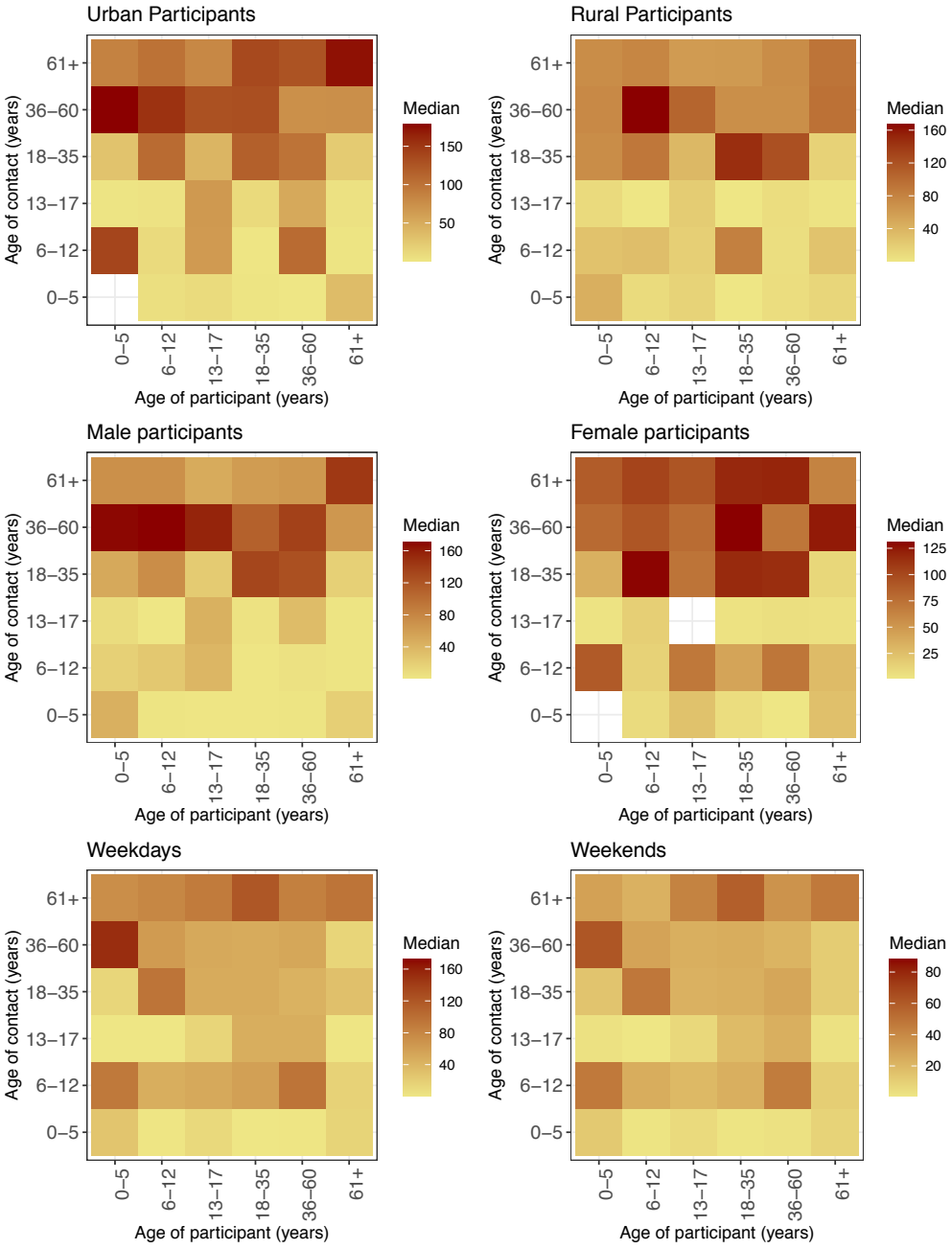


Figure 4.9 Negative binomial GAM of contacts by age group.

The lighter the colour and the higher number defined by the contour, the greater the estimate. The output of the GAM models is given below in table 4.

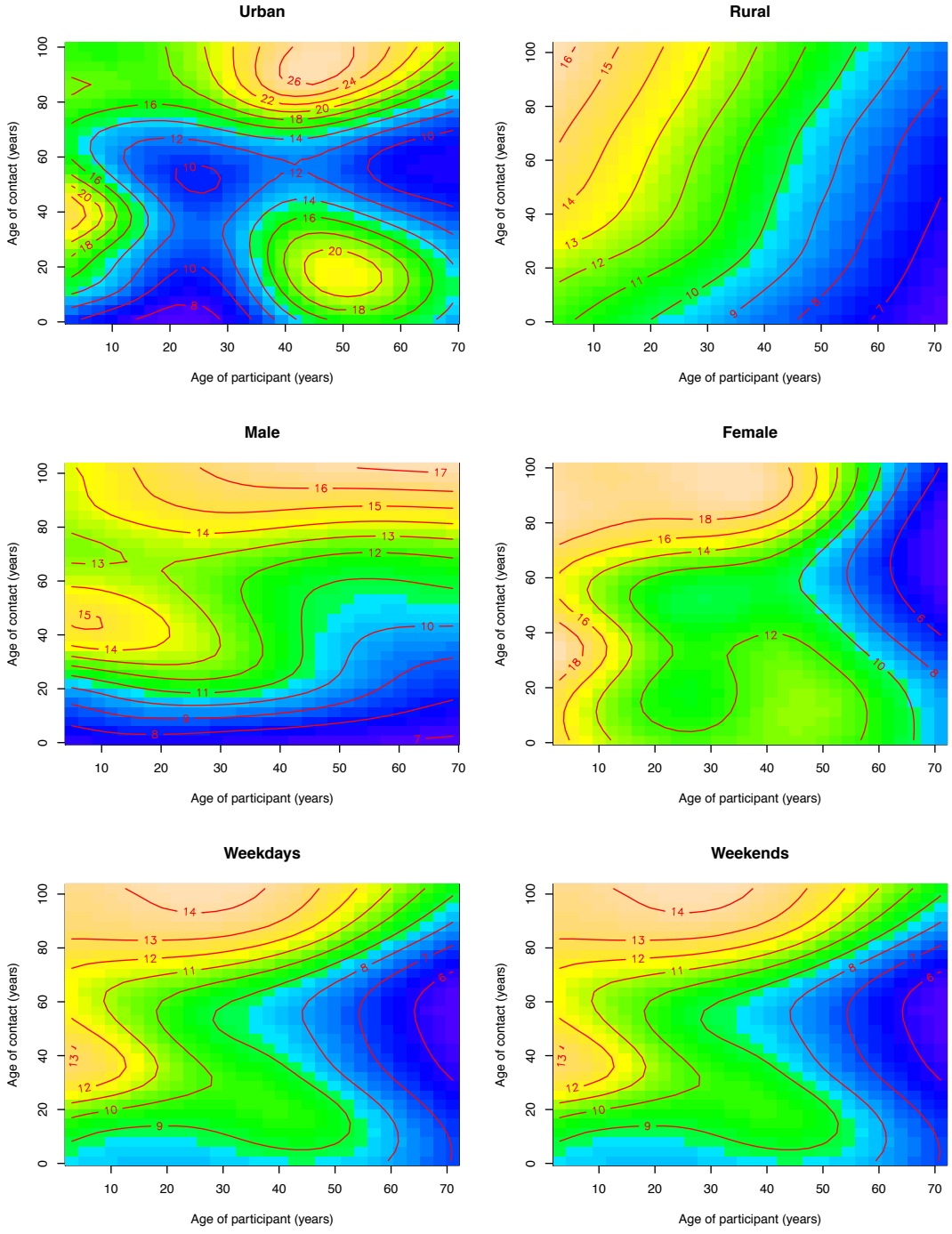


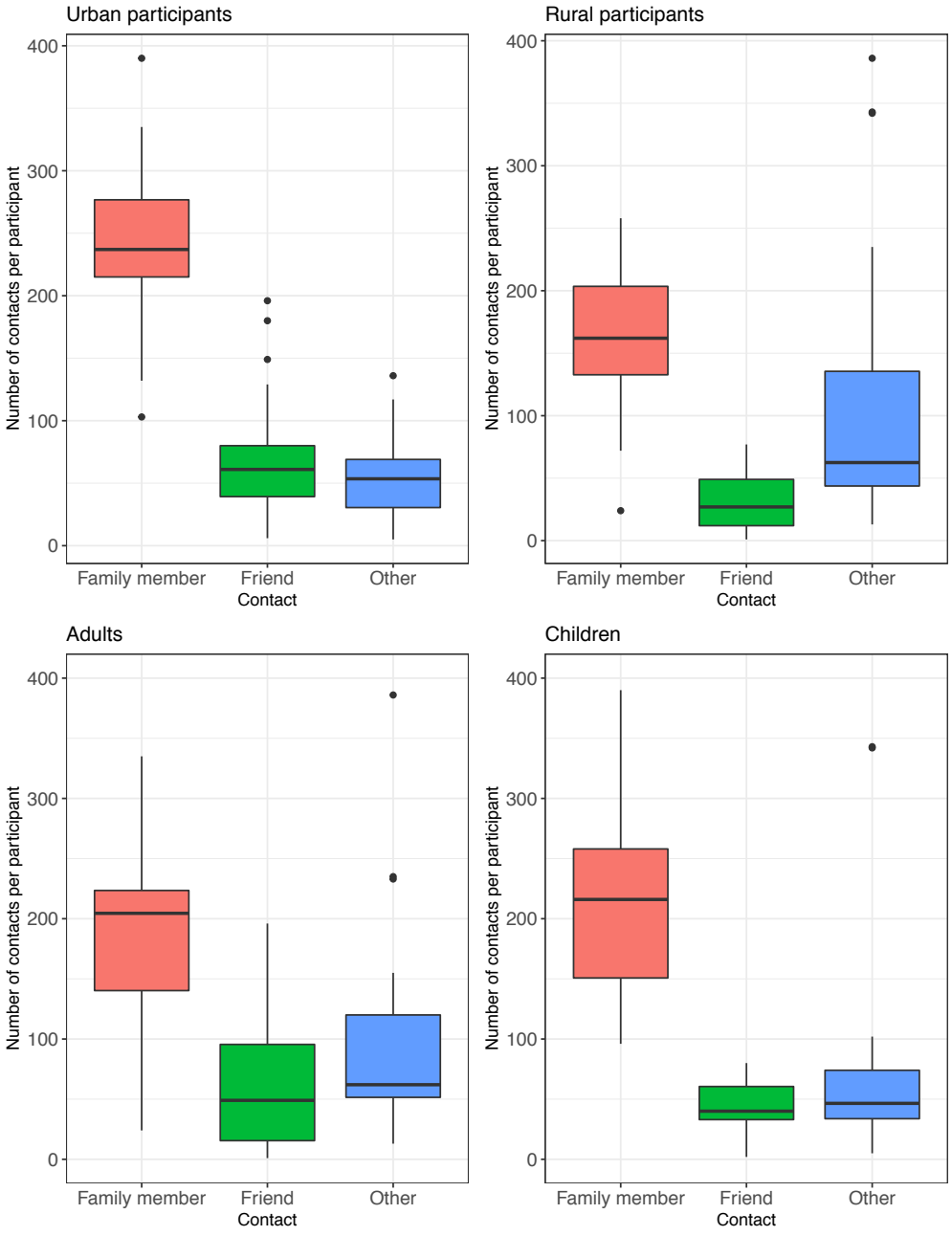
Table 4.1 The output of the smoothed GAM models.

Group	Estimate degrees of freedom	Chi squared test	p value
Urban	15.75	48.63	<0.001
Rural	2.01	34.85	<0.001
Male	6.73	18.48	0.037
Female	11.16	80.75	<0.001
Weekday	8.79	71.45	<0.001
Weekend	10.79	63.6	<0.001

There was strong evidence for a higher number of contacts who were family members compared to friends amongst all groups of participants (figure 4.10).

Per participant, those from urban compared to rural areas met a higher number of contacts at home ($p<0.001$). A higher number of contacts were met both at home, school and work during weekdays compared to weekends ($p<0.001$). Adults had a higher number of non-physical contacts at school or work per participant compared to children however, there was no strong evidence for a difference between the two ($p=0.051$). Children had a higher number of physical contacts at school or work with strong evidence for a difference between the two ($p=0.004$). Children had a higher number of both non-physical and physical contacts per participant in the home compared to adults but with no evidence of a difference between the two ($p=0.77$ and $p=0.867$, respectively).

Figure 4.10 The number of contacts per participant according to whether the contact was a family member, friend or other person.



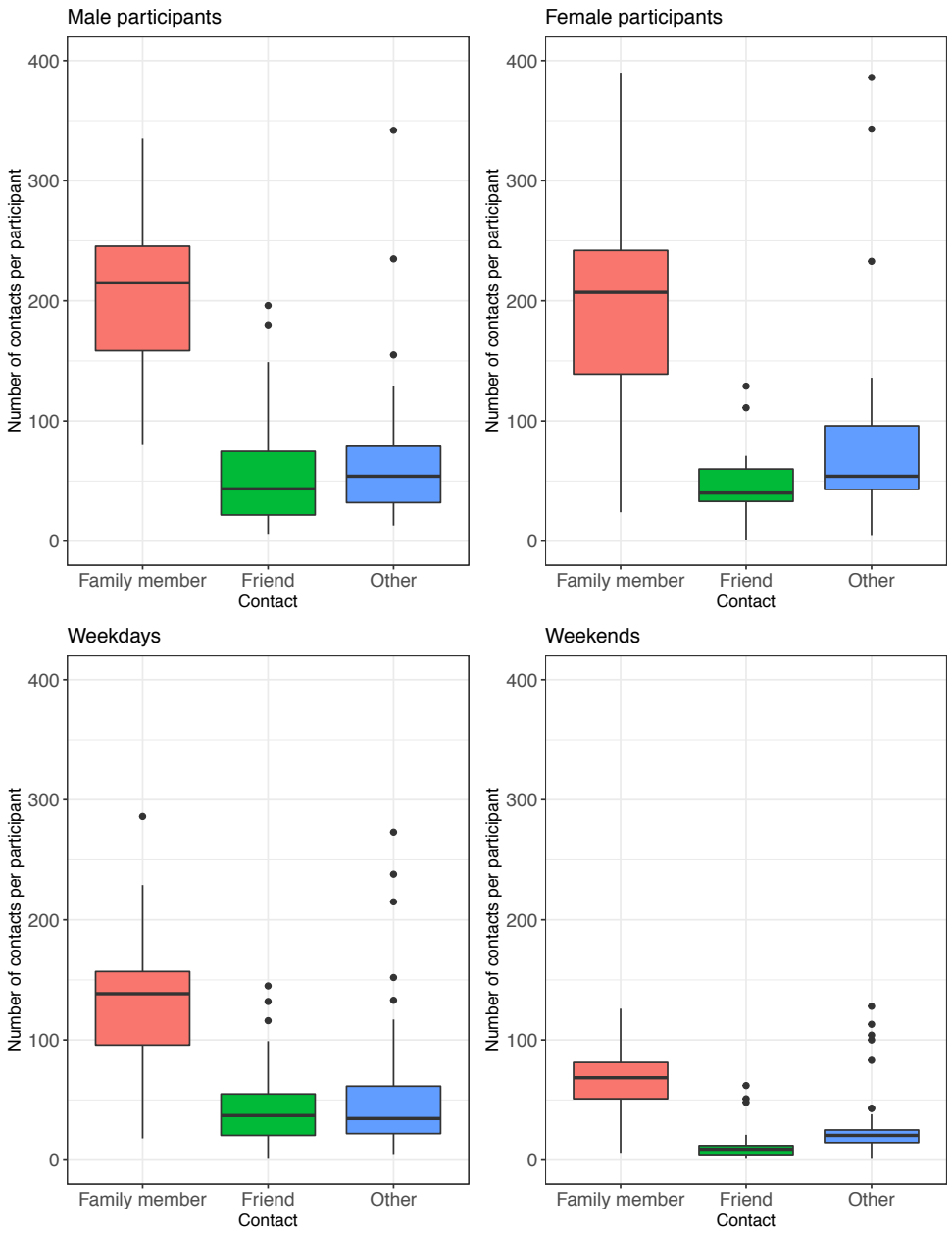
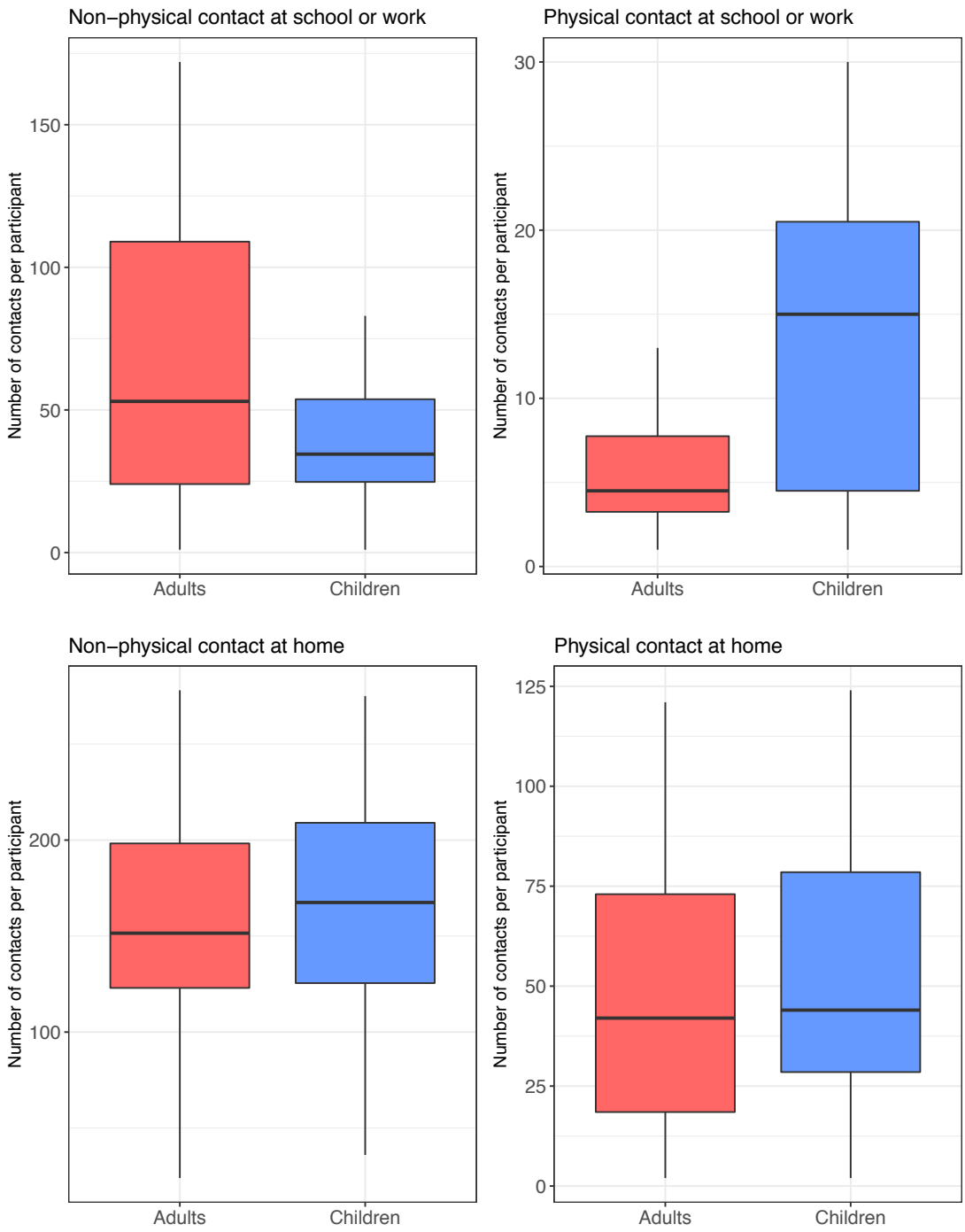

Male participants
Female participants
Weekdays
Weekends
Number of contacts per participant
0
100
200
300
400
Family member
Friend
Other
Contact

Figure 4.11 The number of non-physical compared to physical contacts per participant according to the location and whether the participant was an adult or child.



4.4 DISCUSSION

This study of human movement and contact patterns over a year gives an insight into how these factors may affect the risk of acquisition and transmission of a number of pathogens causing AES in Ha Nam province, northern Vietnam. This work builds on that conducted by Horby et al., 2011 who explored social contacts in Ha Nam through the use of diaries in 2007. This is the first known study to use GPS devices to evaluate human movement patterns in northern Vietnam.

OBJECTIVE 1: TO ASSESS THE ACCEPTABILITY AND FEASIBILITY OF USING GPS DEVICES AND CONTACT PATTERN DIARIES TO UNDERSTAND THE MOBILITY AMONGST HEALTHY PARTICIPANTS LIVING IN HA NAM PROVINCE, NORTHERN VIETNAM

Given the novelty of the movement work in this region there was a need to assess the acceptability, feasibility and quality of data collection using GPS devices. This was not covered by Horby et al., 2011 during their work on social mixing (Horby *et al.*, 2011). Some initial concerns about the GPS device were highlighted during the FGDs. These included privacy; adverse health effects; and whether the device would be uncomfortable or may be lost or broken. Some participants were also concerned about the time it would take to conduct the contact pattern diary. Despite these worries, participants stated they were willing to take part in the study to benefit the health of the community. The concerns about the GPS device were not unique to this setting. FGDs conducted in Peru to determine the acceptability of wearing a GPS device to understand the roles played by humans in the transmission of dengue virus found that participants also expressed apprehension about the effect of the GPS device on their health, their responsibility for the device and caring for it, and the confidentiality of the information and whether the device would audio or video record them (Paz-Soldan *et al.*, 2010). However, pre-and post-survey measures to assess the acceptability of wearing a GPS device amongst young men who have sex with men in New York found that only 8% or less were concerned about losing the GPS, their safety or that it would be uncomfortable to wear (Duncan *et al.*, 2016).

In this study where a different cohort participated in the GPS and contact diaries to those in the FGDs, there were only 31 out of 1,200 reports of people forgetting to wear the device and no devices were lost. Devices were removed on occasion including to

charge the device or whilst sleeping or bathing/washing. One device broke during the study but was replaced. Other studies have found variable rates of compliance. In Peru, 44% of the 126 study participants forgot to wear their device on at least one occasion over a study period of 14-30 days (Paz-Soldan *et al.*, 2010) whereas in New York, just over 10% of 74 participants forgot to wear the GPS device on a daily basis over a period of 7 days (Duncan *et al.*, 2016).

Despite some concerns raised during the FGDs about the length of the contact pattern diaries, the suggestion that female participants may be placed to complete these and that they may be better conducted over the telephone or by the participants themselves rather than the research staff, the return rate for the diaries was good with a diary missing for only one participant for one season. During a study conducted at a scientific conference in Germany 20% (95%CI 11-34%) of the respondents stated that the completion of a contact diary was too much work and only 25% (95%CI 14-38%) stated that they had difficulty remembering their contacts. However, there was improvement with familiarity with the contacts (Smieszek *et al.*, 2016).

The large numbers of different coordinates obtained over the study period, demonstrated that the devices worked well and that participants complied with the study by taking the these outside of the house throughout the year. Additionally, the maps showed that the participants not only took the devices with them whilst moving within their communities but also when they travelled outside of Ha Nam. This showed the dedication the participants had towards the study and assumed that there was acceptance of wearing the device. The reason for this compliance may have been in part due to the quality of the consent process. The participants were assured that the device could not record their voices or recognise their contacts; that it was safe and if uncomfortable to wear they could put it in their pocket. It is also possible that the participants were happy to participate for the benefit of their community as suggested during the FGDs. This might be attributed to the collectivist approach often adopted in Vietnamese society and cited as a potential reason for the successful response to the COVID-19 pandemic (Nguyen, 2020). With nearly 13,000 contacts reported and that the majority of diaries being completed, we can assume that the contact pattern diaries were also accepted.

Although these methods might be deemed acceptable to participants, direct feedback was not obtained from PMC staff and their workload cannot be underestimated. The data from each GPS device needed to be manually downloaded and cleared on return of the device from each participant. The homes of participants also needed to be visited on a daily basis. Although it was feasible in this setting, it is unknown whether this approach would be transferrable.

OBJECTIVE 2: TO COMPARE THE MOBILITY PATTERNS OF DIFFERENT GROUPS OF PARTICIPANTS

The second objective of this study aimed to compare the mobility patterns between healthy adults and children living in rural and urban areas of Ha Nam province during different time periods. There was no evidence for a difference in the overall distances covered between the different urban compared to rural participants, men compared to women and adults compared to children. On average, rural participants travelled further from their commune. This may be due to socio-economic differences but would require further research to investigate this including enquiries about the reasons for travel. Interestingly, the participants tended to spend shorter amounts of time at the same location when travelling outside of Ha Nam. This might suggest that these visits do not involve visiting friends or family at home for periods of days.

Compared to other work, a study in Spain showed that women travel shorter distances compared to men (Lenormand *et al.*, 2015) yet another in the United States showed that gender had less of an impact on human mobility compared to race or ethnicity (Luo *et al.*, 2016). 73% of women in Vietnam are estimated to be part of the labour force (The World Bank, 2020b). Despite this, women continue to do most of the housekeeping and childcare (Vu, *et al*., 2019). Therefore, travel by women for work and family necessities including taking children to school and shopping for groceries may contribute to the absence of a difference in overall mobility between the genders. Given that children travel as much as adults, this might suggest that people in Ha Nam tend to travel as families.

Overall, the distances travelled by participants were significantly greater during weekdays compared to weekends however, by demographic this difference only remained between urban and rural children with rural children travelling further during the weekdays, potentially due to school. We did not see any difference in the overall distances travelled between the seasons. This is also interesting as it might be expected that people travel more during the winter due to the Lunar New Year as is seen in neighbouring China (Gibbs *et al.*, 2020) and potentially less in the summer due to the very warm climate.

OBJECTIVE 3: TO EVALUATE THE TIME SPENT IN AREAS DEEMED TO BE AT RISK OF MOSQUITO BREEDING

The study participants spent more time over the study period in locations with a higher NDVI. This pattern was generally consistent between adults and children, males and females and urban and rural participants. The data therefore suggests that participants were spending longer periods in areas suitable for mosquito breeding. As mosquito breeding and survival is also sensitive to climatic changes (Reisen *et al.*, 2008; Ciota *et al.*, 2014; Upadhyayula *et al.*, 2012), the time of year in addition to the NDVI can influence the likelihood of the population coming into contact with these vectors however, no difference was seen between the time spent in areas of different NDVIs by the participants. In addition to the changes in climate, different mosquito species are active at different times of the day with *Cx. tritaeniorhynchus* biting in the evenings and mornings (Reisen *et al.*, 1976) and *Aedes* species in during the morning and late afternoon (Reinhold, Lazzari and Lahondere, 2018). However, there was no difference between the time spent in different areas of NDVI by time of the day.

OBJECTIVE 4: TO COMPARE THE CONTACT AND BEHAVIOURAL PATTERNS OF PARTICIPANTS

The final objective of the study was to assess the relationship between the behavioural patterns of healthy people in this study and known risk factors for AES. The majority of contact was non-physical e.g. face to face (82%). This proportion was higher than that recorded by Horby et al., 2011 in a previous study in Vietnam (56%) however, it is possible that the behaviours of the population have changed over time with potentially a higher proportion meeting people in the workplace. It is also possible

that there were differences between the participants in terms of their socio-economic and employment status but given that the sample size for this study was much smaller than conducted by Horby et al., 2011 with 48 participants compared to 865, it is difficult to draw conclusions about the differences in behaviour. In this study, the majority of contacts per participant occurred at home compared to school or work which is in keeping with the study by Horby et al., 2011 but also a study conducted in a number of European countries (Mossong *et al.*, 2008). The per participant number of contacts which occurred at home was higher amongst urban compared to rural participants suggesting that the urban participants either lived in households of a larger size or had more episodes of contact with the same people within the home. This differs from a comment made by authors who conducted a study in China and suggested that most urban contacts occur outside the home (Read *et al.*, 2014). Additionally, more contacts per participants occurred at home during weekdays compared to weekends. This was an unexpected finding as during the weekdays we would expect people to spend more time outside of the home, at work or school however, whether this is due to unemployment or shift work would need to be investigated. Across all groups of participants, contact (physical and non-physical combined) occurred more frequently with family members compared to friends. This may reflect the importance of the family unit in this setting in Vietnam.

The raw and modelled contact pattern data showed that the contact between children and adults was more frequent than between children. Additionally, there was evidence of moderate contact between adults. This pattern is similar to that described by Horby et al., 2011 who found moderately intense contact between adults aged 20-65 years representing the working age group and between children aged 0-5 years and adults aged 20-65 years representing contact between children and their parents or grandparents. This pattern differed to that seen in studies in the UK and Europe where during the school term the highest intensity of contact occurred between children of school age and between adults of working age (Eames *et al.*, 2012; Mossong *et al.*, 2008). However, children were more likely to have physical contact in school compared to adults at work.

WHAT DOES THIS MEAN FOR RISK OF INFECTION OF PATHOGENS CAUSING AES AND TRANSMISSION DYNAMICS?

There is evidence that people in Ha Nam spend time in areas conducive to the breeding of the vectors which transmit pathogens such as JEV, DENV and *O. tsutsugamushi*. If these people become infected in Ha Nam, it is therefore possible that they could introduce these pathogens to other areas as a result of their movement patterns as it is thought that a large outbreak of dengue fever in Hanoi in 2009 was due to the rapid movement of the population between communities (Toan do *et al.*, 2013). However, this risk would be increased if they spent more time in areas outside of their home location.

The adult to child contact in this study suggests that there could be an increased risk of pathogens spread by droplets for which adults may not have longstanding immunity without vaccination such as influenza (Bahadoran *et al.*, 2016; Eames *et al.*, 2012) or measles (He *et al.*, 2013). Our data would suggest that contact within the home, between family members would pose the most risk. However, as participants also frequently have contact in other locations such as schools and work, the potential for transmission to others in these settings should be considered whilst bearing in mind the type of contact required for transmission as given that physical contact is more likely to happen amongst children in school than adults in the workplace.

STUDY LIMITATIONS

The logistics required to conduct this study including the cost of the GPS devices resulted in a relatively small sample size, with fewer participants compared to similar studies (Owers *et al.*, 2018; Seto *et al.*, 2012). Although the devices were shared between the participants due to the effect of seasonality on the transmission of a number of pathogens, the data had to be collected within limited time periods. Since this study, I have explored the use of applications on smartphones which enable movement data to be automatically synchronised to a server. However, unfortunately, there are two major drawbacks with this: 1) not all participants would necessarily own a smartphone and they are very expensive to purchase 2) those which provide adequate data security through encryption such as Open Data Kit (Open Data Kit, 2020) only allow for a limited number of coordinates to be obtained.

In addition to the small sample size, this study was only conducted in one semi-rural province. As a result, the results cannot be generalised to the wider population of Vietnam or beyond. The absence of a difference between NDVI measurements was possibly in part due to the resolution of the raster file but also and the absence of large differences in vegetation between the rural and urban areas in this study area. It is also possible that the characteristics of the population were similar in terms of socio-economic status for which data was not collected. This may therefore explain why we did not see many differences between these groups in terms of travel patterns.

Unlike the studies conducted by Anders in southern Vietnam, and Owers et al., 2018 in Brazil, this study did not include participants with the disease or, in this case, the syndrome. It was not possible to recruit participants who had had AES given that many are left with a disability. Not only would this have had practical challenges but also there would have been a risk of introducing bias as behaviour pre- and post-AES may be different. Furthermore, given the relatively low incidence of AES, participants would have to be recruited from geographically diverse areas which may not have been logistically possible. However, this meant that I was unable to look at correlations between disease or infection status and risk of exposure. Additionally, it was unknown whether the participants were having the same contact with a few people or multiple different people as contacts were not given unique identifiers and the duration of contact was not ascertained. This would therefore limit the ability to predict the magnitude and speed at which pathogens may be transmitted.

The study was subject to a degree of responder biases as the contact patterns were reliant on participant recall and not validated. A previous study conducted in the USA found that there were discrepancies between the data recorded in diaries compared to GPS tracking devices (Elgethun *et al.*, 2007). However, to in order to obtain this level of information we would need additional consent and more defined recordings of the locations in which contact happened.

FUTURE CONSIDERATIONS

It would be recommended to repeat this study in environments where there is a higher incidence of AES such in the provinces Lai Chau or Son La. This might also allow us to quantify the degree of cross border movement into Lao PDR as discussed in chapter two. The study could be repeated in an urban population, perhaps Hanoi, to determine the differences in movement and contact patterns. An alternative to the use of NDVI could include tasselled cap wetness and brightness (Longbottom *et al.*, 2017).

The use of different devices to obtain GPS data should be explored. This may improve the efficiency of data collection and allow for a greater number of participants over a wider geographical area to be recruited. If the acquisition of mobile phone data is not feasible, the use of a smaller and more cosmetically acceptable GPS device such as sports watch could be considered if budgets allow. The contact pattern diary interviews could also be conducted on a daily basis via phone calls instead of home visits which would be important if conducting the study in a more rural location.

Finally, healthy participants could be followed-up over time to ascertain whether they developed symptoms of AES or became seropositive for example JEV or DENV.

CONCLUSION

This study provides an initial analysis of the movement and contact patterns of healthy people in Ha Nam province to help give some insight into the potential risk factors for the acquisition and spread of pathogens causing AES. However, given the small sample size and limited geographical area in which the study was conducted, further research is recommended, particularly to understand the risks in areas of high incidence.

CHAPTER 5 THE SEROPREVALENCE OF JAPANESE ENCEPHALITIS VIRUS IN PIGS IN NORTHERN VIETNAM

5.1 INTRODUCTION

JAPANESE ENCEPHALITIS VIRUS (JEV): THE ENZOOTIC CYCLE

JEV a *flavivirus,* occurs in South and South-East Asia (Solomon *et al.*, 2000a) and is the commonest cause of acute encephalitis syndrome (AES) in children in Vietnam (Le *et al.*, 2010). It is transmitted in an enzootic cycle between infected mosquitoes, most commonly the species, *Cx. tritaeniorhynchus*, and animal reservoirs which act as amplifying hosts for the virus including pigs and wild birds (Erlanger *et al.*, 2009); humans are dead-end hosts (Solomon *et al.*, 2000a). *Cx. tritaeniorhynchus* breeds in flooded areas such as rice fields (Keiser *et al.*, 2005) but can also occur in urban environments (Mansfield *et al.*, 2017; Lindahl *et al.*, 2012b). The density of the mosquitoes is influenced by climatic factors including temperature and rainfall (Murty, Rao and Arunachalam, 2010; Tian *et al.*, 2015) and potentially, absolute humidity (Xu *et al.*, 2014). A positive association between the NDVI of rice paddy and both mosquito density and the number of cases of JE has been shown in a study in China (Tian *et al*., 2015). In addition to the enzootic cycle, it has also been proposed that the virus can be transmitted between pigs via the oro-nasal secretions under experimental conditions (Ricklin *et al.*, 2016; Mansfield *et al.*, 2011).

INFECTION IN PIGS

It is suggested that most pigs become infected with the pathogen before the age of six months (Ruget *et al.*, 2018). However, unlike humans, clinical signs are not seen except in pregnant sows, which may abort or give birth to stillborn or infertile piglets thereby contributing to economic losses (Salmon, 1984; Lindahl *et al.*, 2012a).

DIFFERENCES IN THE SEROPREVALENCE IN PIGS

The seroprevalence of JEV in pigs varies by geographic region (Di Francesco *et al.*, 2018; Yamanaka *et al.*, 2010); the age of the pig (Lindahl *et al.*, 2012a; Di Francesco *et al.*, 2018); the season during which testing takes place (Ruget *et al.*, 2018; Lindahl *et al.*, 2012a); the type of farm on which the pig was raised (Di Francesco *et al.*, 2018) and the method of diagnostic test.

STUDIES IN VIETNAM

A number of studies have been conducted in Vietnam to determine the seroprevalence of JEV in pigs. In 1999, three-hundred and fifteen sows which were in the second month of gestation or during lactation, were sampled from state-owned pig farms in the Mekong Delta, southern Vietnam. The farms were all routinely vaccinated against Foot and Mouth Disease and Classical Swine Fever (Lindahl *et al.*, 2012a). Sixty percent of pigs were found to be seropositive to JEV using a commercial indirect IgG ELISA (Shenzhen Lvshiyuan Biotechnology Co. Ltd., Shenzhen, China). The odds of being infected with JEV increased with the age of the sow. However, the month of sampling had no influence on the proportion which were seropositive, suggesting that JEV transmission occurred year-round (Lindahl *et al.*, 2012a). A more recent study conducted in Can Tho city, also in the Mekong Delta detected anti-JEV IgG antibody in 100 and 97% of female pigs over six months of age from urban and rural households, respectively (Lindahl *et al.*, 2013). Samples were taken during the rainy season (Lindahl *et al.*, 2013), defined as between July and September (Lin *et al.*, 2000). Competitive IgG ELISA and IgM MAC ELISA were used to perform serological analyses with specificity validated using Swedish pig sera (Lindahl *et al.*, 2013).

In northern Vietnam, sera from six-hundred and forty-one pigs from thirteen provinces with an average age of 5.3 months were obtained from a slaughterhouse in Hanoi and analysed for the presence of antibodies to flaviviruses using a commercial ELISA Kit (ID screen West Nile competition ELISA kit from ID VET, France). 60.4% (n=387) of the samples were seropositive of which 97.2% were confirmed by microneutralisation tests giving a lower seroprevalence compared to the study conducted by Lindahl et al., 2013 in the Mekong Delta but similar to that conducted in 2012. However, the predicted seroprevalence was lower amongst pigs born in August to October suggesting differences in the seasonality of the circulation of the virus (Ruget *et al.*, 2018) which was not seen in southern Vietnam (Lindahl *et al.*, 2012a).

A recent study conducted across ten provinces in northern, southern and central Vietnam sampled two thousand sera collected from pigs of different age group from large-scale farms as part of a swine influenza programme. The samples were tested using ELISA (VDProf JE Ab ELISA; Median, Chuncheonsi, Korea) giving a

seroprevalence of 74.46% (95%CI 71.84-77.06%) ranging from 60.92% (95%CI 53.20-68.25%) in Binh Duong to 85.93% (95%CI 79.73-91.36%) in Dong Thap both of which are in southern Vietnam (Lee *et al.*, 2019).

STUDIES OUTSIDE VIETNAM

Studies of JEV seroprevalence in pigs have also been performed outside of Vietnam. A study in Cambodia followed fifteen pigs from a farm located in a peri-urban area (Ta Khmau Kandal province, in the suburbs of Phnom Penh) with a density of 191.97 pigs/km^2, and the same number from a farm located in a rural area (Kandal province, 60km from Phnom Penh) with a density 145.69 pigs/km^2 (Di Francesco *et al.*, 2018). The samples were analysed using an indirect ELISA (Duong *et al.*, 2011; Yang *et al.*, 2006). Additionally, in the two weeks prior to the date of the pigs' presumed seroconversion, real-time PCR was used to detect JEV RNA. Results from twenty-nine pigs were available at the end of the study, of which all had seroconverted by the age of six months. However, the average age at infection was 98 days in those from the peri-urban farm compared to 150 days in those from the rural farm where maternal antibodies waned much later. Despite this, the force of infection (the rate at which susceptible pigs become infected) was similar in both farms; 0.061 per day (95%CI 0.034-0.098) in the peri-urban cohort compared to 0.069 per day (95%CI 0.047-0.099) in the rural cohort (Di Francesco *et al.*, 2018) which is in keeping with the findings by Lindahl *et al*. 2013 in southern Vietnam. The authors of the work in Cambodia therefore suggest that their finding contradicts the known risk factors for JEV transmission including proximity to rice paddies which are suitable breeding grounds for the mosquito vector (Keiser *et al.*, 2005) and are feeding environments for wild aquatic birds (Di Francesco *et al.*, 2018). This led them to hypothesise that other wild bird species or domestic animals such as chickens or ducks may act as amplifying reservoirs for JEV in peri-urban and rural areas (Di Francesco *et al.*, 2018).

A study conducted in Lao PDR found seroprevalence in pigs at slaughter to be 74.7% (95%CI 71.5-77.9%) using a haemagglutination inhibition assay. IgM detected using an in-house ELISA from the Armed Forces Research Institute for Medical Sciences (AFRIMS), Bangkok, Thailand showed a peak in infection in June and July corresponding with the start of the wet season and the filing of rice paddies with water

(Conlan *et al.*, 2012). This pattern of seasonality corresponds with that in northern Vietnam, albeit with a lower seroprevalence.

In Bangladesh, where pig-rearing is less common for religious reasons, the seroprevalence is much lower than that in South-East Asia with a study showing 16% and 31% in those less than 12 months of age and aged 12 months or older respectively, as confirmed by the presence of anti-JEV IgG antibody using a commercial ELISA (GENTAUR BVBA – Genoprice, Belgium) (Khan *et al.*, 2014). A discrepancy in seroprevalence was seen in Indonesia with 6% of pigs in Java being seropositive as determined by haemagglutination inhibition assay (HAI) compared to 49% in Bali, Indonesia where the population are predominantly Hindu and the pig density is nearly 100 fold higher (Yamanaka *et al.*, 2010).

In addition to the study by Ruget *et al.*, 2018, other studies have compared diagnostics for assessing seroprevalence. A study in Cambodia showed that 65.7% pigs tested positive by HAI compared to 63.5% by ELISA (Duong *et al.*, 2011). A study in southern Nepal compared the use of a competitive ELISA (C-ELISA) with plaque reduction neutralisation testing (PRNT) (Pant *et al.*, 2006). A total of sixty-one percent of pigs had evidence of antibodies against JEV as detected by C-ELISA. Of those which were seropositive, 70% had evidence of JEV antibodies as detected by PRNT (n=84); and of those which were seronegative, 39.2% had evidence of JEV antibodies as detected by PRNT (n=31) (Pant *et al.*, 2006). Diagnosis of JEV by ELISA can be complicated by cross-reactivity between flaviviruses (Mansfield *et al.*, 2011). However, the higher specificity of PRNT minimises this problem (Mansfield *et al.*, 2017).

THE ASSOCIATION OF THE SEROPREVALENCE OF JEV IN PIGS AND HUMAN INFECTION

The association between the seroprevalence of JEV in pigs and infection in humans remains to be fully determined. In India, a study showed that the highest proportion of pigs which seroconverted to JEV occurred during the same month as the peak epidemic period of JE in humans (Kalimuddin, Narayan and Choudhary, 1982). Additionally, a case-control study from Bali and cross-sectional study from Nepal showed that ownership of a pig was associated with serologically confirmed JE in

humans (Liu *et al.*, 2010; Rayamajhi *et al.*, 2007). However, living in close proximity to pigs was not identified as a risk factor for serologically-confirmed JEV, in a case-control study in Vietnam (Lowry *et al.*, 1998).

THE CONTROL OF JEV IN PIGS

Despite the existence of fewer JEV vaccines for livestock compared to humans, in order to reduce abortion and so improve livestock production, immunisation using either a live-attenuated to inactivated vaccine is recommended (Mansfield *et al.*, 2011). A compartmental model suggested that the annual vaccination of fifty-percent of pigs in Bangladesh will result in an eighty-two percent reduction in the annual incidence (Khan *et al.*, 2014) and since 2007, there have been no official notifications of outbreaks in the pig population in South Korea, which has used a live-attenuated viral strain for a swine vaccination programme for over thirty years (Mansfield *et al.*, 2011; Nah *et al.*, 2015). However, as a result of maternal antibody, the effectiveness of the live-attenuated vaccine is reduced. Additionally, it is expensive to give annual vaccinations to newborn pigs when the turnover is high. As a result of this vaccination is not commonly used (Erlanger *et al.*, 2009). Furthermore, the effect of vaccination on incidence in humans has yet to be established (Khan *et al.*, 2014) particularly, as outbreaks of human disease still occurred in the Republic of Korea despite the vaccination programmes (Nah *et al.*, 2015; Seo *et al.*, 2013). In addition to vaccination, the use of insecticide-treated mosquito nets to cover pigs has been shown to reduce the seroconversion rate in both humans and pigs (Dutta *et al.*, 2011).

THE JUSTIFICATION FOR THE RESEARCH AND THE QUESTIONS TO BE ADDRESSED

The other aspects of this thesis have focussed on the epidemiology of JEV in humans. However, as described, pigs play a key role in the transmission of the virus. Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) and the Vietnam National Institute for Veterinary Research (NIVR) in Hanoi undertook a surveillance project which included the sampling of blood and nasal swabs from pigs for the detection of swine influenza viruses and other respiratory pathogens. This included the collection of serum samples from seventy pigs per month from Van Phuc slaughterhouse in Hanoi. These samples were kindly made available to allow this investigation of the seroprevalence of JEV in pigs in northern

Vietnam. The study by Ruget et al. 2018 which also evaluated the seroprevalence in this region, had not been published at the time of developing this study and conducting the laboratory analysis. However, there are still areas to be researched, including whether differences exist in the seroprevalence between provinces and the importance of the month of sampling; between familial and industrial farms where the former may be in closer proximity to mosquito breeding grounds or other animal reservoirs for JEV such as wild birds (Mansfield *et al.*, 2017) and the latter in areas of increased number of mosquitoes (Lindahl *et al.*, 2012b); the association with environmental factors which are conducive to mosquito breeding and transmission; and finally, an association with human cases of acute encephalitis syndrome (AES) and JE in space and time.

The aim of this study is to evaluate the epidemiology seroprevalence of JEV in pigs in northern Vietnam to potentially guide public health control measures by:

1. determining if there are differences in the seroprevalence of JEV in pigs between provinces and by the month of slaughter in northern Vietnam;
2. ascertaining if there are differences in the seroprevalence of JEV in pigs from familial compared to industrial farms;
3. evaluating if there is evidence of a correlation between the seroprevalence of JEV in pigs and the environmental suitability for *Cx. tritaeniorhynchus*;
4. evaluating if there is evidence of a correlation between the incidence of human cases of AES and JE and seroprevalence of JEV in pigs.

5.2 METHODS

THE COLLECTION OF SAMPLES

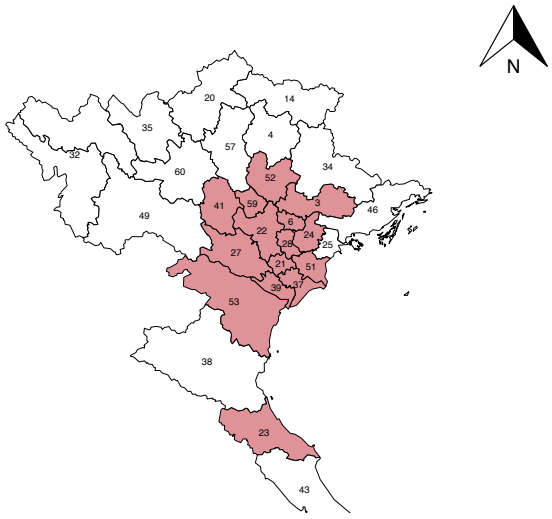
As described, serum samples were provided by CIRAD and NIVR from Van Phuc slaughterhouse. Sample collection commenced in May 2013 was still ongoing at the time this study was undertaken. The pigs were aged 4-6 months at the time of slaughter when the samples were collected. Twenty-five traders operate at the slaughterhouse. For each trader their name, contact details, type of farm (familial or industrial) and location of farms (province and/or district) were collected. One trader

will collect pigs from a number of different farms and therefore potentially reducing the accuracy of the data relating to the location of the origin of the pig (personal communication, Eugenie Baudon). In Vietnam, there are three types of swine farms; familial, company farms and state-owned farms. The familial farms are normally owned by household and are small in size, company farms are owned by foreign or Vietnamese companies and very large; and state-owned farms keep grandparents and great-grandparents of exotic breeds and are also large in size (Baudon *et al.*, 2017).

THE SAMPLING METHODOLOGY

Given that this study includes a spatial component, those samples for which there was no information detailing the location of operation of the trader were excluded leaving a total of 2388 samples for analysis. The number of sera to be sampled was stratified by the number of pigs within regions in northern Vietnam using data from the General Statistics Office of Vietnam (General Statistics Office of Viet Nam) to prevent over-sampling from certain provinces e.g. Hanoi. The regions included the Red River Delta (RRD) and two pre-defined regions outside of the RRD with the provinces shown in figure 5.1. In addition to the required sample size of 1565, a further 129 samples were to be tested to assess the effect of seasonality on JE seroprevalence across northern Vietnam. The addition of 129 samples allowed for a minimum of 30 samples per month from June 2013 to September 2016 to be tested where data on location was available (and stratified by pig density to the extent possible with the samples available).

Figure 5.1 The provinces from which the pigs originated. The provinces in red in the table are within the RRD.



Province name	Province number
Bac Giang	3
Bac Ninh	6
Ha Nam	21
Ha Noi	22
Ha Tinh	23
Hai Duong	24
Hoa Binh	27
Hung Yen	28
Nam Dinh	37
Ninh Binh	39
Phu Tho	41
Thai Binh	51
Thai Nguyen	52
Thanh Hoa	53
Vinh Phuc	59

LABORATORY DIAGNOSTICS

It was decided initially to measure seroprevalence of JEV using a commercial indirect IgG ELISA (LSY-30008 Shenzhen Porcine Encephalitis Virus, Shenzhen Lyvshiyuan Biotechnology Co. Ltd., Shenzhen, China) as this was used previously by NIVR. Swedish and Vietnamese researchers have also published results of seroprevalence in southern Vietnam using the same kits. The authors stated that based on data from the manufacturer, compared to the haemagglutination inhibition test (HI) the ELISA had a relative sensitivity of 0.92 and specificity of 0.79 with no cross-reaction to other significant pathogens such as Porcine reproductive and respiratory syndrome and Porcine Parvovirus (Lindahl *et al.*, 2012a).

Although acute infection is determined by anti-JEV IgM, the titres will wane and other studies of seroprevalence in Vietnam used anti-JEV IgG (Lindahl *et al.*, 2013; Lindahl *et al.*, 2012a). Despite being potentially less specific then plaque reduction neutralization testing (PRNT), the 'gold standard' for the serodiagnosis of flaviviruses (Mansfield *et al.*, 2017), ELISA is easier to perform because it does not require the use of live virus (Maeda and Maeda, 2013). Furthermore, PRNT is not available in Hanoi. Other researchers in Vietnam who used ELISA, developed a Bayesian

framework to estimate the true prevalence of JE from the apparent prevalence by adjusting for the sensitivity and sensitivity. However, there was little difference between the two (true prevalence = 74.46%, 95% credible interval: 73.73-86.41 and apparent prevalence = 73.45%, 95%CI 71.46-75.37) (Lee *et al.*, 2019).

The ELISAs were performed according to manufacturer's instructions, using optical density cut-offs to determine results as follows: greater than or equal to 0.4 to be positive, 0.2-0.4 unclear and less than 0.2, negative (appendix 6.1). However, after testing forty-two samples at the beginning of 2017, NIVR staff reported that more samples than expected were positive. Additionally, it was noticed that the wells continued to change colour after adding stop solution. The staff discussed this problem and sent the results to me whilst making amendments to the timings of procedures and trying different kits without success. Seventy-one samples previously tested using the Shenzhen ELISAs in 2016, were re-tested using the new kits. As shown in table 5.1 below there was discrepancy between the results with thirteen of the twenty-five samples which were negative in 2016 were positive on retesting in 2017.

Table 5.1 Comparison of the testing of porcine JEV IgG antibodies using the Shenzhen ELISAs in 2016 and 2017.

	Samples tested in 2017		
	Positive	Suspected	Negative
Samples tested in 2016			
Positive	8	0	0
Suspected	4	0	3
Negative	13	0	43
Total	25	0	46

I raised the issue with the supplier who felt that the differences may be due to the conditions of the storage of the samples and ELISA kits. However, we had no reason to suspect these were adverse.

As a result of this, I needed to select a different method of testing. I discussed with colleagues in Hanoi the possibility of using HAI however, a colleague based at OUCRU HCMC had previously used a competition enzyme-linked immunosorbent assay (cELISA) from the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia for the testing of pig serum for JEV seroprevalence and I therefore decided to use this. This cELISA measures all antibodies (IgA, IgG and IgM) and is unable to discriminate between these (verbal communication, CSIRO). The ELISA plate was initially coated with JEV antigen in coating buffer and incubated. The test sera diluted to 1 in 10 was then added in duplicate wells after the plate was washed. JEV 989 monoclonal antibody was added to the plates followed by incubation and another wash and the additional of anti-mouse (horseradish peroxidase) HRPO conjugate. The plate was incubated and washed again before adding the tetramethylbenzidine (TMB) substrate, incubating and adding sulphuric acid. The plate was then read with a positive result indicating exposure to JEV or a closely related flavivirus. Sera with percentage inhibition of greater than 50% were classified as positive, less than 40% as negative and 40-50%, suspected positive (appendix 5.1).

Due to the known concerns regarding the potential for cross-reactivity between flaviviruses and hence, specificity of ELISA, I decided to re-test a subset of serum samples by the gold standard method, PRNT (Mansfield *et al.*, 2017). This is also a recommendation by CSIRO who test positive samples for West Nile virus (WNV) and Murray-Valley virus. In Hanoi, there was only provision for conducting microneutralisation testing and not PRNT. Therefore, I sent one-hundred samples to Mahidol University, Center for Vaccine Development, Bangkok under Prof Sutee Yoksan to test for JEV using PRNT. Prof Sutee Yoksan was known to me from a previous collaboration where his team tested human serum samples for anti-JEV and anti-dengue virus. PRNT measures the effect of antibody on virus susceptible cells. A plaque is formed when the virus results in an infection. Serial dilutions of the serum being tested are used and the number of plaques counted to determine the reduction

in virus activity (World Health Organization, 2007a). PRNT was performed at Mahidol University according to the local standard operating procedure (SOP) (appendix 5.2). In summary, LLC-MK2 cells were inoculated with a serum-virus mixture of ten-fold dilutions starting with 1:5 and continuing as required to reach the end point of a fifty percent reduction in plaques. The mixture was left to absorb for ninety minutes at 37°C before adding an overlay medium and incubating at the same temperature in a 5% CO_2 incubator for seven days. After seven days, the number of plaques in each well was counted. Neutralisation was determined by a reduction in plaque count of 50%.

STATISTICAL ANALYSIS

All statistical analysis was conducted using R statistical software (versions 3.6.0 and 3.6.1), (R Core Team, 2019). The data was restructured using the R package 'tidyverse' (Wickham, 2017) with figures constructed using the packages 'ggplot2' (Wickham, 2016), 'viridis' (Garnier, 2018) and 'ggspatial' (Dunnington, 2018), 'sf' (Pebesma, 2018), 'scales' (Wickham, 2018) and 'cowplot' (Wilke, 2019). 95% confidence intervals (CIs) were calculated using the function prop.test().

DESCRIPTIVE ANALYSIS

The number of samples collected per month was shown as panel of bar charts. The number and percentage of the total with 95%CIs of samples collected per province were shown in a table with the percentage also demonstrated in a choropleth map. Choropleths maps were constructed to show the differences in the percentage of pigs from familial and industrial farms; the proportion of land suitable for *Cx. tritaeniorhynchus*, the mean incidence of human cases of AES and JE and the mean percentage of vaccination coverage against JE.

UNIVARIATE ANALYSIS

The seroprevalence of JEV in pigs by the month of slaughter with 95% confidence intervals (CIs) was shown as a bar chart and by province, as a table and choropleth map. A time series plot of the total seroprevalence over the study period was also provided. Differences in seroprevalence were determined using the chi-squared test.

For the single categorical variable 'category of farm', total numbers and percentages including 95%CI were given for pigs which were seropositive and seronegative for JEV. For the remaining continuous variables, means including standard deviations were given.

Logistic regression was performed using the R function glm() used to fit generalised linear models (GLM) (RDocumentation, 2020) to compare the relationship between the JEV seroprevalence (seropositivity compared to seronegativity) in pigs with each of the covariates as below. The R package 'MASS' (Venables and Ripley, 2002) was used to calculate the odds ratio (OR) with 95%CIs for each of the models.

The residuals of the models were tested for the presence of spatial autocorrelation using the Monte-Carlo simulation of Moran's I as described in chapter two using the R package 'spdep' (Bivand, Pebesma and Gomez-Rubio, 2013). To allow for convergence, one-thousand permutations were included in Monte-Carlo simulation of Moran I. If spatial autocorrelation was found to be present, as defined by a p value of less than 0.05, the analysis was re-run using a spatio-temporal INLA latent Gaussian model of the binomial familial and the specification Besag-York-Mollie (BYM) for the spatial random effect. In all model the Random Walk Model of Order1 to account for a temporal random effect as described in chapter two using the R package 'INLA' (Lindgren and Rue, 2015). The fit of the INLA model was defined by the assessment of the posterior estimates of the precision for spatial and temporal components. The estimates of the INLA model were plotted using the function Efxplot from the R package 'ggregplot' (Albery, n.d.).

DATA ACQUISITION

1. **The category of farm from which the pigs originated (familial compared to industrial)**

   This information was obtained directly from the data provided by NIVR.

2. **The percentage of area of each province suitable for *Cx. tritaeniorhynchus***

   A raster file of resolution 5x5km pixels of *Cx. tritaeniorhynchus* suitability was obtained from a paper by Longbottom et al., 2017. The raster is based

constructed from a model which includes covariates associated with the survival of mosquitoes including land surface temperature (LST) (Gap-filled MODIS LST data), Tasselled cap wetness (TCW) and brightness (TCB) (Gap-filled MODIS satellite data), Shuttle Radar Topography Mission (SRTM) elevation and landcover (closed and open shrublands, woody savannas, grasslands, permanent wetlands, croplands, urban/built-up areas and barren/sparsely populated areas (MODIS land cover product).

The mean percentage of area of suitability for the vector within each province (polygon) was extracted as described in chapter two with weighting by human population density.

3. The incidence of human AES and JE; and JE vaccination coverage

As described in chapter two, the monthly incidence of human AES was obtained from the GDPM (Ministry of Health Vietnam General Department of Preventive Medicine, 2019). Populations in each of the provinces were obtained using the R packages 'gso' (Choisy and Contamin, 2019c) which uses data from the General Statistics Office of Vietnam (General Statistics Office of Viet Nam) and merged by year using the R package 'poseid' (Choisy and Contamin, 2019d). The incidence of JE was obtained from sentinel site suspected meningoencephalitis surveillance data which was provided by the Expanded Programme on Immunization (EPI), World Health Organization (WHO), Hanoi. Cases presenting to the National Hospital for Pediatrics in Hanoi with suspected meningoencephalitis with a positive anti-JEV IgM as tested by ELISA were diagnosed with JE. Data on JE vaccination coverage in humans was included due to its potential for interaction with human incidence of JE or AES and was provided by the National Institute of Hygiene and Epidemiology (NIHE), Hanoi (National Institute of Hygiene and Epidemiology, 2018).

The datasets were merged on province of pig origin and time (month) of slaughter/sample collected with the covariates: incidence of human AES, incidence of human JE and JE vaccination coverage. The suitability for *Cx. tritaeniorhynchus* was matched on province only as there is no time component. Table 5.2 outlines the datasets used.

Table 5.2 The datasets used, their unit of time and source.

Dataset	Unit of time	Spatial unit	Source	Year	Resolution
Result of cELISA	NA	Individual (pig)	OUCRU-Hanoi/NIVR	2013-2016	NA
Result of PRNT	NA	Individual (pig)	Mahidol University, Bangkok	2013-2016	NA
Time of sample collection Province of pig origin Category of farm	NA	Individual (pig)	CIRAD/NIVR	2013-2016	NA
Environmental suitability for *Cx. tritaeniorhynchus*	Fixed	Provincial	Longbottom et al., 2017	2017	5x5km
Population density	As needed for the covariate for which it is weighted	Provincial	WorldPop	2009	100m
Population	Yearly	Provincial	GSO	2013-2016	NA
Incidence of AES	Monthly	Provincial	GSO	2013-2016	NA
Incidence of JE	Monthly	Provincial	WHO	2013-2016	NA
JE vaccination coverage	Yearly	Provincial	NIHE	2013-2016	NA

MULTIVARIATE ANALYSIS

Given the presence of spatio-temporal autocorrelation in the majority of univariate analyses, all multivariate analyses were performed the same INLA methods as described for the univariate models.

Four multivariate models with an outcome of seroprevalence were constructed using the covariates as below. The incidence of human JE and AES were also fitted in separate models due to the potential collinearity (we would expect JE cases to be included as part of AES surveillance). As the category of farm was available only for four-hundred and twenty pigs, models without this variable were required to account for the total number of samples.

seroprevalence of JEV in pigs ~ category of farm + environmental suitability for Cx. tritaeniorhynchus + incidence of human JE per 100,000 population + percentage of coverage of JE vaccination

seroprevalence of JEV in pigs ~ category of farm + environmental suitability for Cx. tritaeniorhynchus + incidence of AES per 100,000 population + percentage of coverage of JE vaccination

seroprevalence of JEV in pigs ~ environmental suitability for Cx. tritaeniorhynchus + incidence of human JE per 100,000 population + percentage of coverage of JE vaccination

seroprevalence of JEV in pigs ~ environmental suitability for Cx. tritaeniorhynchus + incidence of AES per 100,000 population + percentage of coverage of JE vaccination

The estimates of each INLA model were plotted using the function Efxplot from the R package 'ggregplot' and the Deviance Information Criterion (DIC) and Watanabe-Akaike information criterion (WAIC) given to assess the models with the best fit including those with and without the category of farm. A choropleth map of the estimate of seroprevalence was constructed for the model with the best fit.

SENSITIVITY AND SPECIFICITY OF THE C-ELISA

The sensitivity, specificity, positive and negative predictive value of the cELISA were calculated manually with 95% confidence intervals determined using the R function prop.test().

ETHICS

The original collection of sera was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB) (reference UW 12-416) under the investigator Eugenie Baudon.

5.3 RESULTS

DESCRIPTIVE ANALYSIS OF THE COVARIATES

1398 samples of what were tested. Following the removal of duplicate samples and those in which the sample ID did not match the animal ID there were 1391 samples for analysis. Of these samples 63.5% (95%CI 60.9-66%, n=883) were seropositive, 2.7% (1.9-3.7%, n=37) were suspected seropositive and 33.9% (31.4-36.4%, n=471) were seronegative. Those which were suspected to be seropositive were removed from the analysis leaving a final 1354 samples.

Samples were obtained from pigs from fifteen provinces in northern Vietnam with a median of 32 samples per province ranging from five samples in Bac Ninh and Ha Tinh to 383 samples in Hanoi (figure 5.2). Eight-hundred and forty samples (62.0%, 95%CI 59.4-64.6%) samples were from the Red River Delta region. The dates of samples ranged from June 2013 to September 2016 with a median number of 35 samples per month (figure 5.3). The highest percentage of familial farms were seen in Ha Nam province (81.6%, 95%CI 71.6-88.8%). The highest percentage of farms which were industrial were in Bac Giang, Bac Ninh, Ha Tinh, Hai Duong, Hoa Binh, Nam Dinh and Thai Binh (100%) (figure 5.4). The mean proportion of area suitable for *Cx. tritaeniorhynchus* using the raster file from Longbottom et al., 2017 ranged

from 0.25 in Hoa Binh to 0.64 in Bac Ninh (figure 5.5). The mean monthly incidence per 100,000 population of human cases of AES by province ranged from zero in Bac Ninh, Ha Tinh, Hung Yen, Nam Dinh and Thai Nguyen to 0.209 (SD=0.274) in Bac Giang (figure 5.6), and the mean monthly incidence per 100,000 population of JE ranged from 0.043 (SD=0.015) in Thanh Hoa to 0.302 (SD=0.068) in Hoa Binh (figure 5.7).

Figure 5.2 The percentage of samples analysed by province.

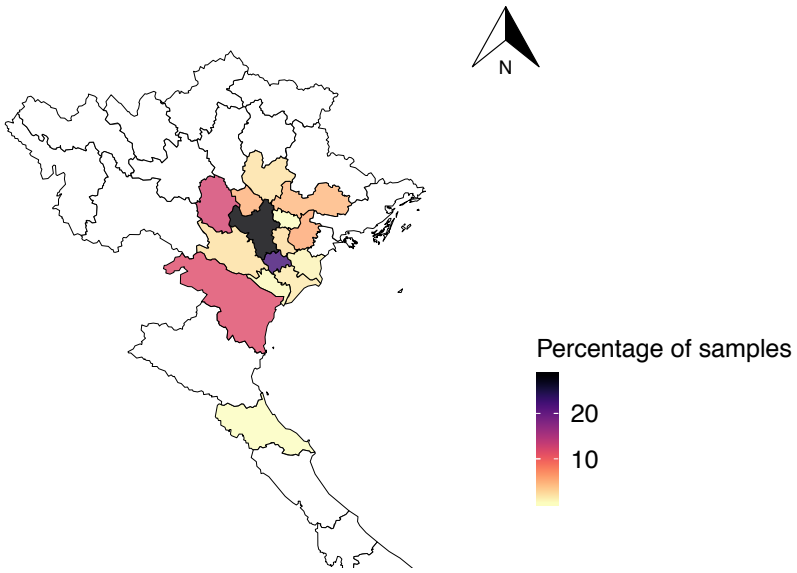


Figure 5.3 The number of samples included in the analysis by month and year.

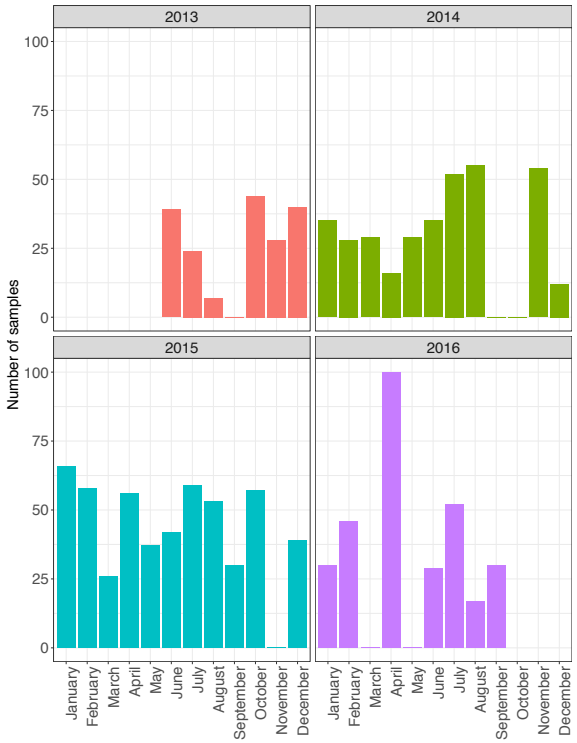


Figure 5.4 The percentage of samples from pigs by farm category of origin.

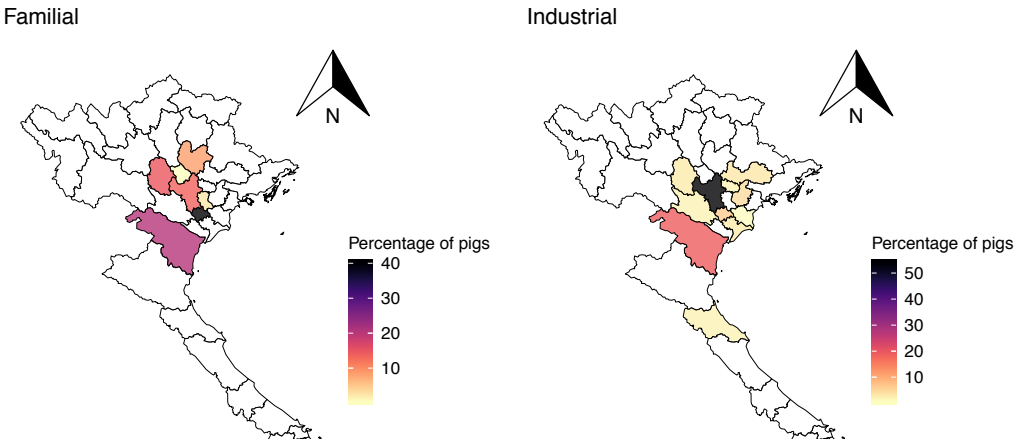


Figure 5.5 The mean proportion of area suitable for *Cx. tritaeniorhynchus* weighted by human population density.

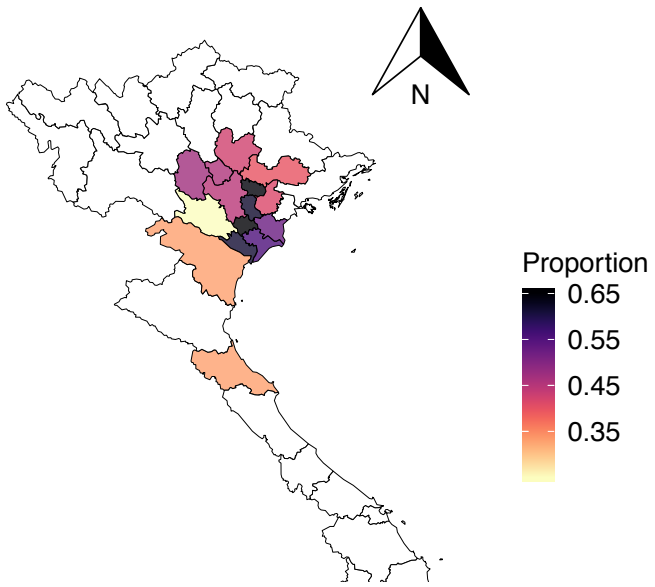


Figure 5.6 The mean monthly incidence per 100,000 population of human AES over the study period.

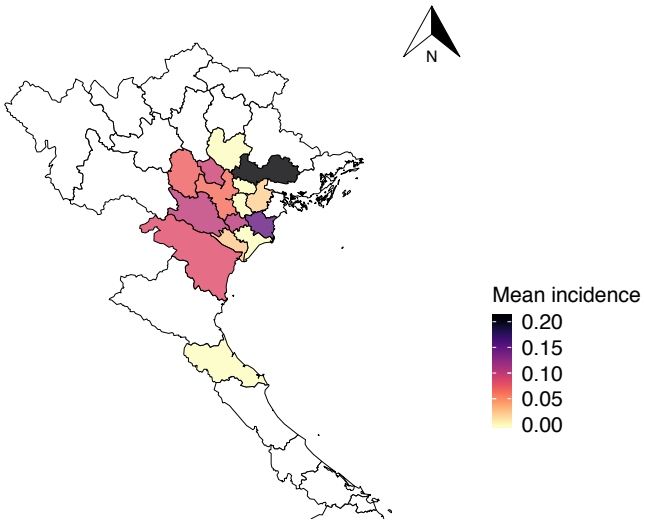
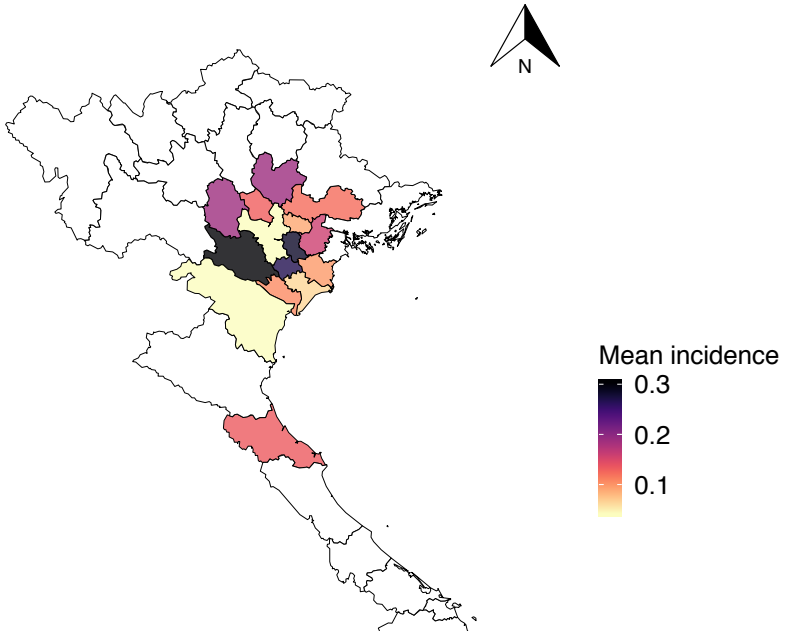


Figure 5.7 The mean incidence of JE by province.



In the years that vaccination against JE was performed, coverage ranged from 88.97% in Ha Tinh in 2013 to 100% in Ha Noi in 2013.

THE PERCENTAGE OF SEROPOSITIVE SAMPLES BY PROVINCE

The percentage of pigs which were seropositive by province ranged from 30.8, 10.4-61.1%) in Thai Nguyen to 100% in Bac Ninh (6.3, 100%) (figure 5.8 and table 5.3). The percentage of pigs from the Red River Delta which were seropositive was 70.7% (95%CI 67.5-73.8%, n=594) compared to other provinces (56.2%, 95%CI 51.8-60.6%, n=289). There was strong evidence for a difference in seroprevalence between provinces from the Red River Delta and other provinces as determined by the chi-squared test ($p<0.001$).

Figure 5.8 The percentage of pigs which were seropositive for JEV by province.

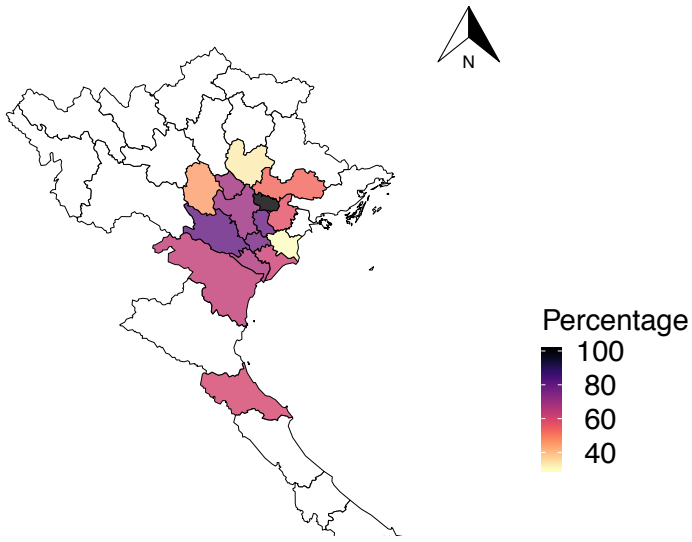


Table 5.3 Percentage of pigs seropositive for JEV by province.

	Positive (N=883) N (%, 95%CI)	**Total (N=1354)**	**p value[a]**
Province			< 0.001
Bac Giang	33 (53.2, 40-65.8%)	62	
Bac Ninh	5 (100.0, 46.3, 100%)	5	
Ha Nam	231 (76.5, 71.2-81.1%)	302	
Ha Noi	267 (69.7, 64.8-74.2%)	383	
Ha Tinh	3 (60.0, 17.0-92.7%)	5	
Hai Duong	43 (57.3, 45.4-68.5%)	75	
Hoa Binh	23 (79.3, 59.7-91.3%)	29	
Hung Yen	25 (78.1, 59.6-90.1%)	32	
Nam Dinh	13 (61.9, 38.7-81.0%)	21	
Ninh Binh	6 (66.7, 30.9-91%)	9	
Phu Tho	76 (45.2, 37.6-53.1%)	168	
Thai Binh	4 (30.8, 10.4-61.1%)	13	
Thai Nguyen	9 (33.3, 17.2-54%)	27	
Thanh Hoa	99 (63.5, 55.3-70.9%)	156	
Vinh Phuc	46 (68.7, 56-79.1%)	67	

*The difference in the percentage of samples which were seropositive between provinces

calculated using the chi-squared test.

DIFFERENCES IN THE SEROPREVALENCE OF JEV BY MONTH OF SAMPLING

There was strong evidence for a difference between number of pigs seropositive and seronegative at different months of slaughter as determined by the chi-squared test ($p<0.001$). The highest percentage of pigs being seropositive at slaughter was in the month of August (85.6% (95%CI 78.2-90.1%)) and the lowest in March (30.9 (95%CI 19.5-45.0%)) (figure 5.9). There was however, no evidence of an increase or decrease in trend over the study period (figure 5.10).

Figure 5.9 The percentage of pigs seropositive at slaughter by month. The error bars indicate the 95% confidence intervals.

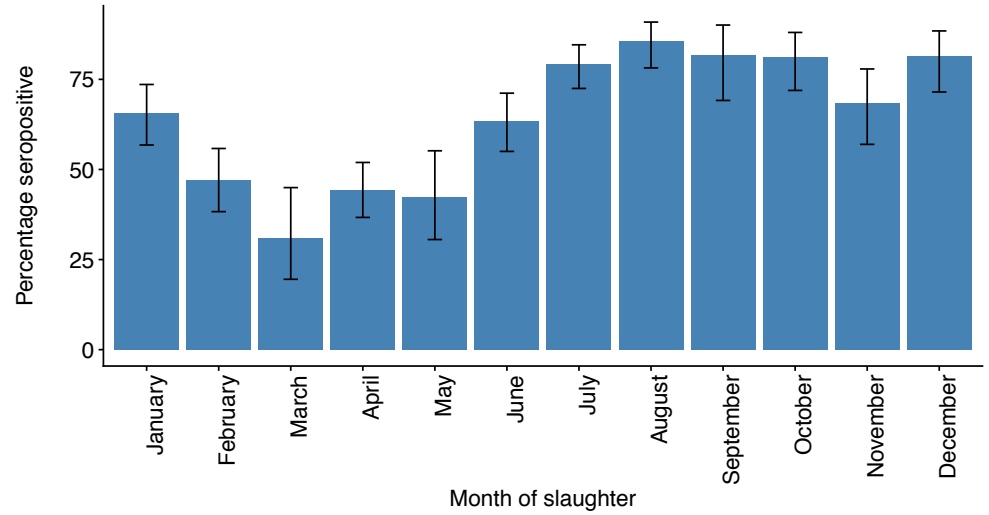
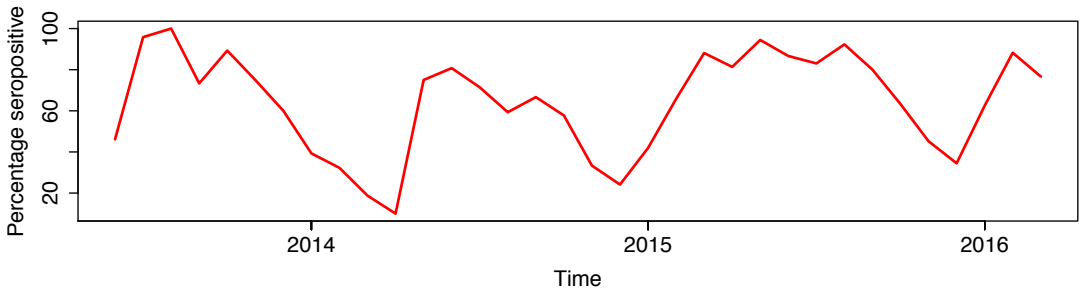


Figure 5.10 The percentage of pigs which were seropositive over the study period.



The univariate analysis using GLM showed there was no difference in the seroprevalence between pigs from an industrial compared to familial farm with no evidence of spatial autocorrelation (table A5.1, appendix 5.3). After taking into account the temporal autocorrelation, the INLA model showed that those from an industrial farm were less likely to be seropositive with a mean posterior value of -0.139 and the absence of the crossing of zero in the range between the 2.5 and 97.5 percentile (-0.224- (-0.054) (table A5.2, appendix 5.3 and figure 5.11, below). This correlation was also seen in multivariate models 1 and 2 (figure 5.11).

There was strong evidence for a positive correlation between the proportion of land suitable for *Cx. tritaeniorhynchus* and seropositivity (OR=7.15, 95%CI 2.53-20.51) with evidence of both spatial autocorrelation (table A5.3, appendix 5.3). The univariate INLA model however, did not show any evidence of correlation (estimate = 0.425 (-0.185-1.063) (table A5.4, appendix 5.3 and figure 5.11). This absence remained in multivariate models (figure 5.11).

Incidence of human AES but not incidence of human JE was positively correlated with seropositivity in the univariate logistic regression models (OR=3.23, 95%CI (1.61-7.29) OR=1.53, 95%CI 0.34-7.40) with both analyses showing evidence of spatial autocorrelation (tables A5.5 and A5.7, appendix 5.3). There was no correlation with the incidence of human JE and the seropositivity of JEV in pigs in the univariate multivariate analyses however, the incidence of human AES was negatively associated in model 2, and positively associated in model 4 with seropositivity (tables A5.6 and A5.18 and tables A5.11-A5.14, appendix 5.3; and figure 5.11).

The univariate logistic regression analysis using GLM showed no evidence of a correlation between JE vaccination coverage and seropositivity (OR=1.00, 95%CI 1.00-1.01) but with evidence of spatial autocorrelation (table A5.9, appendix 5.3). This absence of a correlation remained in the INLA univariate and multivariate models (tables A5.9 and A5.11-A5.14, appendix 5.3 and figure 5.11). The DIC and WAIC

results for the models were lower in those in which the category of farm was included (table 5.4).

Figure 5.11 The posterior means of the INLA univariate and multivariate models by each covariate with an outcome of number of cases of meningitis. The dot represents the posterior mean value and bars represent the 95% credible intervals. The presence of an asterix (*) indicates 95% credible intervals which do not cross zero.

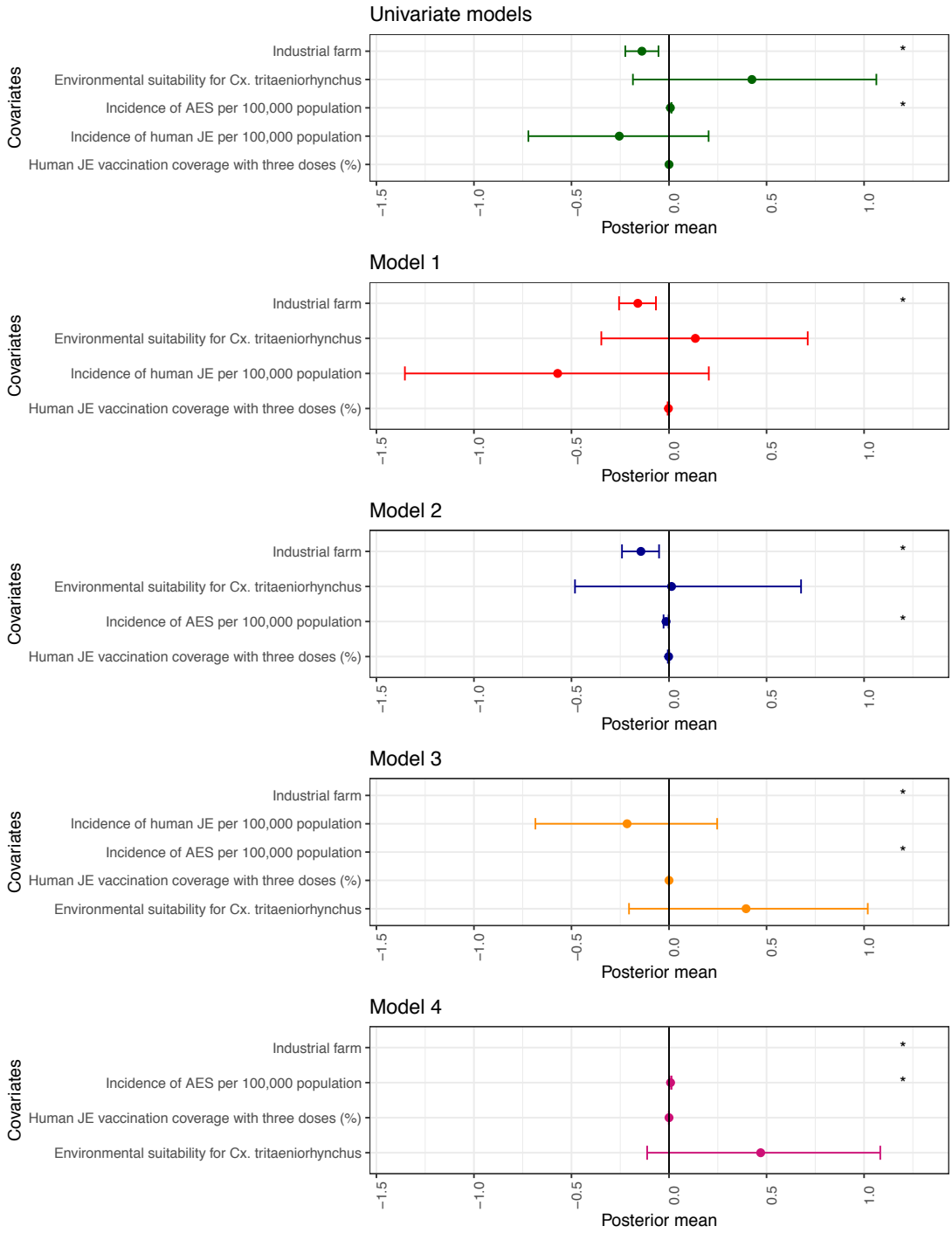


Table 5.4 The DIC and WAIC for each multivariate INLA model.

Model	DIC	WAIC
1	492.11	493.09
2	487.96	489.10
3	1725.07	1729.27
4	1718.91	1722.81

THE COMPARISON BETWEEN C-ELISA AND PRNT

Table 5.5 The correlation between cELISA and PRNT results

		PRNT	
		Positive	Negative
cELISA	Positive	22	26
	Negative	8	35
	Total	30	61

PRNT results were available for ninety-seven samples (for three samples the identification codes are incorrect and could not be processed). Compared to PRNT, the sensitivity of cELISA was 73.3% (95%CI 53.87.), specificity, 57.4% (95%CI 44.1-69.7%) (table 5.5).

5.4 DISCUSSION

GENERAL RESULTS

This study examines the seroprevalence of JEV in pigs at six months of age in northern Vietnam, the association with environmental risk factors and human incidence of AES and JE.

Over a study period of three years, sixty-four percent of pigs in this region were seropositive for JE at six months of age. This shows that despite a reduction in the incidence of human AES as detailed in chapter two, there is ongoing transmission of JEV between the zoonotic reservoir and mosquitoes.

This percentage of seropositive pigs however, is very similar to that obtained by Ruget et al., 2018 in who sampled pigs of a similar age in northern Vietnam. It is however lower than that in a study by Lindahl et al., 2013 where 97-100% of pigs were seropositive but were from southern Vietnam and of an older age. As pigs get older, their chance of being infected would increase. Additionally, in southern Vietnam, JEV transmission is likely to occur year-round (Lindahl *et al.*, 2012a) compared to northern Vietnam where transmission is seasonal thereby potentially reducing the likelihood of exposure to the virus depending on when the pigs were born (Ruget *et al.*, 2018). Despite this, the result was however, similar to that obtained by a different study in southern Vietnam which sampled older pigs (sows), where 60% were seropositive (Lindahl *et al.*, 2012a).

The seroprevalence of JEV in this study was also similar to that of studies conducted in some countries outside Vietnam including Lao PDR (74.7%), Nepal (61%) and Cambodia (65.7%). However, was higher than that in Bangladesh (16%) and Indonesia (6% in Java and 49% in Bali). Given the likelihood that the environment for the *Cx. tritaeniorhynchus* vector is suitable in all of these locations, it is possible that the differences in seroprevalence are due to socio-cultural factors. The pig herd in Vietnam has been reported to be the largest in Southeast Asia (Lemke, 2008) however, although pig rearing does occur in some ethnic minority communities in

Bangladesh, for religious reasons, social stigma may be associated with this practice (Nahar *et al.*, 2013) and potentially also in Java, Indonesia where the pigs density is much lower than in neighbouring Bali.

A difference in seroprevalence was seen between provinces in northern Vietnam in this study. This might suggest a variation in the transmission of the virus between areas however, as the number of samples tested in some provinces was very small e.g. five, this produced very wide confidence intervals. Differences in seroprevalence by month of slaughter is likely to be due to the differences in climate in northern Vietnam. Northern Vietnam experiences warm and humid summers and cooler winters (Thai *et al.*, 2015) which influences the life cycle of *Cx. tritaeniorhynchus* and hence, transmission of the virus (Reisen, Aslamkhan and Basio, 1976; Longbottom *et al.*, 2017). This could therefore explain the highest seroprevalence in August corresponding with infection in June or July after losing their maternal antibody which normally occurs at 4-6 months of age (Scherer, Moyer and Izumi, 1959).

The multivariate analysis showed that pigs from industrial farms were less likely to be seropositive for JEV compared to pigs from familial farms in both the univariate and multivariate analyses. This might suggest that familial farms were located in areas where there was higher transmission of JEV however, without knowing where the locations of these farms within the province and hence the proximity to environmental areas suitability for *Cx. tritaeniorhynchus* or whether the pigs in one the familial farms spent more time outside or less time under ITNs it is difficult to understand the reasons for this. This contrasts with other studies which have shown an association between *Cx. tritaeniorhynchus* and urban areas (Lindahl *et al.*, 2012b) or no difference between peri-urban and rural farms (Cappelle *et al.*, 2016).

The covariates including the proportion of land which was suitable for *Cx. tritaeniorhynchus*, the incidence of human cases of JE, and vaccination coverage against JE did not show any association with the seropositivity of JEV in pigs in the univariate and multivariate INLA models. The association between the incidence of JE in humans and the proximity to pigs or the seroprevalence of JEV in pigs, has yet to be fully determined with studies showing different results (Kalimuddin, Narayan and

Choudhary, 1982; Konno *et al.*, 1966; Liu *et al.*, 2010; Lowry *et al.*, 1998; Rayamajhi *et al.*, 2007). It might also suggest that animals other than pigs for example, birds, may play a larger role in the transmission of JEV in Vietnam as has been hypothesised previously (Lord, Gurley and Pulliam, 2015).

Clinical studies in Vietnam have shown that JE accounts for the highest proportion of cases CNS infections in children (Le *et al.*, 2010; Ho Dang Trung *et al.*, 2012). Although we do not know the age distribution of the AES surveillance data in our study, we might assume that a number of these cases are due to JE as discussed in other chapters. If this is the case, it may account for the positive correlation between the seropositivity in pigs and the incidence of human AES seen in one of the multivariate models. However, an absence of a correlation between the incidence of human JE and seropositivity in pigs may be due to the aggregation of the data into provinces hence subjecting the study to ecological fallacy, and the low numbers of human cases.

SPECIFICITY AND SENSITIVITY OF THE C-ELISA

Although not listed as an objective in this study, the results of the cELISA were compared to PRNT to validate this test. The low specificity of the cELISA (57%) may suggest that there is cross-reactivity with other flaviviruses, a known problem in humans (Koraka *et al.*, 2002) but also potentially in pigs (Lindahl *et al.*, 2012a). Other flaviviruses which infect pigs may include WNV (Escribano-Romero *et al.*, 2015) and MVEV (New South Wales Government, 2016).

STUDY LIMITATIONS AND FUTURE IDEAS

As the majority of covariates are provided at the provincial level, this study is subject to ecological fallacy (Piantadosi, Byar and Green, 1988). The results of the models therefore have to be interpreted with some caution as these cannot be applied at the individual level. The covariate data was also subjected to assumptions and biases. Despite one province being assigned to each sample, traders may collect pigs from several provinces (verbal communication, Eugenie Baudon). Furthermore, the human surveillance data, particularly that for AES may be subject to reporting biases based

on the case definition of AES; and selection bias as those cases which do not present to a healthcare centre would be missed as discussed in chapter two. Without the GPS coordinates of the farm, it is not possible to establish the proportion of land suitable for *Cx. tritaeniorhynchus* within the range of flight of the mosquitoes. Similarly, without the GPS coordinates of the homes of human cases of JE or AES, we cannot deduce the distance between humans and pigs. The collection of these would therefore be recommended for future studies.

To avoid both the problem of ecological fallacy and some of the potential biases and assumptions, a prospective longitudinal study with pigs sampled over serial time points might be beneficial. This would allow for the time at which the pigs seroconvert to be determined whilst also collecting data on covariates at a smaller unit level. A correlation between the timing of pig and human seroconversion may help guide public health measures for example, advising unvaccinated people when they should avoid close contact with pigs; when it would be most cost-effective to vaccinate pigs; or when to use insecticide-treated nets for pig pens. Given that there is still the possibility that vector-free transmission of JEV in pigs occurs (Ricklin *et al.*, 2016) a correlation between the density of infected mosquitoes and rate of seroconversion could also be explored in addition to sampling oro-nasal secretions from pigs in northern Vietnam for the presence of the virus.

INLA models are recommended to account for spatio-temporal autocorrelation, particularly are these are able to rapidly produce results (Ross, Hooten and Koons, 2012; Beguin *et al.*, 2012). However, other methods also exist (Dormann *et al.*, 2007) which could be explored to optimise the fit of the model. Although not shown in this thesis, separate models were constructed including the covariates: normalised difference vegetation index (NDVI); mean maximum and minimum temperature; mean absolute and relative humidity; mean rainfall; mean hours of sunshine and mean elevation. A combination of these covariates accounting for co-linearity were used in place of the covariate 'environmental suitability for *Cx. tritaeniorhynchus*'. However, in the multivariate INLA models, none showed an association with the seropositivity with the exception of NDVI which was positively correlated in selected multivariate models. An association between climatic variables and seropositivity was not

identified however, this may have been due to the similarities in climate between the provinces.

Despite ELISA being a commonly used method for similar studies, the low specificity and sensitivity of the cELISA results in difficulties drawing firm conclusions about the results. We may be both under-estimating the proportion of pigs which were seropositive for JEV or incorrectly attributing the seroprevalence to JEV when another pathogen may be involved. Unfortunately, due to time, logistics and finances, it would have been very challenging to re-test all samples using PRNT. However, this could be considered for future studies and might include testing for other flaviriviruses.

In view of the limitations of this current study, advice regarding public health control measures such as changes to vaccination policies against human JE would not be recommended. As most pigs are still slaughtered at six months of age, a full cost-effective analysis should be under-taken prior to considering the implementation of vaccination of pigs.

CONCLUSION

The results of this study are comparable to others within the region and demonstrate that there is ongoing transmission of JEV between mosquitoes and pigs despite a reduction in human AES over time as shown in chapter two. However, given the low specificity and sensitivity of cELISA and the ecological study design, it would be recommended to conduct an individual longitudinal study and to use PRNT for testing samples prior to providing advice regarding public health control measures.

DISCUSSION

The aim of this thesis was to understand the epidemiology for, and risk factors associated with central nervous system (CNS) infections in Vietnam. The thesis uses a variety of methodologies to answer this, exploring zoonotic, entomological and socio-demographic risks to account for multiple causes. It commences with a literature review of the epidemiology of CNS infections in Vietnam and the surrounding region. This provides an understanding of the different aetiologies and their unique and over-lapping risk factors. The literature review is followed by an ecological study which uses national surveillance data for AES, meningitis and a variety of pathogens known to cause CNS infections to explore their spatio-temporal patterns and associations with environmental, socio-demographic and zoonotic risk factors. However, due to ecological fallacy, no conclusions could be made about causation. The second results chapter therefore explores the use of a pilot study to understand the feasibility and acceptability of conducting a case control study to understand the risk factors for AES in northern Vietnam. As the transmission dynamics of many pathogens causing AES are influenced both by human mobility and contract patterns, the third results chapter looks at both of these factors in a small cohort of the community in Ha Nam province. The fourth chapter focusses only on JE and the importance of the zoonotic reservoir by looking at associations between seroprevalence in pigs with incidence of AES and JE in humans. Whilst using the results to make assumptions about the possible aetiologies in those in whom a cause was not identified, suggestions are made about how methods could be adapted and expanded to undertake future and possibly larger, studies and in some cases, guide public health measures.

THE AETIOLOGY OF CNS INFECTIONS IN VIETNAM

Despite the economic development and improvements in the health status of the population in Vietnam (The World Bank, 2020a; Takashima *et al.*, 2017), CNS infections continue to be a burden, particularly in terms of morbidity. In adults, the most common cause is *S. suis*, a bacterium which is associated with the consumption of raw pig blood and viscera which often results in a meningitis and can leave patients with a hearing impairment (Ho Dang Trung *et al.*, 2012; Wertheim *et al.*, 2009b; van Samkar *et al.*, 2015; Huong *et al.*, 2019; Huong *et al.*, 2018). However, in children the most common cause is JEV, a flavivirus transmitted by the bite of infected *Culex*

mosquitoes, with pigs and wild birds acting as amplifying hosts (Le *et al.*, 2010; Solomon *et al.*, 2000a). JEV remains prevalent despite the implementation of vaccination against JEV in the EPI since 1997 (Yen *et al.*, 2010; Yen *et al.*, 2015). Other less common causes of CNS infections in Vietnam include the viruses DENV, enterovirus, HSV, VZV, measles, mumps, rubella and CMV, and the bacteria *S. pneumoniae*, *M. tuberculosis, H. influenza* type b and *N. meningitidis* (Tan le *et al.*, 2014; Le *et al.*, 2010; Ho Dang Trung *et al.*, 2012; Taylor *et al.*, 2012). In addition to the infectious causes, non-infectious causes of encephalitis are starting to be identified in Vietnam, including the autoimmune anti-NMDA receptor encephalitis (Nguyen Thi Hoang *et al.*, 2017) and possible toxicity from the consumption of lychees (Paireau *et al.*, 2012b). However, a number of studies both in Vietnam and on a global level have not been able to identify a pathogen in many cases despite extensive diagnostics (Tan le *et al.*, 2014; Le *et al.*, 2010; Ho Dang Trung *et al.*, 2012; Taylor *et al.*, 2012; Granerod *et al.*, 2010b). The reason for this is likely to be multifactorial. Case definitions for AES often have a low specificity which may result in a number of patients in whom an infectious agent cannot be found (Granerod *et al.*, 2010b). For example, an encephalopathy can often be confused with encephalitis or meningitis where the former causes a change in conscious state without inflammation of the brain or meninges, due to an infection occurring outside the CNS, a neurological disease such as epilepsy or other neurological manifestations such as a stroke or psychiatric disorder (Quist-Paulsen *et al.*, 2019). Additionally, the timing of the sample can influence the detection of the pathogen with samples taken too early in the course of the illness leading to false negative results due to a lower detection rate of viral nucleic acid (Davies *et al.*, 2005). Finally, it may not always be possible to identify novel pathogens (Granerod *et al.*, 2010b). In Vietnam, it is quite possible that novel causes contribute to the undiagnosed CNS infections. Le et al., 2010 suggested that a number of cases of encephalitis of unknown aetiology in children may be due to unknown or rarer pathogens such as Banna virus and in separate study of CSF samples obtained from patients with CNS infection between 1999 to 2009, a novel cyclovirus was detected by PCR (Tan le *et al.*, 2013). A number of studies have been performed to try to identify novel pathogens in CNS infections for example by using next generation sequencing (Brown, Bharucha and Breuer, 2018) and one of the aim of the Vietnam Initiative on Zoonotic Infections (VIZIONS) was to try to understand the aetiology of CNS infections of unknown origin using multiple diagnostics (Rabaa *et al.*, 2015).

in addition to novel pathogens it is also possible that some of the causes of CNS infections in Vietnam are due to pathogens which have been identified as common causes of CNS infections in neighbouring countries. These include *O. tsutsugamushi*, *R. typhi* and *Leptospira* species (Dittrich *et al.*, 2015; Tarantola *et al.*, 2014). Given the evidence for the existence of these pathogens in Vietnam (Nadjm *et al.*, 2014; Hamaguchi *et al.*, 2015b; Thai *et al.*, 2006; Van *et al.*, 1998) it possible that they contribute to the cause of CNS infections too. As well as novel pathogens, emerging and re-emerging pathogens such as Nipah virus, CHIKV and ZIKV have either been found in humans in Vietnam or in humans or animal reservoirs in the region (Cappelle *et al.*, 2020; Quyen *et al.*, 2017; Quan *et al.*, 2018). Although these are not thought to be currently contributing to human disease in Vietnam, it would be worthwhile testing for these pathogens in those with an CNS infection in whom an aetiology has not be found.

FINDINGS FROM THE RESEARCH AND HOW CAN THESE BE USED TO DESIGN FUTURE STUDIES

A number of studies have been conducted in Vietnam looking at the aetiology of CNS infections (Tan le et al., 2014, Le et al., 2010, Ho Dang Trung et al., 2012, Taylor et al., 2012). Additionally, some have focused on risk factors for particular pathogens such as *S. suis* (Nghia *et al.*, 2011). However, there was a need to understand the risk factors for CNS infections as a group whilst also aiming to understand the possible aetiology of those in whom a pathogen had not been detected. Despite being an ecological study, the results from chapter two can be used to make suggestions about the possible causes of CNS infections in Vietnam. There is a clear effect of seasonality of AES in northern but not southern Vietnam, with peaks in incidence in the summer months. This finding in addition to the positive association with temperature and absolute humidity in multivariate models which account for spatial and temporal autocorrelation could imply that many cases are caused by diseases caused by vectors whose life-cycle is dependent on climate conditions (Reisen *et al.*, 2008; Tian *et al.*, 2015; Yang *et al.*, 2014; Kuo *et al.*, 2017). Non-vector-borne diseases such as influenza and measles also show seasonality however, their patterns did not correlate with those of AES whereas peaks of incidence of JE from sentinel were seen in the same month as peaks of incidence of AES. The hypothesis that many cases are due to vector-borne diseases is further supported by the positive

association of incidence of AES with NDVI at a lag of one month where this higher density of biomass may provide a suitable habitat for mosquito breeding (Tian *et al.*, 2015; Longbottom *et al.*, 2017). However, as with all assumptions, further studies would be needed to determine whether this is true.

Despite, a national reduction in cases of AES and meningitis over time, increases in incidence of CNS infections have been seen in the northern provinces which border Lao PDR and those in the central highlands. These are economically poorer provinces with a number of ethnic minority groups (Rheinlander *et al.*, 2010; Tuyen, 2015; Giang, Wang and Yan, 2014). Many pathogens are associated with poverty in Vietnam, particularly those causing respiratory infections (Hoa, Hojer and Persson, 1997) and enteric diseases (Kelly-Hope *et al.*, 2007) and it is known that uptake of vaccination in poorer populations in Vietnam is lower (Kien *et al.*, 2017; Dao *et al.*, 2016). Accounting for the seasonality and climatic associations it could therefore be suggested that a number of cases in these provinces are due to JE. This may be attributed to missed vaccination in Vietnamese population or that cases are from Lao PDR where the vaccine was implemented more recently (World Health Organization, 2015b), particularly as it is known at Laotian cross the border to seek medical care (Durham, 2017). However, case control or cohort studies with laboratory confirmation would be needed to confirm this.

Undertaking a case control study in this setting would require careful planning. The pilot study conducted at NHTD revealed a number of challenges and lessons were learnt. Firstly, it is important to consider using a case definition for either AES or meningitis which is appropriate for the level of healthcare routinely provided. Case definitions which are based solely on clinical criteria may be suitable for resource-limited settings where diagnostics and imaging are not available. However, these are often lack specificity and may include CNS manifestations which are not AES or meningitis (Granerod *et al.*, 2010b). The results of the pilot study showed that lumbar punctures are undertaken in many hospitals in northern Vietnam and also done relatively promptly after presentation. Therefore, case definitions for studies of CNS infections in Vietnam, should include diagnostics and where possible, imaging, to improve specificity. Additionally, the spatio-temporal patterns of incidence should be considered in the design, ensuring that recruitment takes place during the season of

peak incidence, is geographically representative and that recruitment is sufficiently stratified by age.

Home visits were feasible and accepted by the population in the Red River Delta however, if the study was to focus on provinces in northwest Vietnam which are at higher risk of CNS infections close collaboration with those who have permission to visit these communities would be required. Additionally, to overcome the problem of finding matched controls in a hospital setting as was demonstrated during the pilot study, the recruitment of community controls could be considered but this would need advanced planning to obtain permissions from the relevant authorities. Finally, given the success of using electronic data capture at the homes of participants, there may be a role for using this to collect clinical data, particularly if multiple healthcare settings are involved from which paper CRFs cannot easily be returned on a regular basis.

The questionnaire completed at the home of the participants covered a number of potential risk factors for AES and was similar to that used in a case control study in India (Singh *et al.*, 2016). Despite its length, it was accepted by the participants and there were no major problems with completion. Additionally, the results from the FGDs with rural participants did not suggest that there were any particular zoonotic or entomological risk factors which were missed. Therefore, a similar questionnaire could feasibly be implemented for a future study.

Questions about recent travel were included in the questionnaire for the pilot case control study however, the results from the GPS devices in chapter four highlight the need to address human movement patterns as a risk factor for both acquisition and transmission of pathogens causing CNS infections such as DENV (Adams and Kapan, 2009; Wesolowski *et al.*, 2015b). Although many participants from Ha Nam province spent most of their time within their commune, there was evidence of travel to Hanoi and to other regions of Vietnam. Such movement becomes a particularly relevant risk factor if there is travel to areas of high incidence of pathogens causing CNS infections and may which may help to determine to the aetiology. In addition to movement, the results from Ha Nam showed the potential effect of human contact patterns in the transmission dynamics of pathogens causing AES such as enterovirus,

influenza virus and measles virus (Cauchemez *et al.*, 2008; Eames *et al.*, 2012; Metcalf *et al.*, 2009). More in-depth questioning around social contact including the age of those with whom contact has occurred should be incorporated into future studies. This may also help to determine cause, particularly if others within the household are unwell whilst bearing in mind differences in susceptibility to pathogens as a result of age or vaccination status.

Despite the recruiting a small cohort of participants in Ha Nam, the study demonstrated that the wearing of a GPS device to track movements and completing a contact pattern diary over a year was feasible in this setting. It would therefore be beneficial to use these methods in provinces where a higher incidence of AES is seen such as Son La or Lai Chau in the northwest and where there is cross-border movement between Vietnam and Lao PDR. However, a number of factors would need to be considered before implementing this. Firstly, the acceptability of undertaking such a study would need to be ascertained with a new population. Secondly, if this was to be performed on a larger scale, an alternative method for recording the GPS coordinates might be considered given the logistics required to download and clean the data from each device. More technologically advanced methods such as using apps on smartphone might be a possibility. Thirdly, it might be worth exploring conducting the contact pattern diary interviews over the telephone with data entered by staff directly into an electronic form on a tablet or smartphone. This may be more convenient for the participants but would also allow the near real-time access to the data. Finally, although this was a study on risk factors for AES infections, these methods could equally be applied to populations in this region to understand the transmission dynamics of a number pathogens, particularly in outbreak situations such as the current pandemic caused by SARS-CoV-2 where understanding movement and social contact patterns can influence public health policy (Prem *et al.*, 2020; Jarvis *et al.*, 2020).

A number of pathogens causing CNS infections have a zoonotic reservoir and therefore the utilisation of data from animals can be equally as important as humans. The final results chapter analysed serum samples from pigs from northern Vietnam and showed that almost two-thirds sample were seropositive to JEV at six months of age. Although this study did not demonstrate a positive association between the

incidence of JE in humans and seroprevalence of JEV in pigs, the effect of ecological fallacy and low numbers of cases of JE may have affected the result as may have the poor specificity of the cELISA. However, the evidence of continued infection of the zoonotic reservoir suggests that improvements in living standards and subsequent reduction of mosquito breeding sites has been insufficient in preventing transmission of the virus. Given that risk of living in close proximity to pigs and the acquisition of JE in other settings is inconclusive (Liu *et al.*, 2010; Rayamajhi *et al.*, 2007; van den Hurk, Ritchie and Mackenzie, 2009; Solomon, 2006) studies to determine the individual risk in northern Vietnam would be beneficial. However, the impact of this on public health policy is unknown as it is unlikely that populations would change practices around keeping livestock and there may be little economic justification for implementing vaccination in pigs, particularly when they are often slaughtered at six months of age (Erlanger *et al.*, 2009). Control of JE in Vietnam may therefore be better managed through revisions of the vaccination policy in children. A study conducted in Ha Tay province, northern Vietnam demonstrated that 96.2% of children within the target population, received two or more doses of vaccine during a mass JE immunisation campaign with a subsequent reduction in incidence of JE. The authors proposed that JE vaccine policy should be changed to include children aged 5-9 years in a catch-up campaign with a fourth dose given to those who received three doses during the first 2-3 years of life (Yen *et al.*, 2015). The adoption of a similar system in areas of high incidence of AES, such as the provinces bordering Lao PDR should be explored whilst recognising that the campaigns must be accessible to and accepted by all communities.

RECOMMENDATIONS

This thesis has addressed a number of gaps in the knowledge of the epidemiology of CNS infections in Vietnam. However, is has also identified areas where further research is needed based on the literature review and results chapters. Table 6.1 below makes key suggestions about how these could be addressed.

Table 6.1 Remaining knowledge gaps in the understanding of the epidemiology of CNS infections in Vietnam and recommendations for further research.

Remaining knowledge gaps	**Recommendations for further research**
The true contribution of pathogens detected in neighbouring countries but not reported as common causes of CNS infections in Vietnam.	To test for pathogens including: *O. tsutsugamushi*, *Leptospira* species and *R. typhi* in future studies.
The contribution of JEV to the cases of AES reported under the national surveillance system	To conduct a seroprevalence survey of JEV amongst cases of AES
The reasons for continued high incidence of AES in certain provinces in Vietnam, particularly those in the northwest, bordering Lao PDR.	To conduct studies of the aetiology and risk factors for AES in selected provinces such as Son La and Lai Chau including cross-border movement between Vietnam and Lao PDR.
The impact of human movement patterns on the acquisition and transmission of pathogens causing CNS infections.	To repeat the human movement study, potentially in a range of provinces e.g. rural and urban with high and low incidence of JE. To follow-up participants up over time to determine if they seroconvert to pathogens such as JEV and DENV (a cohort study).
The impact of human contact patterns on the acquisition and transmission of pathogens.	To repeat the contact pattern study, potentially in a range of provinces e.g. rural and urban with high and low incidence of JE. To follow-up participants up over time to determine if they seroconvert to pathogens such as JEV and DENV (a cohort study).

The role of pigs in the risk of human JE in Vietnam	To conduct studies evaluating the time of seroconversion of JEV in pigs and whether living in close proximity to pigs at the time of seroconversion is a risk factor for infection with JEV in humans.

CONCLUSION

This thesis used a data from a number of sources to understand the risk factors for CNS infections in Vietnam. JE remains the most common cause in children despite implementation of vaccination in the EPI yet in adults, the most common cause is the bacterium, *S. suis*. Surveillance data shows evidence of seasonality of AES in northern Vietnam with peaks in the summer months and positive associations of both AES and meningitis with temperature and humidity. This might suggest that many cases of CNS infections are due to vector-borne diseases, potentially JE. However, further studies are required to confirm or refute this suggestion building on the lessons learnt from the pilot case control study conducted in Hanoi.

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APPENDICES

APPENDIX 2.1

Supplementary figures and tables for chapter 2

Figure A2.1 Decomposition of time series of the national incidence of AES, meningitis, dengue, ILI, *S. suis*, measles, leptospirosis and HFMD from 1998-2016.

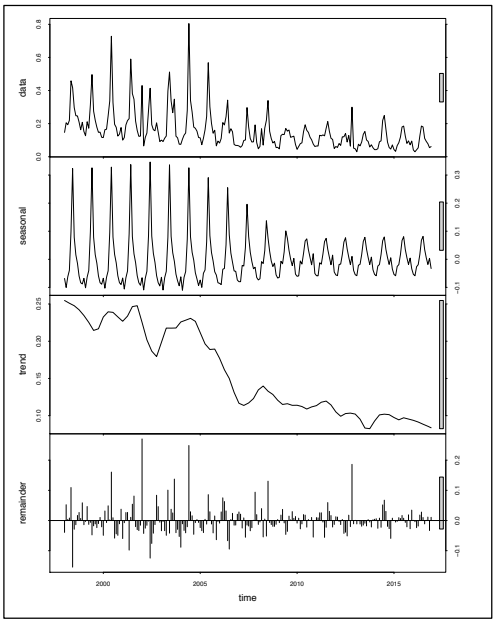
Data: time series of raw data

Seasonal: seasonal component of the time series

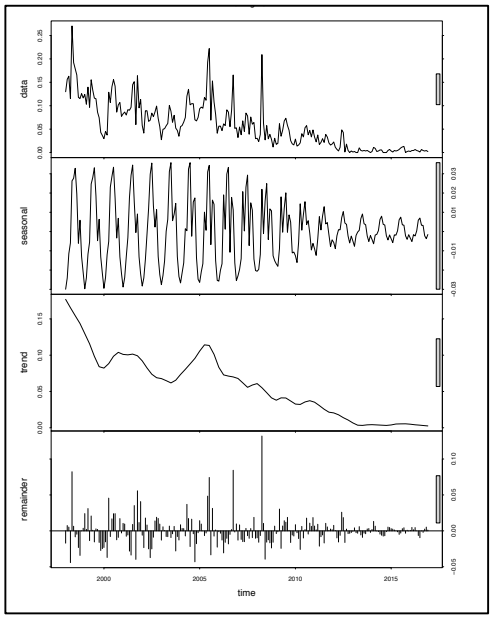
Trend: trend of the time series

Remainder: time series minus the seasonal and trend components

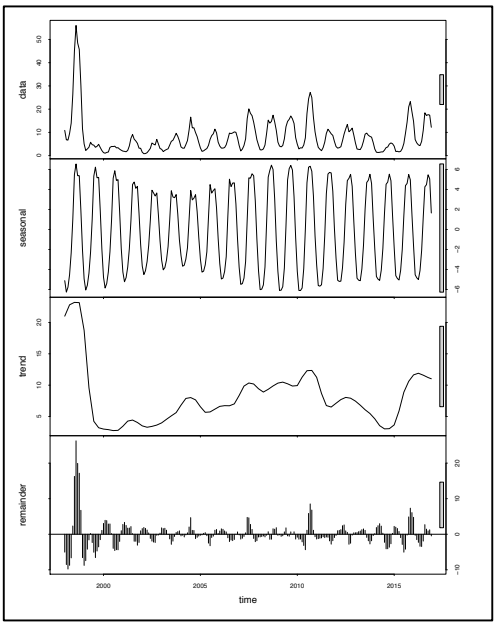
AES



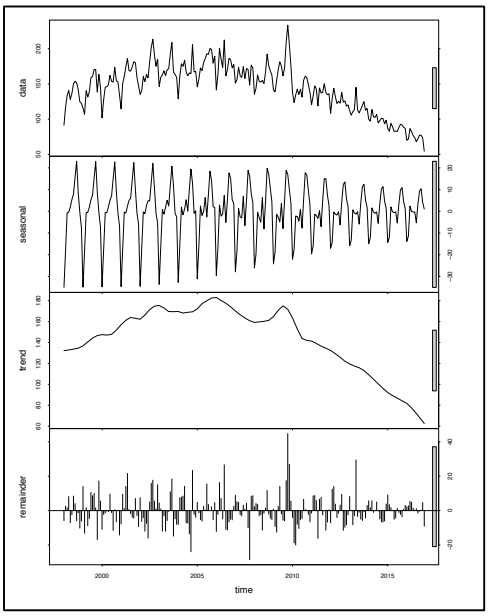
Meningitis



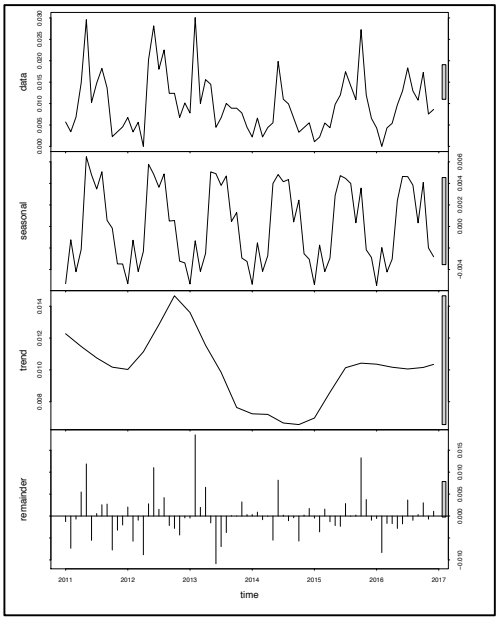
Dengue



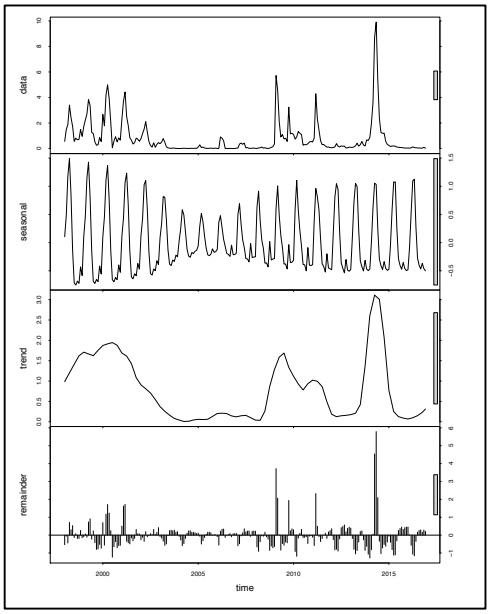
ILI



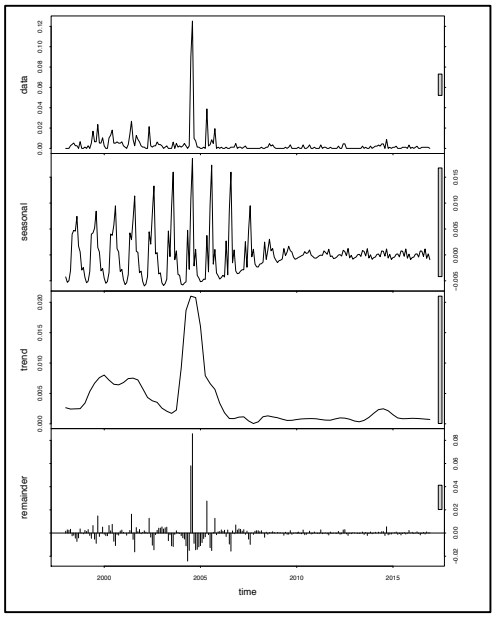
S. suis



Measles



Leptospirosis



HFMD

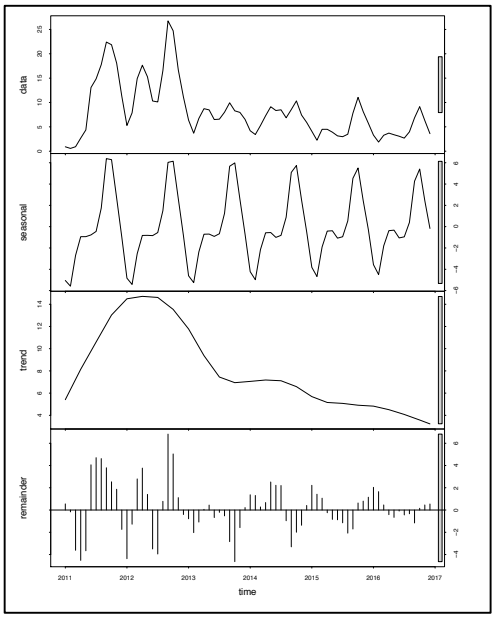


Table A2.1 Negative binomial model with an outcome of incidence of AES and time fitted as a linear predictor.

	Estimate	**SE**	**p value**	**Monte-Carlo Moran's I of the residuals**
Time (1:228) (linear)	-5.379×10^{-2}	8.537×10^{-4}	<0.001	Statistic = 0.076 p value <0.001

Table A2.2 Negative binomial model with an outcome of number of cases of AES and year fitted as a categorical covariate.

Year	Estimate	SE	p value	Monte Carlo Moran's I of the residuals
1998	NA	NA	NA	Statistic = 0.077
1999	-0.152	0.028	<0.001	p value <0.001
2000	-0.008	0.028	0.788	
2001	-0.030	0.028	0.294	
2002	-0.194	0.028	<0.001	
2003	-0.093	0.028	0.001	
2004	-0.010	0.028	0.715	
2005	-0.219	0.028	<0.001	
2006	-0.395	0.028	<0.001	
2007	-0.635	0.029	<0.001	
2008	-0.538	0.029	<0.001	
2009	-0.624	0.029	<0.001	
2010	-0.719	0.029	<0.001	
2011	-0.591	0.029	<0.001	
2012	-0.683	0.029	<0.001	
2013	-0.962	0.029	<0.001	
2014	-0.727	0.029	<0.001	
2015	-0.750	0.029	<0.001	
2016	-0.851	0.029	<0.001	

Table A2.3 Negative binomial model with an outcome of number of cases of AES and month fitted as a categorical covariate.

Month	**Estimate**	**SE**	**p value**	**Monte Carlo Moran's I of the residuals**
January	NA	NA	NA	Statistic = 0.074
February	-0.160	0.023	<0.001	p value <0.001
March	0.265	0.023	<0.001	
April	0.340	0.023	<0.001	
May	0.813	0.023	<0.001	
June	1.324	0.022	<0.001	
July	0.929	0.023	<0.001	
August	0.658	0.023	<0.001	
September	0.466	0.023	<0.001	
October	0.222	0.023	<0.001	
November	0.252	0.023	<0.001	
December	-0.036	0.023	0.123	

Table A2.4 Negative binomial model with an outcome of number of cases of AES and harmonic terms fitted as covariates.

Harmonic regression terms	**Estimate**	**SE**	**p value**	**Monte Carlo Moran's I of the residuals**
Annual (sin(2*pi*month/12))	-0.155	0.007	<0.001	Statistic = 0.072 p value <0.001
Bi-annual (sin(4*pi*month/12))	0.011	0.007	0.093	Statistic = 0.072 p value <0.001
Quarterly (sin(8*pi*month/12))	-0.013	0.007	0.060	Statistic = 0.072 p value <0.001

Table A2.5 Negative binomial model with an outcome of number of cases of meningitis and time fitted as a linear predictor.

	Estimate	**SE**	**p value**	**Monte-Carlo Moran's I of the residuals**
Time (1:228) (linear)	-0.166	0.001	<0.001	Statistic = 0.067 p value <0.001

Table A2.6 Negative binomial model with an outcome of number of cases of meningitis and year fitted as a categorical covariate.

Year	Estimate	SE	p value	Monte Carlo Moran's I of the residuals
1998	NA	NA	NA	Statistic = 0.066
1999	-0.399	0.035	<0.001	p value <0.001
2000	-0.442	0.035	<0.001	
2001	-0.354	0.035	<0.001	
2002	-0.692	0.035	<0.001	
2003	-0.901	0.035	<0.001	
2004	-0.513	0.035	<0.001	
2005	-0.214	0.035	<0.001	
2006	-0.665	0.035	<0.001	
2007	-0.921	0.036	<0.001	
2008	-1.034	0.036	<0.001	
2009	-1.216	0.036	<0.001	
2010	-1.337	0.036	<0.001	
2011	-1.652	0.037	<0.001	
2012	-2.209	0.040	<0.001	
2013	-3.795	0.058	<0.001	
2014	-3.449	0.052	<0.001	
2015	-3.207	0.049	<0.001	
2016	-3.467	0.052	<0.001	

Table A2.7 Negative binomial model with an outcome of number of cases of meningitis and month fitted as a categorical covariate

Month	Estimate	SE	p value	Monte Carlo Moran's I of residuals
January	NA	NA	NA	Statistic = 0.061
February	0.028	0.034	0.408	p value <0.001
March	0.167	0.034	<0.001	
April	0.372	0.033	<0.001	
May	0.432	0.033	<0.001	
June	0.565	0.033	<0.001	
July	0.601	0.033	<0.001	
August	0.346	0.033	<0.001	
September	0.314	0.034	<0.001	
October	0.314	0.035	<0.001	
November	0.011	0.034	0.745	
December	-0.094	0.034	0.006	

Table A2.8 Negative binomial model with an outcome of number of cases of meningitis and harmonic terms fitted as covariates

Harmonic regression terms	**Estimate**	**SE**	**p value**	**Monte Carlo Moran's I of residuals**
Annual (sin(2*pi*month/12))	-0.076	0.010	<0.001	Statistic = 0.061 p value <0.001
Bi-annual (sin(4*pi*month/12))	-0.011	0.010	0.238	Statistic = 0.061 p value <0.001
Quarterly (sin(8*pi*month/12))	0.071	0.010	<0.001	Statistic = 0.061 p value <0.001

Table A2.9 Univariate negative binomial generalized additive models with an outcome of the number of cases of AES.

Variable	Estimate	SE	p value	Monte Carlo Moran's I of the residuals
Maximum temperature (°C)				
No lag	-0.014	0.004	<0.001	Statistic = 0.075, p<0.001
Lag-1	-0.029	0.004	<0.001	Statistic = 0.072, p<0.001
Lag-2	-0.051	0.004	<0.001	Statistic = 0.070, p<0.001
Minimum temperature (°C)				
No lag	-0.003	0.004	<0.001	Statistic = 0.069, p<0.001
Lag -1	-0.004	0.004	<0.001	Statistic = 0.069, p<0.001
Lag -2	-0.056	0.003	<0.001	Statistic = 0.067, p<0.001
Relative humidity (%)				
No lag	0.028	0.003	<0.001	Statistic = 0.072, p<0.001
Lag -1	0.040	0.003	<0.001	Statistic = 0.070, p<0.001
Lag -2	0.041	0.003	<0.001	Statistic = 0.071, p<0.001
Absolute humidity (g/m^3)				
No lag	-0.027	0.003	<0.001	Statistic = 0.071, p<0.001
Lag -1	-0.032	0.003	<0.001	Statistic = 0.070, p<0.001
Lag -2	-0.049	0.003	<0.001	Statistic = 0.068, p<0.001

Rainfall (mm)				
No lag	2.363×10^{-4}	5.758×10^{-5}	<0.001	Statistic = 0.078,
Lag-1	5.246×10^{-4}	5.512×10^{-5}	<0.001	p<0.001
Lag-2	4.696×10^{-4}	5.610×10^{-5}	<0.001	Statistic = 0.079,
Lag-3	4.897×10^{-4}	5.642×10^{-5}	<0.001	p<0.001
				Statistic = 0.080,
				p<0.001
				Statistic = 0.080,
				p<0.001
Sunshine (hours)				
No lag	5.239×10^{-4}	1.839×10^{-4}	0.004	Statistic = 0.080,
Lag-1	5.702×10^{-4}	1.811×10^{-4}	0.002	p<0.001
Lag-2	-2.000×10^{-3}	1.784×10^{-4}	<0.001	Statistic = 0.077,
				p<0.001
				Statistic = 0.075,
				p<0.001
NDVI				
No lag	0.799	0.078	<0.001	Statistic = 0.077,
Lag-1	0.530	0.078	<0.001	p<0.001
Lag-2	0.188	0.078	0.016	Statistic = 0.077,
				p<0.001
				Statistic = 0.078,
				p<0.001
Elevation (weighted by population density)	1.345×10^{-3}	4.433×10^{-5}	<0.001	Statistic = 0.059, p<0.001
Number of pigs per 100,000 population	1.814×10^{-5}	7.456×10^{-7}	<0.001	Statistic = 0.064, p<0.001
Poverty rate (%)	2.081×10^{-2}	7.854×10^{-4}	<0.001	Statistic = 0.045, p<0.001
Number of hospitals per 1000km^2	-0.036	0.002	<0.001	Statistic = 0.078, p<0.001
JE vaccination coverage (%)	2.547×10^{-3}	3.057×10^{-4}	<0.001	Statistic = 0.072, p<0.001
Incidence of meningitis	0.975	0.045	<0.001	Statistic = 0.072, p<0.001
Incidence of dengue	-3.147×10^{-3}	5.92×10^{-4}	<0.001	Statistic = 0.075, p<0.001

Incidence of *S. suis*	1.321	0.369	<0.001	Statistic = 0.146, p<0.001
Incidence of ILI	1.838×10^{-3}	5.511×10^{-5}	<0.001	Statistic = 0.057, p<0.001
Incidence of measles	1.12×10^{-2}	2.01×10^{-3}	<0.001	Statistic = 0.077, p<0.001
Incidence of leptospirosis	0.711	0.144	<0.001	Statistic = 0.077, p<0.001
Incidence of HFMD	-1.9×10^{-2}	2.0×10^{-3}	<0.001	Statistic = 0.139, p<0.001

Table A2.10 Univariate negative binomial generalized additive models with an outcome of the number of cases of meningitis.

Variable	Estimate	SE	p value	Monte Carlo Moran's I of the residuals
Maximum temperature (°C)				
No lag	-0.021	0.005	<0.001	Statistic = 0.050, p<0.001
Lag-1	-0.022	0.005	<0.001	Statistic = 0.050, p<0.001
Lag-2	-0.037	0.005	<0.001	Statistic = 0.049, p<0.001
Minimum temperature (°C)				
No lag	-0.039	0.005	<0.001	Statistic = 0.049, p<0.001
Lag -1	-0.030	0.005	<0.001	Statistic = 0.049, p<0.001
Lag -2	-0.049	0.005	<0.001	Statistic = 0.049, p<0.001
Relative humidity (%)	0.033	0.004	<0.001	Statistic = 0.048, p<0.001
No lag	0.003	0.004	<0.001	Statistic = 0.048, p<0.001
Lag -1	0.033	0.003	<0.001	Statistic = 0.049, p<0.001
Lag -2				
Absolute humidity (g/m^3)	-0.026	0.004	<0.001	Statistic = 0.050, p<0.001
No lag	-0.024	0.004	<0.001	Statistic = 0.050, p<0.001
Lag -1	-0.037	0.004	<0.001	Statistic = 0.050, p<0.001
Lag -2				
Rainfall (mm)				
No lag	-3.249×10^{-4}	1.241×10^{-4}	0.009	Statistic = 0.052, p<0.001
Lag-1	-1.372×10^{-4}	1.158×10^{-4}	0.236	Statistic = 0.052, p<0.001
Lag-2	7.571×10^{-6}	1.008×10^{-4}	0.94	Statistic = 0.053, p<0.001
Lag-3	-3.275×10^{-5}	1.164×10^{-4}	0.778	Statistic = 0.053, p<0.001

Sunshine (hours) No lag Lag-1 Lag-2	1.998×10^{-5} -1.815×10^{-4} -5.368×10^{-4}	2.418×10^{-4} 2.381×10^{-4} 2.346×10^{-4}	0.934 0.446 0.022	Statistic = 0.052, p<0.001 Statistic = 0.052, p<0.001 Statistic = 0.052, p<0.001
NDVI No lag Lag-1 Lag-2	 0.827 0.537 0.206	 0.105 0.106 0.106	 <0.001 <0.001 0.052	 Statistic = 0.052, p<0.001 Statistic = 0.053, p<0.001 Statistic = 0.053, p<0.001
Elevation (weighted by population density)	1.140×10^{-3}	5.759×10^{-5}	<0.001	Statistic = 0.054, p<0.001
Number of pigs per 100,000 population	1.636×10^{-5}	1.047×10^{-6}	<0.001	Statistic = 0.045, p<0.001
Poverty rate (%)	0.012	0.001	<0.001	Statistic = 0.047, p<0.001
Number of hospitals per 1000km^2	-0.009	0.003	0.002	Statistic = 0.054, p<0.001
JE vaccination coverage (%)	-1.228×10^{-3}	3.667×10^{-4}	<0.001	Statistic = 0.057, p<0.001
Incidence of dengue	6.197×10^{-4}	6.083×10^{-4}	0.308	Statistic = 0.052, p<0.001
Incidence of *S. suis*	1.869	0.610	0.002	Statistic = 0.034, p<0.001
Incidence of ILI	1.143×10^{-3}	6.694×10^{-5}	<0.001	Statistic = 0.050, p<0.001
Incidence of measles	0.010	0.003	<0.001	Statistic = 0.052, p<0.001
Incidence of leptospirosis	-0.064	0.188	0.733	Statistic = 0.052, p<0.001
Incidence of HFMD	-0.010	0.004	0.009	Statistic = 0.032, p<0.001

Table A.211 Multivariate model 1 with an outcome of the number of cases of AES.

	Mean	**SD**	**0.025quant**	**0.975quant**
(Intercept)	-5.105	0.601	-6.279	-3.919
Max.temp	0.079	0.011	0.057	0.101
Max.temp.lag_minus1	0.024	0.013	-0.001	0.05
Max.temp.lag_minus2	-0.018	0.01	-0.039	0.002
Relative.humidity	0.021	0.006	0.009	0.032
Relative.humidity.lag_minus1	0.006	0.004	-0.002	0.014
Relative.humidity.lag_minus2	0.001	0.003	-0.006	0.007
Rainfall	0	0	0	0.001
Rainfall.lag_minus1	0	0	0	0.001
Rainfall.lag_minus2	0	0	0	0
Rainfall.lag_minus3	0	0	0	0
Sunshine	0.001	0	0	0.002
Sunshine.lag_minus1	0.001	0	0	0.002
Sunshine.lag_minus2	-0.002	0	-0.003	-0.001
NDVI	0.119	0.122	-0.12	0.359
NDVI.lag_minus1	0.226	0.115	0.001	0.452
NDVI.lag_minus2	-0.287	0.114	-0.51	-0.064
Elevation	0.001	0.001	0	0.002
Number_of_pigs	0	0	0	0
Poverty_rate	-0.013	0.004	-0.021	-0.006
Number_hospitals	0.028	0.017	-0.005	0.061
JE.vaccination	0.003	0.001	0.002	0.004
Size for the nbinomial observations (1/overdispersion)	0.48	0.01	0.461	0.501
Precision for idregion.x (iid component)	1.29	0.246	0.873	1.836
Precision for idregion.x (spatial component)	1888.01	1852.812	127.696	6774.671
Precision for year	36.15	15.566	15.796	75.465
Precision for month	43.89	22.925	13.611	101.28

Table A2.12 Multivariate model 2 with an outcome of the number of cases of AES.

	Mean	**SD**	**0.025quant**	**0.975quant**
(Intercept)	-3.555	0.581	-4.692	-2.411
Min.temp	0.033	0.007	0.019	0.046
Min.temp.lag_minus1	0.036	0.007	0.022	0.05
Min.temp.lag_minus2	-0.008	0.007	-0.022	0.006
Relative.humidity	0.016	0.006	0.005	0.028
Relative.humidity.lag_minus1	0.005	0.004	-0.002	0.012
Relative.humidity.lag_minus2	-0.002	0.003	-0.007	0.004
Rainfall	0	0	0	0.001
Rainfall.lag_minus1	0	0	0	0.001
Rainfall.lag_minus2	0	0	0	0
Rainfall.lag_minus3	0	0	0	0
Sunshine	0.003	0	0.002	0.004
Sunshine.lag_minus1	0.001	0	0	0.002
Sunshine.lag_minus2	-0.002	0	-0.003	-0.002
NDVI	0.204	0.121	-0.034	0.443
NDVI.lag_minus1	0.226	0.115	0	0.451
NDVI.lag_minus2	-0.333	0.114	-0.557	-0.109
Elevation	0.001	0.001	0	0.002
Number_of_pigs	0	0	0	0
Poverty_rate	-0.014	0.004	-0.021	-0.006
Number_hospitals	0.03	0.017	-0.004	0.063
JE.vaccination	0.002	0.001	0.001	0.004
Size for the nbinomial observations (1/overdispersion)	0.478	0.01	0.459	0.498
Precision for idregion.x (iid component)	1.276	0.24	0.854	1.795
Precision for idregion.x (spatial component)	1890.514	1852.65	128.545	6778.354
Precision for year	37.587	17.05	16.065	81.103
Precision for month	27.239	13.92	9.215	62.478

Table A2.13 Multivariate model 3 with an outcome of the number of cases of AES.

	Mean	**SD**	**0.025quant**	**0.975quant**
(Intercept)	-3.078	0.556	-4.168	-1.986
Absolute.humidity	0.053	0.009	0.036	0.07
Absolute.humidity.lag_minus1	0.028	0.011	0.005	0.05
Absolute.humidity.lag_minus2	-0.017	0.009	-0.035	0
Relative.humidity	0.005	0.006	-0.007	0.017
Relative.humidity.lag_minus1	0.004	0.004	-0.005	0.012
Relative.humidity.lag_minus2	0.001	0.003	-0.005	0.008
Rainfall	0	0	0	0
Rainfall.lag_minus1	0	0	0	0
Rainfall.lag_minus2	0	0	0	0
Rainfall.lag_minus3	0	0	0	0
Sunshine	0.002	0	0.001	0.003
Sunshine.lag_minus1	0.001	0	0	0.002
Sunshine.lag_minus2	-0.002	0	-0.003	-0.001
NDVI	0.155	0.121	-0.083	0.394
NDVI.lag_minus1	0.263	0.115	0.038	0.489
NDVI.lag_minus2	-0.291	0.114	-0.514	-0.067
Elevation	0.001	0.001	0	0.002
Number_of_pigs	0	0	0	0
Poverty_rate	-0.013	0.004	-0.021	-0.005
Number_hospitals	0.031	0.017	-0.003	0.064
JE.vaccination	0.003	0.001	0.002	0.004
Size for the nbinomial observations (1/overdispersion)	0.48	0.01	0.461	0.5
Precision for idregion.x (iid component)	1.27	0.25	0.863	1.84
Precision for idregion.x (spatial component)	1891.83	1854.05	127.283	6784.46
Precision for year	36.76	15.76	16.109	76.5
Precision for month	60.23	32.95	18.554	143.88

Table A2.14 Multivariate model 4 with an outcome of the number of cases of AES.

	Mean	SD	0.025quant	0.975quant
(Intercept)	-4.967	0.598	-6.135	-3.788
Max.temp	0.075	0.011	0.054	0.097
Max.temp.lag_minus1	0.021	0.013	-0.004	0.046
Max.temp.lag_minus2	-0.018	0.01	-0.038	0.002
Relative.humidity	0.02	0.006	0.009	0.031
Relative.humidity.lag_minus1	0.005	0.004	-0.003	0.014
Relative.humidity.lag_minus2	0.002	0.003	-0.005	0.008
Rainfall	0	0	0	0.001
Rainfall.lag_minus1	0	0	0	0.001
Rainfall.lag_minus2	0	0	0	0
Rainfall.lag_minus3	0	0	0	0
Sunshine	0.001	0	0	0.002
Sunshine.lag_minus1	0.001	0	0	0.002
Sunshine.lag_minus2	-0.002	0	-0.003	-0.001
NDVI	0.103	0.121	-0.135	0.341
NDVI.lag_minus1	0.21	0.114	-0.014	0.435
NDVI.lag_minus2	-0.327	0.113	-0.549	-0.104
Elevation	0.001	0.001	0	0.002
Number_of_pigs	0	0	0	0
Poverty_rate	-0.013	0.004	-0.021	-0.006
Number_hospitals	0.031	0.017	-0.003	0.064
JE.vaccination	0.003	0.001	0.002	0.004
Incidence_meningitis	0.79	0.089	0.618	0.969
Incidence_S.suis	2.093	0.618	0.946	3.376
Size for the nbinomial observations (1/overdispersion)	0.488	0.01	0.467	0.508
Precision for idregion.x (iid component)	1.302	0.253	0.883	1.875
Precision for idregion.x (spatial component)	1758.788	1798.758	123.423	6493.097
Precision for year	39.079	16.289	16.996	79.824
Precision for month	44.552	23.954	14.076	105.399

Table A2.15 Multivariate model 5 with an outcome of the number of cases of AES.

	Mean	SD	0.025quant	0.975quant
(Intercept)	-3.528	0.575	-4.654	-2.396
Min.temp	0.032	0.007	0.019	0.046
Min.temp.lag_minus1	0.033	0.007	0.018	0.047
Min.temp.lag_minus2	-0.008	0.007	-0.022	0.006
Relative.humidity	0.016	0.006	0.005	0.027
Relative.humidity.lag_minus1	0.004	0.004	-0.003	0.011
Relative.humidity.lag_minus2	-0.001	0.003	-0.006	0.005
Rainfall	0	0	0	0.001
Rainfall.lag_minus1	0	0	0	0.001
Rainfall.lag_minus2	0	0	0	0
Rainfall.lag_minus3	0	0	0	0
Sunshine	0.003	0	0.002	0.004
Sunshine.lag_minus1	0.001	0	0	0.002
Sunshine.lag_minus2	-0.002	0	-0.003	-0.002
NDVI	0.182	0.121	-0.055	0.419
NDVI.lag_minus1	0.208	0.114	-0.016	0.433
NDVI.lag_minus2	-0.374	0.114	-0.597	-0.15
Elevation	0.001	0.001	0	0.002
Number_of_pigs	0	0	0	0
Poverty_rate	-0.013	0.004	-0.021	-0.006
Number_hospitals	0.032	0.017	-0.002	0.065
JE.vaccination	0.003	0.001	0.002	0.004
Incidence_meningitis	0.808	0.09	0.634	0.988
Incidence_S.suis	2.082	0.619	0.934	3.368
Size for the nbinomial observations (1/overdispersion)	0.485	0.01	0.466	0.506
Precision for idregion.x (iid component)	1.294	0.244	0.869	1.822
Precision for idregion.x (spatial component)	1763.729	1802.07	124.054	6504.102
Precision for year	41.894	16.407	16.821	80
Precision for month	30.184	15.107	9.832	67.737

Table A2.16 Multivariate model 6 with an outcome of the number of cases of AES.

	Mean	SD	0.025quant	0.975quant
(Intercept)	-3.062	0.551	-4.143	-1.978
Absolute.humidity	0.052	0.009	0.035	0.069
Absolute.humidity.lag_minus1	0.023	0.011	0.001	0.046
Absolute.humidity.lag_minus2	-0.017	0.009	-0.035	0.001
Relative.humidity	0.005	0.006	-0.007	0.017
Relative.humidity.lag_minus1	0.004	0.004	-0.005	0.012
Relative.humidity.lag_minus2	0.002	0.003	-0.004	0.008
Rainfall	0	0	0	0
Rainfall.lag_minus1	0	0	0	0
Rainfall.lag_minus2	0	0	0	0
Rainfall.lag_minus3	0	0	0	0
Sunshine	0.002	0	0.001	0.003
Sunshine.lag_minus1	0.001	0	0	0.002
Sunshine.lag_minus2	-0.002	0	-0.003	-0.001
NDVI	0.137	0.121	-0.1	0.374
NDVI.lag_minus1	0.246	0.114	0.022	0.471
NDVI.lag_minus2	-0.33	0.113	-0.553	-0.108
Elevation	0.001	0.001	0	0.002
Number_of_pigs	0	0	0	0
Poverty_rate	-0.013	0.004	-0.02	-0.005
Number_hospitals	0.033	0.017	0	0.066
JE.vaccination	0.003	0.001	0.002	0.004
Incidence_meningitis	0.791	0.09	0.619	0.97
Incidence_S.suis	2.115	0.619	0.966	3.4
Size for the nbinomial observations (1/overdispersion)	0.487	0.01	0.467	0.508
Precision for idregion.x (iid component)	1.306	0.247	0.866	1.83
Precision for idregion.x (spatial component)	1973.409	1897.418	124.007	6982.787
Precision for year	40.461	16.311	17.698	80.571
Precision for month	61.325	34.393	18.626	149.451

Table A2.17 Multivariate model 1 with an outcome of the number of cases of meningitis.

	Mean	SD	0.025quant	0.975quant
(Intercept)	-3.577	0.695	-4.942	-2.215
Max.temp	0.05	0.014	0.023	0.076
Max.temp.lag_minus1	0.061	0.017	0.028	0.095
Max.temp.lag_minus2	-0.039	0.013	-0.065	-0.013
Relative.humidity	0.012	0.008	-0.003	0.027
Relative.humidity.lag_minus1	-0.014	0.006	-0.025	-0.003
Relative.humidity.lag_minus2	0.006	0.004	-0.003	0.014
Rainfall	0	0	0	0
Rainfall.lag_minus1	0	0	0	0
Rainfall.lag_minus2	0	0	0	0.001
Rainfall.lag_minus3	0	0	-0.001	0
Sunshine	-0.002	0.001	-0.003	0
Sunshine.lag_minus1	-0.001	0.001	-0.003	0
Sunshine.lag_minus2	0.001	0.001	0	0.002
NDVI	0.24	0.174	-0.101	0.583
NDVI.lag_minus1	0.076	0.167	-0.251	0.403
NDVI.lag_minus2	-0.068	0.164	-0.389	0.253
Elevation	0	0.001	-0.001	0.002
Number_pigs	0	0	0	0
Poverty_rate	0.004	0.006	-0.007	0.015
Number_hospitals	-0.01	0.022	-0.055	0.033
JE.vaccination	-0.001	0.001	-0.003	0
Size for the nbinomial observations (1/overdispersion)	3.26×10^{-1}	1.00×10^{-2}	0.307	3.47×10^{-1}
Precision for idregion.x (iid component)	1.02	2.18×10^{-1}	0.653	1.51
Precision for idregion.x (spatial component)	1.86×10^{3}	1.84×10^{3}	129.499	6.71×10^{3}
Precision for year	5.00	1.79	2.217	9.14
Precision for month	1.70×10^{-4}	1.74×10^{4}	905.438	6.36×10^{4}

Table A2.18 Multivariate model 2 with an outcome of the number of cases of meningitis.

	Mean	SD	0.025quant	0.975quant
(Intercept)	-2.748	0.687	-4.095	-1.399
Min.temp	0.041	0.012	0.019	0.064
Min.temp.lag_minus1	0.055	0.013	0.03	0.081
Min.temp.lag_minus2	-0.027	0.01	-0.048	-0.007
Relative.humidity	0.006	0.008	-0.01	0.021
Relative.humidity.lag_minus1	-0.01	0.005	-0.02	-0.001
Relative.humidity.lag_minus2	0.003	0.004	-0.004	0.01
Rainfall	0	0	0	0
Rainfall.lag_minus1	0	0	0	0
Rainfall.lag_minus2	0	0	0	0
Rainfall.lag_minus3	0	0	-0.001	0
Sunshine	-0.001	0.001	-0.002	0.001
Sunshine.lag_minus1	0	0.001	-0.002	0.001
Sunshine.lag_minus2	0	0.001	-0.001	0.001
NDVI	0.29	0.174	-0.051	0.632
NDVI.lag_minus1	0.112	0.166	-0.215	0.438
NDVI.lag_minus2	-0.074	0.164	-0.395	0.248
Elevation	0	0.001	-0.001	0.002
Number_pigs	0	0	0	0
Poverty_rate	0.004	0.006	-0.007	0.015
Number_hospitals	-0.007	0.022	-0.052	0.036
JE.vaccination	-0.002	0.001	-0.003	0
Size for the nbinomial observations (1/overdispersion)	3.26×10^{-1}	1.00×10^{-2}	0.307	3.47×10^{-1}
Precision for idregion.x (iid component)	1.05	2.25×10^{-1}	0.649	1.53
Precision for idregion.x (spatial component)	2.35×10^{3}	2.41×10^{3}	223.932	8.76×10^{3}
Precision for year	5.07	1.82	2.244	9.30
Precision for month	2.19×10^{4}	2.21×10^{4}	1722.147	8.10×10^{4}

Table A2.19 Multivariate model 3 with an outcome of the number of cases of meningitis.

	Mean	**SD**	**0.025quant**	**0.975quant**
(Intercept)	-1.792	0.708	-3.18	-0.403
Absolute.humidity	0.039	0.012	0.015	0.063
Absolute.humidity.lag_minus1	0.043	0.017	0.01	0.077
Absolute.humidity.lag_minus2	-0.026	0.012	-0.049	-0.002
Relative.humidity	-0.005	0.008	-0.021	0.012
Relative.humidity.lag_minus1	-0.01	0.006	-0.022	0.001
Relative.humidity.lag_minus2	0.003	0.004	-0.005	0.011
Rainfall	0	0	-0.001	0
Rainfall.lag_minus1	0	0	-0.001	0
Rainfall.lag_minus2	0	0	0	0.001
Rainfall.lag_minus3	0	0	-0.001	0
Sunshine	-0.001	0.001	-0.002	0
Sunshine.lag_minus1	-0.001	0.001	-0.002	0.001
Sunshine.lag_minus2	0	0.001	-0.001	0.002
NDVI	0.278	0.174	-0.063	0.619
NDVI.lag_minus1	0.127	0.166	-0.199	0.454
NDVI.lag_minus2	-0.056	0.164	-0.378	0.266
Elevation	0	0.001	-0.001	0.002
Number_pigs	0	0	0	0
Poverty_rate	0.004	0.006	-0.007	0.015
Number_hospitals	-0.009	0.022	-0.054	0.034
JE.vaccination	-0.001	0.001	-0.003	0
Size for the nbinomial observations (1/overdispersion)	3.27×10^{-1}	0.01	0.307	3.47×10^{-1}
Precision for idregion.x (iid component)	1.03	0.22	0.638	1.50
Precision for idregion.x (spatial component)	1.92×10^{3}	1873.09	130.52	6.86×10^{3}
Precision for year	4.89	1.83	2.262	9.35
Precision for month	1.88×10^{4}	18315.94	1303.893	6.75×10^{4}

Table A2.20 Multivariate model 4 with an outcome of the number of cases of meningitis.

	Mean	SD	0.025quant	0.975quant
(Intercept)	-3.629	0.694	-4.991	-2.269
Max.temp	0.051	0.014	0.024	0.077
Max.temp.lag_minus1	0.061	0.017	0.027	0.094
Max.temp.lag_minus2	-0.04	0.013	-0.066	-0.014
Relative.humidity	0.013	0.008	-0.002	0.028
Relative.humidity.lag_minus1	-0.014	0.006	-0.025	-0.003
Relative.humidity.lag_minus2	0.006	0.004	-0.002	0.015
Rainfall	0	0	0	0
Rainfall.lag_minus1	0	0	0	0
Rainfall.lag_minus2	0	0	0	0.001
Rainfall.lag_minus3	0	0	-0.001	0
Sunshine	-0.002	0.001	-0.003	0
Sunshine.lag_minus1	-0.001	0.001	-0.002	0
Sunshine.lag_minus2	0.001	0.001	0	0.002
NDVI	0.236	0.174	-0.105	0.578
NDVI.lag_minus1	0.072	0.166	-0.254	0.399
NDVI.lag_minus2	-0.073	0.164	-0.394	0.248
Elevation	0	0.001	-0.001	0.002
Number_pigs	0	0	0	0
Poverty_rate	0.004	0.006	-0.007	0.016
Number_hospitals	-0.013	0.022	-0.058	0.03
JE.vaccination	-0.001	0.001	-0.003	0
Incidence_HFMD	-0.013	0.005	-0.022	-0.004
Size for the nbinomial observations (1/overdispersion)	3.27×10^{-1}	1.00×10^{-2}	0.307	3.48×10^{-1}
Precision for idregion.x (iid component)	1.01	2.24×10^{-1}	0.65	1.52
Precision for idregion.x (spatial component)	1.92×10^{3}	1.87×10^{3}	137.017	6.85×10^{3}
Precision for year	4.80	1.74	2.171	8.92
Precision for month	1.80×10^{4}	1.80×10^{4}	1160.942	6.60×10^{4}

Table A2.21 Multivariate model 5 with an outcome of the number of cases of meningitis.

	Mean	SD	0.025quant	0.975quant
(Intercept)	-2.797	0.687	-4.145	-1.448
Min.temp	0.042	0.012	0.019	0.065
Min.temp.lag_minus1	0.055	0.013	0.03	0.081
Min.temp.lag_minus2	-0.028	0.01	-0.048	-0.007
Relative.humidity	0.007	0.008	-0.009	0.022
Relative.humidity.lag_minus1	-0.01	0.005	-0.02	-0.001
Relative.humidity.lag_minus2	0.003	0.004	-0.004	0.01
Rainfall	0	0	0	0
Rainfall.lag_minus1	0	0	0	0
Rainfall.lag_minus2	0	0	0	0
Rainfall.lag_minus3	0	0	-0.001	0
Sunshine	-0.001	0.001	-0.002	0.001
Sunshine.lag_minus1	0	0.001	-0.001	0.001
Sunshine.lag_minus2	0	0.001	-0.001	0.001
NDVI	0.288	0.174	-0.053	0.629
NDVI.lag_minus1	0.109	0.166	-0.217	0.436
NDVI.lag_minus2	-0.079	0.164	-0.4	0.243
Elevation	0	0.001	-0.001	0.002
Number_pigs	0	0	0	0
Poverty_rate	0.004	0.006	-0.007	0.015
Number_hospitals	-0.01	0.022	-0.055	0.033
JE.vaccination	-0.001	0.001	-0.003	0
Incidence_HFMD	-0.013	0.005	-0.022	-0.004
Size for the nbinomial observations (1/overdispersion)	3.27×10^{-1}	1.00×10^{-2}	0.307	3.48×10^{-1}
Precision for idregion.x (iid component)	1.03	2.24×10^{-1}	0.662	1.53
Precision for idregion.x (spatial component)	1.90×10^{3}	1.87×10^{3}	129.234	6.82×10^{3}
Precision for year	4.91	1.77	2.163	9.03
Precision for month	1.84×10^{4}	1.82×10^{4}	1285.886	6.67×10^{4}

Table A2.22 Multivariate model 6 with an outcome of the number of cases of meningitis.

	Mean	SD	0.025quant	0.975quant
(Intercept)	-1.849	0.707	-3.234	-0.461
Absolute.humidity	0.039	0.012	0.015	0.063
Absolute.humidity.lag_minus1	0.043	0.017	0.01	0.077
Absolute.humidity.lag_minus2	-0.026	0.012	-0.05	-0.003
Relative.humidity	-0.004	0.008	-0.02	0.013
Relative.humidity.lag_minus1	-0.01	0.006	-0.022	0.001
Relative.humidity.lag_minus2	0.003	0.004	-0.005	0.011
Rainfall	0	0	-0.001	0
Rainfall.lag_minus1	0	0	-0.001	0
Rainfall.lag_minus2	0	0	0	0.001
Rainfall.lag_minus3	0	0	-0.001	0
Sunshine	-0.001	0.001	-0.002	0
Sunshine.lag_minus1	0	0.001	-0.002	0.001
Sunshine.lag_minus2	0	0.001	-0.001	0.002
NDVI	0.274	0.174	-0.066	0.615
NDVI.lag_minus1	0.125	0.166	-0.201	0.451
NDVI.lag_minus2	-0.061	0.164	-0.382	0.261
Elevation	0	0.001	-0.001	0.002
Number_pigs	0	0	0	0
Poverty_rate	0.004	0.006	-0.007	0.016
Number_hospitals	-0.011	0.022	-0.056	0.032
JE.vaccination	-0.001	0.001	-0.003	0
Incidence_HFMD	-0.012	0.005	-0.022	-0.003
Size for the nbinomial observations (1/overdispersion)	3.27×10^{-1}	1.00×10^{-2}	0.308	3.48×10^{-1}
Precision for idregion.x (iid component)	1.02	2.18×10^{-1}	0.639	1.49
Precision for idregion.x (spatial component)	2.18×10^{3}	2.10×10^{3}	189.223	7.79×10^{3}
Precision for year	4.83	1.74	2.159	8.91
Precision for month	2.08×10^{4}	2.01×10^{4}	1723.643	7.42×10^{4}

13HN ICF Cases V4.2 04AUG16

13HN: UNDERSTANDING RISK FACTORS FOR ENCEPHALITIS

(BRAIN INFLAMMATION)

Information Sheet For Cases

One Copy For Study Records And One Copy To Be Given To The Participant

Please note that you can refer to "I" if consent if being obtained from the participant themselves or "your child/relative" if consent is obtained from a representative on behalf of the participant

You are being invited to participate in a study looking at risk factors for acute encephalitis syndrome, an inflammation of the brain. Approximately 2000 people will be part of this study. Please read this information sheet carefully or have someone read it to you. Please ask the study staff to explain any information that you are not sure about. It is your choice whether to participate in the study and please do not feel obliged do so. If you would like to take part you will be asked to sign or place a thumbprint on the last page. You will be given a copy of this form for your personal records.

You will be taking part in a practice study before we conduct a larger study. The information we collect from you is therefore very important to us as it will help us to see if there are any problems and will also be used for our larger study.

Why is this study being done?

Acute Encephalitis Syndrome (AES) is a syndrome of brain inflammation or swelling. Patients may die or be left disabled. There are around two thousand cases of AES in Viet Nam every year. Although Japanese encephalitis, a virus spread by mosquitoes is a common cause of AES in children and Streptococcus suis, a bacteria associated with eating or preparing raw pork meat is a common cause in adults, for half of the patients or more, we do not know the cause of their AES. However, we do know that most of the cases occur during the warmer seasons and therefore we think that mosquitoes or other insects are spreading diseases, which cause AES in northern Viet Nam.

This study therefore aims to look at the reasons why people might or might not get AES including where the person lives and their risks in and around their home and work environment and their contact with animals.

Who is doing this study?

This study is being performed by a team from:
The Oxford University Clinical Research Unit (OUCRU) in Ha Noi
The University of Liverpool, United Kingdom
The Wellcome Trust Liverpool Glasgow Centre for Global Health Research, United Kingdom
The National Hospital of Tropical Diseases, Ha Noi

What will happen to me in this study and what are the risks?

You have been chosen to take part because the doctors and nurses think that you may have inflammation/swelling of the brain (acute encephalitis syndrome). We would like to look at the factors, which may or may not have played a role in you getting this syndrome.

With your permission another member of the study team will visit your house within the four weeks after you are discharged from hospital. We will ask for your phone number so we can contact you to arrange a time. If you are willing, we will also ask for a phone number from a relative/friend who may be able to help contact you in case

we are unable to. During the visit, we will ask you to answer a questionnaire, which a member of the study team will go through with you. This will include questions about your background, house, ways in which you prevent mosquito bites and your contact with animals and the environment. The study team member will also obtain more information by walking around the house and taking photos. He/she will also find the location of the house using the mobile phone and plot this on a map, possibly using an online programme which is accessible to the public. If you would like more detail, please inform us and we can show these questions to you. You are able to refuse to answer any questions or restrict access of the study team member to any parts of your house and surroundings.

We will also use the results from blood tests and any fluid taken from around your spine, which your doctors and nurses have performed as part of your medical care in hospital. This is to look for any bacteria, viruses and other infections which might be causing your brain inflammation. There are some risks with taking blood and fluid from around the spine which your doctor or nurse should explain to you before the procedure.

We will then keep your blood and fluid from around the spine in a freezer and test it again to look for anything else that might be causing this problem. Because these tests will only be done once every couple of months, they will not be useful for your care and you will not be given the results of these tests. In the future the sample may be tested again to look at your immunity, for any evidence of poisons and to look at your genetics. Looking at the genetic testing involves looking at the DNA in your cells. This helps us to understand why you became unwell whereas someone else may not have. Some of these samples may be sent abroad for testing. You can opt out of this part whilst still taking part in the rest of the study. We will also take some information from yourself or your medical records about your symptoms any treatment you received before you came to hospital and your diagnosis information about your discharge from hospital.

We will also collect some information about your from your medical records when you arrived at hospital. Additionally, we will ask you to help complete a questionnaire

about your health when you go home and 3 months afterwards which is also optional. At 3 months time will contact you by telephone.

What will happen to the samples / information taken?

We will keep your blood and fluid from around the spine in a freezer and test it again to look for any other infections. In the future the sample may be tested again to look at your immunity, for any evidence of poisons and to look at your genetics. The samples will be kept for at least ten years, possibly longer.

The information from the questionnaire will be examined and we will use maps and information from around your house to look at other possible risks for AES.

The results will be written as a report or thesis for the University of Liverpool and will be published in a journal, which will be made available to the public. The results will also be available in Vietnamese through the OUCRU website and the Hospital for Tropical Diseases website. The results will also be available for the Ministry of Health in Viet Nam. You will not be able to be identified in these results with the exception of pictures of your house and the dot on a map to show the location of your house.

The results will also be made available to the public through workshops about AES in the community or schools and possibly via the radio or other forms of media.

What is the benefit of participating?

There is no intended personal benefit to taking part however, the benefit will be to the community and people of Viet Nam by help us to understand the risks associated with AES.

You will be reimbursed 100,000 VND for the time spent answering the questionnaire.

What happens if I decline participation or change my mind later on?

You have the right to decline participation or withdraw from the study or any part of the study at any time. Please let us know if you wish to do so. You do not need to give an explanation for this. You can request for your results to be destroyed at any time. There will be no effect to your clinical management.

Can someone else decide to take the patient out of the study?

The research team doctors can withdraw you from the study, if there is a problem with the information collected from you. The Universities and ethics committees can also stop the study.

Confidentiality

You will be given an anonymous code instead of your name, which will be used for the questionnaire and the samples of your blood and fluid from around the spine. Details linking you to this code will be kept in a separate place. All consent forms will be kept in a locked room. Only those who are involved with running the study will have access to these documents which will be kept for 3 years and then put on an electronic system. The information from the questionnaire will be recorded on a mobile phone and transferred to a computer. However, this information will be kept very securely.

However, it is possible that you may be identified from the location of your house which will be shown as a dot on a map and from photos taken around your house which with your permission will be used in publications and presentations. Your name will not be linked to this information. Your house will be located using Google Earth which is an online (and not private database).

Whom do I contact if I have questions or complaints?

If you have questions about the study you may contact: *To be decided*

If you have questions about being in a research study, please contact the Research Ethics Board of the NHTD at (phone number) the Clinical Trials Unit at (phone number).

INFORMED CONSENT FORM FOR CASES

RISK FACTORS FOR AND SPATIO-TEMPORAL DISTRIBUTION OF CASES OF ACUTE ENCEPHALITIS SYNDROME OF SUSPECTED INFECTIOUS AETIOLOGY IN THE RED RIVER DELTA REGION, NORTHERN VIET NAM: A FEASIBILITY STUDY

- I freely give my permission to join this study.
- I understand that I have the right to withdraw from the study. I will be given a copy of this signed consent form to keep for my reference.

COMPULSORY FOR STUDY RECRUITMENT	
1. I have read this information sheet or it has been read to me and I understand it.	
2. I have been able to ask questions about the study.	
3. I understand that my participation in the study is voluntary and I do not have to answer questions if I do not wish to do so.	
4. I am happy for the research assistants to look around my house to obtain further information relating the questionnaire. I understand that if there is any area of the house or garden I do not wish them to look at, this is my decision.	
5. I give permission to use results from my investigations in hospital (including blood tests and fluid taken from around the spine) for the study. I also give permission to use information from my medical records.	
6. I give permission for the study to store and test the samples of my blood and fluid taken from around the spine for infections and other possible causes of encephalitis. I understand that I will not receive the results of these tests.	

7. I understand that can withdraw from the study at any time. If I chose to withdraw, I do not need to give a reason and it will not affect me in any manner.	
8. I understand that I can ask for access to the information I provide or have this information destroyed if I wish.	
9. I understand that I will be given an anonymous code instead of my name which will be used for the questionnaire data. However, I understand that I may be identifiable from photographs taken around my house and the location of my house shown as a dot on a map.	
10. I understand that in order for the study team to locate and use my address on a map they will have to use an online programme which is accessible to the public.	
11. I agree for the questionnaire data collected from me to be used in future research publications and presentations without my name.	
12. I agree to take part in the above study.	
OPTIONAL FOR STUDY RECRUITMENT	
13. I agree for my sample of blood or fluid from around the spine to be sent abroad for testing where required.	
14. I agree for my sample of blood or fluid from around the spine to be used for genetic testing.	
15. I give permission to use photos of my house and surroundings and the location of my house on a map, in future presentations and publications without my name.	

16. I am happy to complete a questionnaire relating to my health at discharge from hospital and after 3 months (by telephone).	

Participant ID: 13HN-[__|__]-[__|__|__]

Name of participant (Please Print): _____

By signing/marking my name here, I confirm what is written above (aged 16 years and above)

Signature of Participant	*Full Name*	*Date of Signature*
*x*_____	*x*_____	___/____/_____

If the participant is under 16 years old or cannot give consent themselves, his/her representative will sign this form on his/her behalf:

Signature	*Full Name*	*Relationship to participant*	*Date of Signature*
*x*_____	*x*_____	*x*_____	___/____/___

Children aged from 12 to 15 signs below:

Signature	*Full Name of Child*	*Date of Signature*
*x*_____	*x*_____	___/____/_____

I, the undersigned, have fully explained the relevant information of this program to the person named above and will provide her/him with a copy of this signed and dated informed consent form.

Investigator/Designee Signature *x*_____	*Full Name* *x*_____	*Date of Signature* ___/____/_____

If the person giving consent cannot read the form themselves, a witness must be present and sign here:

I was present throughout the entire informed consent process with the participant. This form was read accurately to the volunteer, all questions from the volunteer were answered and the volunteer has agreed to take part in the research.

Witness Signature *x*_____	*Full Name* *x*_____	*Date of Signature* ___/____/_____

13HN ICF CONTROLS V4.2 04AUG16

13HN: UNDERSTANDING RISK FACTORS FOR ENCEPHALITIS

(BRAIN INFLAMMATION)

Information Sheet For Controls

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You will be taking part in a practice study before we conduct a larger study. The information we collect from you is therefore very important to us as it will help us to see if there are any problems and will also be used for our larger study.

Why is this study being done?

Acute Encephalitis Syndrome (AES) is a syndrome of brain inflammation or swelling. Patients may die or be left disabled. There are around two thousand cases of AES in Viet Nam every year. Although Japanese encephalitis, a virus spread by mosquitoes is a common cause of AES in children and Streptococcus suis, a bacteria associated with eating or preparing raw pork meat is a common cause in adults, for half of the patients or more, we do not know the cause of their AES. However, we do know that most of the cases occur during the warmer seasons and therefore we think that mosquitoes or other insects are spreading diseases, which cause AES in northern Viet Nam.

This study therefore aims to look at the reasons why people might or might not get AES including where the person lives and their risks in and around their home and work environment and their contact with animals.

Who is doing this study?

This study is being performed by a team from:

The Oxford University Clinical Research Unit (OUCRU) in Ha Noi
The University of Liverpool, United Kingdom
The Wellcome Trust Liverpool Glasgow Centre for Global Health Research, United Kingdom
The National Hospital of Tropical Diseases, Ha Noi

What will happen to me in this study and what are the risks?

You have been chosen to take part we are looking for healthy people from the community who are relatives of patients admitted to the hospital with other infections to compare your risk factors to those of patients with AES.

With your permission another member of the study team will visit your house within the next four weeks. We will ask for your phone number so we can contact you to arrange a time. If you are willing, we will also ask for a phone number from a relative/friend who may be able to help contact you in case we are unable to. During the visit, we will ask you to answer a questionnaire, which a member of the study team will go through with you. This will include questions about your background, house, ways in which you prevent mosquito bites and your contact with animals and the environment. The study team member will also obtain more information by walking

around the house and taking photos. He/she will also find the location of the house using the mobile phone and plot this on a map. If you would like more detail, please inform us and we can show these questions to you. You are able to refuse to answer any questions or restrict access of the study team member to any parts of your house and surroundings.

What will happen to the samples / information taken?

The information from the questionnaire will be examined and we will use maps and information from around your house to look at other possible risks for AES.

The results will be written as a report or thesis for the University of Liverpool and will be published in a journal, which will be made available to the public. The results will also be available in Vietnamese through the OUCRU website and the Hospital for Tropical Diseases website. The results will also be available for the Ministry of Health in Viet Nam. You will not be able to be identified in these results with the exception of pictures of your house and the dot on a map to show the location of your house.

The results will be also be made available to the public through workshops about AES in the community or schools and possibly via the radio or other forms of media.

What is the benefit of participating?

There is no intended personal benefit to taking part however, the benefit will be to the community and people of Viet Nam by help us to understand the risks associated with AES.

You will be reimbursed 100,000 VND for the time spent answering the questionnaire.

What happens if I decline participation or change my mind later on?

You have the right to decline participation or withdraw from the study or any part of the study at any time. Please let us know if you wish to do so. You do not need to give an explanation for this. You can request for your results to be destroyed at any time. There will be no effect to your management.

Can someone else decide to take the patient out of the study?

The research team doctors can withdraw you from the study, if there is a problem with the information collected from you. The Universities and ethics committees can also stop the study.

Confidentiality

Individual information about you will be kept confidential. You will be given an anonymous code, which will be used for the questionnaire. Details linking yourself to this code will be kept in a separate place. All consent forms will be kept in a locked room. Only those who are involved with the study will have access to these documents which will be kept for 3 years and then put on an electronic system. The information from the questionnaire will be recorded on a mobile phone and transferred to a computer. This information will be kept very securely.

However, it is possible that you may be identified from the location of your house which will be shown as a dot on a map and from photos taken around your house which with your permission will be used in publications and presentations. Your name will not be linked to this information. Your house will be located using Google Earth which is an online (and not private database).

Whom do I contact if I have questions or complaints?

If you have questions about the study you may contact: *To be decided*

If you have questions about being in a research study, please contact the Research Ethics Board of the NHTD at (phone number) the Clinical Trials Unit at (phone number).

- I freely give my permission to join this study.
- I understand that I have the right to withdraw from the study. I will be given a copy of this signed consent form to keep for my reference.

COMPULSORY FOR STUDY RECRUITMENT	
1. I have read this information sheet or it has been read to me and I understand it.	
2. I have been able to ask questions about the study.	
3. I understand that my participation in the study is voluntary and I do not have to answer questions if I do not wish to do so.	
4. I am happy for the research assistants to look around my house to obtain further information relating the questionnaire. I understand that if there is any area of the house or garden I do not wish them to look at, this is my decision.	
5. I understand that can withdraw from the study at any time. If I chose to withdraw, I do not need to give a reason and it will not affect me in any manner.	
6. I understand that I can ask for access to the information I provide or have this information destroyed if I wish.	
7. I understand that I will be given an anonymous code instead of my name which will be used for the questionnaire data. However, I understand that I may be identifiable from photographs taken around my house and the location of my house shown as a dot on a map.	
8. I understand that in order for the study team to locate and use my address on a map they will have to use an online programme which is accessible to the public.	
9. I agree for the questionnaire data collected from me to be used in future research publications and presentations without my name.	

10. I agree to take part in the above study	

OPTIONAL FOR STUDY RECRUITMENT	
2. I give permission to use photos of my house and surroundings and the location of my house on a map, in future presentations and publications without my name.	

Participant ID: 13HN-[__|__]-[__|__|__]

Name of participant (Please Print): _____

By signing/marking my name here, I confirm what is written above.

Signature of Participant: *x*_____	*Participant Full Name:* *x*_____	*Date of Signature:* ____/____/_____

If the participant does not have capacity to consent or is aged under 16 years, a representative must sign below on his/her behalf

Signature of Participant: *x*_____	*Full Name:* *x*_____	*Relationship to Participant* _____	*Date of Signature:* ____/____/____

If the participant is aged 12-15 years, he/she must give assent and sign below

Signature of Participant: *x*_____	*Participant Full Name:* *x*_____	*Date of Signature:* ____/____/_____

If the person giving consent cannot read the form themselves, a witness must be present and sign here:

I was present throughout the entire informed consent process with the participant. This form was read accurately to the volunteer, all questions from the volunteer were answered and the volunteer has agreed to take part in the research.

Witness Signature *x*_____	*Witness Full Name* *x*_____	*Date of Signature* ___/____/_____

Confirmation of study staff:

By signing/marking my name here, I confirm that I have fully explained the relevant information of this study to the person named above and will provide her/him with a copy of this signed and dated informed consent form.

Signature of study staff x_____	Full Name: x_____	Date of Signature: ____/____/_____

OxTREC 42-15 QU HOSPSTAFFCASES v2.0 15MAR16

Risk factors for and spatio-temporal distribution of cases of acute encephalitis syndrome of suspected infectious aetiology in the Red River Delta region, northern Viet Nam: a feasibility study

FEEDBACK QUESTIONNAIRE FOR STUDY STAFF (HOSPITAL CASES)

To be completed at the end of each week

Participant ID: 13HN-[__|__]-[__|__|__]

Date (day-month-year): [__|__]-[__|__|-[__|__|[__|__|

Week beginning (day-month-year): [__|__]-[__|__|-[__|__|[__|__|

Please list all potential cases of acute encephalitis syndrome who were not successfully recruited into the study. Please tick all applicable columns for reasons why the participant was not recruited.

Number	Lived outside catchment area (specify province)	Did not have fever	Did not have impaired consciousness	Did not have seizures	Had another cause of encephalopathy (e.g. secondary to sepsis or electrolyte imbalance – please specify)	Had post-operative meningitis/ encephalitis	Patient refused to participate	Absence of relative from who to take consent (in the case of a patient without capacity)	Already participated in the study	Other reason (specify)
1										
2										
3										
4										
5										
6										

If the participant refused to consent for the study please indicate his/her reason why

Name of person completing form

Signature

OxTREC 42-15 QU HOSPSTAFFCASES v2.0 15MAR16

Risk factors for and spatio-temporal distribution of cases of acute encephalitis syndrome of suspected infectious aetiology in the Red River Delta region, northern VietNam: a feasibility study

FEEDBACK QUESTIONNAIRE FOR STUDY STAFF (HOSPITAL - CONTROLS)

To be completed at the end of each week

Participant ID: 13HN-[__|__]-[__|__|__]

Date (day-month-year): [__|__]-[__|__|-[__|__|[__|__|

How many patients of potential controls did you see this week?

How long did it take you to identify a suitable relative of the patient – give shortest time and longest time?

What were the reasons for unsuccessful recruitment of the relatives

	Number of patients approached
No relatives of the matched age	
No relatives of the matched gender	

	Number of relatives approached	Reason why (where applicable)
Refused to participate		
Had a past medical history of AES		
Had a current medical history of AES		
Lives in the house of someone with a current history of AES		
Lives in the house of someone with a past history of AES		
Refused permission for the study team to visit their home		
Had no method of contact to provide the study team to visit their home		

No one available to give consent of their behalf (in the case of relatives who lack capacity)		
Already participated in the study (or has a family member who already participated in the study)		

If the participant refused to consent for the study please indicate his/her reason why

Please list any other problems or concerns

Name of person completing form

Signature

Date

13HN OxTREC 42-15 CRF V3.0 05SEP16

1. Patient Information

1a) Date of birth *(dd/mm/yyyy)* : __/__/____

1b) Date of admission to NHTD (*dd/mm/yyyy)*: __/__/____

1c) Date of admission to initial hospital (dd/mm/yyyy) : __/__/____

2. Eligibility:

Please indicate 'unknown' if the result is not known or at the time of consent, the patient specified that he/she did not want this information to be used

Inclusion Criteria (essential)

2a) Is the patient currently living in a province in the Red River Delta region? *(Ha Noi, Hai Phong, Bac Ninh, Ha Nam, Hai Duong, Hung Yen, Nam Dinh, Ninh Binh, Thai Binh, Vinh Phuc)*

□ Yes□ No □ Unknown

If yes, please specify

□ Bac Ninh □ Ha Nam □ Ha Noi □ Hai Duong
□ Hai Phong □ Hung Yen □ Nam Dinh □ Ninh Binh
□ Thai Binh □ Vinh Phuc

2b) Has the patient had a acute onset of fever +/- 10 days of admission to initial hospital?

□ Yes□ No □ Unknown

Inclusion criteria (At least one of c-f)

2c) Has the patient had a change in mental status (including symptoms such as confusion, disorientation, coma, change in behaviour or speech)?

□ Yes□ No □ Unknown

If yes, please specify:
_____

2d) Has the patient had a new onset of seizures (excluding simple febrile seizures)?

□ Yes□ No □ Unknown

2e) Has the patient had increase in irritability (in children only)?

□ Yes□ No □ Unknown □ Not applicable

2f) Does the patient have focal neurological signs– (complete for those aged 2 years and over only)?

□ Yes□ No □ Unknown □ Not applicable

If yes, please specify **(tick all that apply)**:

o Paresis of a limb **o** Abnormality of vision

o Paresis of the side of the face **o** Abnormality of hearing

o Abnormality of speech **o** Imbalance

o Other (specify):
_____

Exclusion Criteria

2g) Do you suspect that the patient has an encephalopathy due to a non-infectious cause other than acute encephalitis syndrome e.g. brain tumour, cerebrovascular accident, electrolyte imbalance?

□ Yes□ No □ Unknown
If yes, please specify_____

2h) Do you suspect that the patient has an encephalopathy due to a infectious cause other than acute encephalitis syndrome e.g. sepsis due to pneumonia?

□ Yes□ No □ Unknown
If yes, please specify_____

2i) Does the patient have post-surgical meningitis/encephalitis?

□ Yes□ No □ Unknown

3. Demographic Information

3a) Gender at birth

□ Male □ Female □ Unknown

3b) Date the patient first experienced symptoms *(dd/mm/yyyy)*: __/__/____

□ Unknown

3c) Date the patient first sought medical care for the symptoms *(dd/mm/yyyy)*: __/__/____

□ Unknown

4. Antibiotics, antivirals, antifungals and steroids given prior to admission

Please complete for all medications known otherwise leave blank

4a) Medication 1

i. Name: _____

ii. Location given

□Hospital □Health care centre □Pharmacy

□Other (specify): _____

□Unknown

□ Unable to answer (participant's decision)

iii. Date commenced (dd/mm/yyyy): __/__/____ □Unknown

□ Unable to answer (participant's decision)

iv. Date stopped (dd/mm/yyyy): __/__/____ □Unknown

□ Unable to answer (participant's decision)

4b) Medication 2

i. Name: _____

ii. Location given

□Hospital □Health care centre □Pharmacy

□Other (specify): _____

□Unknown

□ Unable to answer (participant's decision)

iii. Date commenced (dd/mm/yyyy): __/__/____ □Unknown

□ Unable to answer (participant's decision)

iv. Date stopped (dd/mm/yyyy): __/__/____ □Unknown

□ Unable to answer (participant's decision)

4c) Medication 3

i. Name: _____

ii. Location given

□Hospital □Health care centre □Pharmacy

□Other (specify): _____

□Unknown

iii. Date commenced (dd/mm/yyyy): __/__/____ □Unknown

iv. Date stopped (dd/mm/yyyy): __/__/____ □Unknown □ Not stopped by time of discharge

4d) Medication 4

i. Name: _____

ii. Location given

□Hospital □ Health care centre □ Pharmacy

□Other (specify): _____ □ Unknown

iii. Date commenced (dd/mm/yyyy): __/__/____ □ Unknown

iv. Date stopped (dd/mm/yyyy): __/__/____ □ Unknown □ Not applicable

4e) Medication 5

i. Name: _____

ii. Location given

□Hospital □Health care centre □Pharmacy

□Other (specify): _____

□Unknown

iii. Date commenced (dd/mm/yyyy): __/__/____ □Unknown

iv. Date stopped (dd/mm/yyyy): __/__/____ □Unknown □ Not applicable

Medication 6

i. Name: _____

ii. Location given

□Hospital □Health care centre □Pharmacy

□Other (specify): _____

□Unknown

iii. Date commenced (dd/mm/yyyy): __/__/____ □Unknown

iv. Date stopped (dd/mm/yyyy): __/__/____ □Unknown □ Not applicable

5. Current History: General

5a) Admitted to:

□ ICU □ Non-ICU Ward □Unknown

5b) Is the patient known to be HIV positive?

□ Yes □ No

□ Unknown □ Unable to answer (participant's decision)

If yes please give CD4 count _____

□ Unknown

5c) Past medical history

Diabetes

□ Yes □ No □ Unknown

Previous meningitis or encephalitis

□ Yes □ No □ Unknown

Family member with a history meningitis or encephalitis

□ Yes □ No □ Unknown

1. Tuberculosis

   □ Yes currently being treated (specify type e.g. pulmonary, abdominal etc)

   _____

   □ Yes previously treated (specify type e.g. pulmonary, abdominal etc)

   _____

   □ Never

   □ Unknown
2. Alcohol consumption

   □ Yes □ No □ Unknown

   If yes specify how much (e.g. _____

6) Glasgow Coma Score

6a). Glasgow Coma Score on admission to initial hospital *For adults and children ≥ 2 years only*

Eyes	___/4	□Unknown
Verbal	___/5	□Unknown
Motor	___/6	□Unknown
Total	___/15	□Unknown

6b) AVPU score on admission to initial hospital *For children < 2 years only*

□ Alert □Voice □ Pain □ Unresponsive □Unknown

6c) Glasgow Coma Score on admission to NHTD *For adults and children ≥ 2 years only*

Eyes	___/4	□Unknown
Verbal	___/5	□Unknown
Motor	___/6	□Unknown
Total	___/15	□Unknown

6d) AVPU score on admission *For children < 2 years only*

□ Alert □Voice □ Pain □ Unresponsive □Unknown

6e) Was the patient intubated on admission to NHTD?

□ Yes □ No □ Unknown

7. LABORATORY RESULTS ***Please record all sets of results***

7a). First lumbar puncture

i. CSF Sample taken date *(dd/mm/yyyy)*: ____/____/_____ □ Unknown

ii. Location of lumbar puncture □ NHTD □ Other site □ Unknown

iii. CSF cell count

- Date tests completed *(dd/mm/yyyy)*: ____/____/_____
  □ Not done/unknown
- CSF white cell count Result |__|__|__|__|.|__|__| x cells/µl
  □ Not done/unknown
- CSF neutrophils Result |__|__| . |__| % □ Not done/unknown
- CSF lymphocytes Result |__|__| . |__| %
  □ Not done/unknown
- CSF eosinophils Result |__|__| . |__| %
  □ Not done/unknown

iv) Biochemistry

- Date tests completed (dd/mm/yyyy): ____/____/_____ □ Not done/unknown
  - CSF Protein Result |__|__| . |__| g/L □ Not done/unknown
  - Glucose Result |__|__| . |__| mmol/L □ Not done/unknown

v. Gram Stain

□ Positive

□ Gram positive □ Gram negative □ Fungi

□ Other

□ Cocci □ Cocci

specify:_____

□ Rods □ Rods

□ Negative

□ Not done/unknown

vi. Culture

□ Positive

□ *Streptococcus suis* □ *Streptococcus pneumonia*

□ *Neisseria meningitides* □ *Staphylococcus aureus*

□ *Escherichia coli* □ *Cryptococcus spp*

□ Other (specify):

_____

□ Negative

□ Not done/unknown

7b). Second lumbar puncture

i. CSF Sample taken date *(dd/mm/yyyy)*: ____/____/_____ □ Unknown

ii. Location of lumbar puncture □ NHTD □ Other site □ Unknown

iii. CSF cell count

- Date tests completed *(dd/mm/yyyy)*: ____/____/_____
  □ Not done/unknown
- CSF white cell count Result |__|__|__|__|.|__|__| x cells/µl
  □ Not done/unknown
- CSF neutrophils Result |__|__| . |__| % □ Not done/unknown
- CSF lymphocytes Result |__|__| . |__| %
  □ Not done/unknown
- CSF eosinophils Result |__|__| . |__| %
  □ Not done/unknown
-

iv) Biochemistry

- Date tests completed (dd/mm/yyyy): ____/____/_____ □ Not done/unknown
  - CSF Protein Result |__|__| . |__| g/L □ Not done/unknown
  - Glucose Result |__|__| . |__| mmol/L □ Not done/unknown

v. Gram Stain

□ Positive

□ Gram positive □ Gram negative □ Fungi

□ Other

□ Cocci □ Cocci

specify:_____

□ Rods □ Rods

□ Negative

□ Not done/unknown

vi. Culture

□ Positive

□ *Streptococcus suis* □ *Streptococcus pneumonia*

□ *Neisseria meningitides* □ *Staphylococcus aureus*

□ *Escherichia coli* □ *Cryptococcus spp*

□ Other (specify):

_____

□ Negative

□ Not done/unknown

7c). Third lumbar puncture

i. CSF Sample taken date *(dd/mm/yyyy)*: ____/____/_____ □ Unknown

ii. Location of lumbar puncture □ NHTD □ Other site □ Unknown

iii. CSF cell count

- Date tests completed *(dd/mm/yyyy)*: ____/____/_____
  □ Not done/unknown
- CSF white cell count Result |__|__|__|__|.|__|__| x cells/µl
  □ Not done/unknown
- CSF neutrophils Result |__|__| . |__| % □ Not done/unknown
- CSF lymphocytes Result |__|__| . |__| %
  □ Not done/unknown
- CSF eosinophils Result |__|__| . |__| %
  □ Not done/unknown
-

iv) Biochemistry

- Date tests completed (dd/mm/yyyy): ____/____/_____ □ Not done/unknown
  - CSF Protein Result |__|__| . |__| g/L □ Not done/unknown
  - Glucose Result |__|__| . |__| mmol/L □ Not done/unknown

v. Gram Stain

□ Positive

□ Gram positive □ Gram negative □ Fungi

□ Other

□ Cocci □ Cocci

specify:_____

□ Rods □ Rods

□ Negative

□ Not done/unknown

vi. Culture

□ Positive

□ *Streptococcus suis* □ *Streptococcus pneumonia*

□ *Neisseria meningitides* □ *Staphylococcus aureus*

□ *Escherichia coli* □ *Cryptococcus spp*

□ Other (specify):

_____

□ Negative

□ Not done/unknown

8. Pathogen testing

Pathogen	**Sample**	**Date of test**	**Location of test**	**Type of test**	**Result**
Herpes Simplex virus 1 *Subsequent test*	□ CSF □Blood □Other_____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □Other (specify) _____ □ Unknown	□ Positive □Negative □ Unknown
Herpes Simplex virus 2 *Initial test*	□ CSF □Blood □Other_____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □Other_____ □ Unknown	□ Positive □Negative □ Unknown
Herpes Simplex virus 2 *Subsequent test*	□ CSF □Blood □Other_____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □Other_____ □ Unknown	□ Positive □Negative □ Unknown
Japanese encephalitis virus	□ CSF □Blood □Other_____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ Serology □Other_____ □ Unknown	□ Positive □Negative □ Unknown
Japanese encephalitis virus	□ CSF □Blood □Other_____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ Serology □Other_____ □ Unknown	□ Positive □Negative □ Unknown

Pathogen	Sample	Date of test	Location of test	Type of test	Result
Dengue virus	□ CSF □Blood □Other_____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ Serology □ NS1 □Other_____ □ Unknown	□ Positive □Negative □ Unknown
Mycobacterium tuberculosis	□ CSF □Blood □ Other(specify): _____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ZN stain □Gene Expert □Culture □Other_____ □ Unknown	□ Positive □Negative □ Unknown
Streptococcus suis	□ CSF □Blood □ Other(specify): _____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □Culture □Other_____ □ Unknown	□ Positive □Negative □ Unknown
Streptococcus pneumoniae	□ CSF □Blood □ Other(specify): _____	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □Culture □Other _____ □ Unknown	□ Positive □Negative □ Unknown

Pathogen	**Sample**	**Date of test**	**Location of test**	**Type of test**	**Result**
Neisseria meningitides	□ CSF □Blood □ Other(specify): _______________	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □Culture □Other _____________ □ Unknown	□ Positive □Negative □ Unknown
Other (specify) ____________	□ CSF □Blood □ Other(specify): _______________	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □ Serology □Culture □Other _____________ □ Unknown	□ Positive □Negative □ Unknown
Other (specify) ___________	□ CSF □Blood □ Other(specify): _______________	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □ Serology □Culture □Other _____________ □ Unknown	□ Positive □Negative □ Unknown
Other (specify) ___________	□ CSF □Blood □ Other(specify): _______________	____/____/_____ □ Unknown	□ NHTD □ Other site □ Unknown	□ PCR □ Serology □Culture □Other _____________ □ Unknown	□ Positive □Negative □ Unknown

9. Blood cultures

9a) First Blood culture

i. Date sample taken *(dd/mm/yyyy)*: ____/____/_____

□ Not done □ Unknown

ii. Location of test □ NHTD □ Other site □ Unknown

iii. Pathogen grown

□ *Streptococcus suis* □ *Streptococcus pneumonia*

□ *Neisseria meningitides* □ *Staphylococcus aureus*

□ *Escherichia coli* □ *Cryptococcus spp*

□ Other (specify):

_____

□ Negative

□ Unknown

CRF completed by:_____

_____

Name

Signature

on *(dd/mm/yyyy)***:** __/__/

10a) Date of discharge or death *(dd/mm/yyyy)* : __/__/_____

□ Unknown

10b) Diagnosis at discharge:

a. ICD-10 Code 1: |__|__|__| . |__|__| ……
b. ICD-10 Code 2: |__|__|__| . |__|__| ……
c. ICD-10 Code 3: |__|__|__| . |__|__| ……
d. ICD-10 Code 4: |__|__|__| . |__|__| ……
e. ICD-10 Code 5: |__|__|__| . |__|__| ……
f. ICD-10 Code 6: |__|__|__| . |__|__| ……

□ Unknown

10c) Outcome at discharge *(please check only one)*:

□ Discharge to home

□ Transferred to another hospital

□ Died in hospital

□ Discharged to die

□ Discharged without permission

□ Unknown

□ Other

FOR CHILDREN AGED 15 YEARS AND YOUNGER ONLY

LIVERPOOL OUTCOME SCORE AT DISCHARGE
(TO BE ANSWERED BY THE PARENT
OR LEGAL GUARDIAN)

1. Date of completion *(dd/mm/yyyy)* : __/__/_____
2. Relationship of person with child (e.g. mother/aunt): _____

Answer each question by ticking the box applicable.

Ask the parent/guardian the following questions:

For some of these questions, you ask the parent or caregiver how the child compares with how
they were immediately before the illness (irrespective of length of time in hospital).

1. Speech or communication

Compared with before the illness, is the child's speech or communication:

☐ The same as before (5)

☐ Changed or reduced (3)

☐ Not speaking or communicating (2)

Score_____

2. Feeding

The child's feeding is:

☐ The same as before illness (5)

☐ Occasionally needs help (3)

☐ Always needs more help (2)
Score_____

3. Leaving Alone

Before the illness, could this child be left alone without coming to harm?

☐ No (5)

If **Yes**, can this child now be left alone?

☐ Yes (5)

☐ Yes briefly in familiar environment (3)

☐ No (2)
Score_____

4. Behaviour

Compared with before the illness do the caregivers think the child's behaviour is altered?

☐ No completely normal (5)

☐ Gets angry easily (4)

☐ Other behavioural problems (4)

☐ Severely abnormal (2)
Score_____

If abnormal give details: _____

5. Recognition

Could the child recognise their family members, other than their main carer, before the illness?

☐ No (5)

If **Yes**, can this child now recognise their family members, other than their main carer?

☐ Yes (5)

☐ Some (3)

☐ None (2) Score
_____

6. School and working

Before the illness, was the child at school or working?

If **Yes**, do the carers think the child will go back to school or work?

☐ Yes (5)

☐ No (3)

If **No,** do the carers think the child will still able to do the same tasks at home, follow the same routine, or play normally?

☐ Yes (5)

☐ Not able to (3)

_____

7. Epilepsy/ Seizures

Did the child have any seizures during this illness?

☐ No (5)

If **Yes**, is the child still having seizures?

☐ No seizures and not on anti-epileptic drugs (5)

☐ No seizures and on anti-epileptic drugs (4)

☐ Yes still having seizures (3)

☐ Yes, seizures most days (2)
Score_____

8. Dressing

Can other children of this age dress themselves?

☐ No (5)

If **Yes**, can the child dress themselves since their illness?

☐ Yes, the same as before illness (5)

☐ Occasionally needs extra help (3)

☐ Needs more help than before (2)
Score_____

9. Bladder and Bowel control

Is urinary and faecal continence:

☐ The same as before the illness (5)

☐ Occasionally needs more help or occasionally is incontinent (4)

☐ Needs more help or is incontinent of bowel or bladder (2)

Score _____

10. Hearing

Does the parent think this child's hearing is:

☐ Normal (5)

☐ Reduced in one or both ears (4)

☐ Cannot hear at all (3) Score_____

Observation of the child's abilities

Answer these questions based on what the caregiver says.

11. Sitting

Could the child sit before the illness?

☐ No (5)

If **Yes**, observe, can this child sit?

☐ Yes independently (5)

☐ Needs help (3)

☐ Not at all (2)
Score_____

12. Standing up

Could the child get from sitting to standing before the illness?

☐ No (5)

If **Yes**, observe, can the child get from sitting to standing?

☐ Yes, independently (5)

☐ Needs help (3)

☐ Not at all (2)

Score_____

13. Walking

Could the child walk before the illness?

☐ No (5)

If **Yes**, observe this child walking 5 metres across room. The child walks:

☐ Normally (5)

☐ Abnormally, but independently +/- crutches/stick (3)

☐ Not able to walk (2)
Score _____

14. Hands on head

Put both your hands on your head, and ask the child to copy you. Child is:

☐ Too young (5)

☐ Normal both hands (5)

☐ Abnormal one or both hands (4)

☐ Unable one or both hands (3)
Score_____

15. Picking Up

Ask child to pick up pea-sized ball of paper or small coin. Child is:

☐ Too young (5)

☐ Normal pincer grasp both hands (5)

☐ Unable one hand (3)

☐ Abnormal one hand or both hands (3)

☐ Unable both hands (2)
Score_____

Any other Comments: _____

The Final Liverpool Outcome Score is the lowest number scored for any question single question

Score interpretation:

5 = Full recovery

4 = Minor sequelae with no effect, or only minor effects, on physical function; or personality change; or on medication.

3 = Moderate sequelae mildly affecting function, probably compatible with independent living

2 = Severe sequelae, impairing function sufficient to make patient dependent

1 = Death

Liverpool Outcome Score completed by:

_____ _____

Name *Signature*

on *(dd/mm/yyyy)*: __/__/_____

FOR CHILDREN AGED 15 YEARS AND YOUNGER ONLY

LIVERPOOL OUTCOME SCORE AT 3 MONTHS AFTER DISCHARGE
(TO BE ANSWERED BY THE PARENT
OR LEGAL GUARDIAN)

Answer each question. Circle or underline the correct answer, and write the score in the column. If the parent/guardian does not wish to answer or does not know please indicate this.

1. Date of completion *(dd/mm/yyyy)* : __/__/_____
2. Relationship of person with child (e.g. mother/aunt): _____

Ask the parent/guardian the following questions:

For some of these questions, you ask the parent or caregiver how the child compares with how
they were immediately before the illness (irrespective of length of time in hospital).

1. Speech or communication

Compared with before the illness, is the child's speech or communication:

□ The same as before (5)

□ Changed or reduced (3)

□ Not speaking or communicating (2)

Score _____

2. Feeding

The child's feeding is:

□ The same as before illness (5)

□ Occasionally needs help (3)

□ Always needs more help (2)
Score_____

3. Leaving Alone

Before the illness, could this child be left alone without coming to harm?

□ No (5)

If **Yes**, can this child now be left alone?

□ Yes (5)

□ Yes briefly in familiar environment (3)

□ No (2)
Score_____

4. Behaviour

Compared with before the illness do the caregivers think the child's behaviour is altered?

□ No completely normal (5)

□ Gets angry easily (4)

□ Other behavioural problems (4)

□ Severely abnormal (2)
Score_____

If abnormal give details: _____

5. Recognition

Could the child recognise their family members, other than their main carer, before the illness?

□ No (5)

If **Yes**, can this child now recognise their family members, other than their main carer?

□ Yes (5)

□ Some (3)

□ None (2)
Score _____

6. School and working

Before the illness, was the child at school or working?

If **Yes**, do the carers think the child will go back to school or work?

□ Yes (5)

□ No (3)

If **No,** do the carers think the child will still able to do the same tasks at home, follow the
same routine, or play normally?

□ Yes (5)

□ Not able to (3)

_____

7. Epilepsy/ Seizures

Did the child have any seizures during this illness?

□ No (5)

If **Yes**, is the child still having seizures?

□ No seizures and not on anti-epileptic drugs (5)

□ No seizures and on anti-epileptic drugs (4)

□ Yes still having seizures (3)

□ Yes, seizures most days (2)
Score_____

8. Dressing

Can other children of this age dress themselves?

□ No (5)

If **Yes**, can the child dress themselves since their illness?

□ Yes, the same as before illness (5)

□ Occasionally needs extra help (3)

□ Needs more help than before (2)
Score_____

9. Bladder and Bowel control

Is urinary and faecal continence:

□ The same as before the illness (5)

□ Occasionally needs more help or occasionally is incontinent (4)

□ Needs more help or is incontinent of bowel or bladder (2)

Score _____

10. Hearing

Does the parent think this child's hearing is:

□ Normal (5)

□ Reduced in one or both ears (4)

□ Cannot hear at all (3)

Score_____

Observation of the child's abilities

Answer these questions based on what the caregiver says.

11. Sitting

Could the child sit before the illness?

□ No (5)

If **Yes**, observe, can this child sit?

□ Yes independently (5)

□ Needs help (3)

□ Not at all (2)

Score_____

12. Standing up

Could the child get from sitting to standing before the illness?

□ No (5)

If **Yes**, observe, can the child get from sitting to standing?

□ Yes, independently (5)

□ Needs help (3)

□ Not at all (2)

Score_____

13. Walking

Could the child walk before the illness?

□ No (5)

If **Yes**, observe this child walking 5 metres across room. The child walks:

□ Normally (5)

□ Abnormally, but independently +/- crutches/stick (3)

□ Not able to walk (2)
Score _____

14. Hands on head

Put both your hands on your head, and ask the child to copy you. Child is:

□ Too young (5)

□ Normal both hands (5)

□ Abnormal one or both hands (4)

□ Unable one or both hands (3)
Score_____

15. Picking Up

Ask child to pick up pea-sized ball of paper or small coin. Child is:

□ Too young (5)

□ Normal pincer grasp both hands (5)

□ Unable one hand (3)

□ Abnormal one hand or both hands (3)

□ Unable both hands (2)
Score_____

Any other Comments: _____

The Final Liverpool Outcome Score is the lowest number scored for any question single question

Score interpretation:

5 = Full recovery

4 = Minor sequelae with no effect, or only minor effects, on physical function; or personality change; or on medication.

3 = Moderate sequelae mildly affecting function, probably compatible with independent living

2 = Severe sequelae, impairing function sufficient to make patient dependent

1 = Death

Liverpool Outcome Score completed by:

_____

_____

Name *Signature*

on *(dd/mm/yyyy)*: __/__/_____

FOR ADULTS AGED 16 YEARS AND OVER ONLY

EXTENDED GLASGOW OUTCOME SCALE AT DISCHARGE
(TO BE ANSWERED BY THE PARENT
OR LEGAL GUARDIAN IF THE PATIENT LACKS CAPACITY)

1	**Death**	**D**
2	**Vegetative State**	**VS**
3	**Lower severe disability**	**SD-**
4	**Upper severe disability**	**SD+**
5	**Lower moderate disability**	**MD-**
6	**Upper moderate disability**	**MD+**
7	**Lower good recovery**	**GR-**
8	**Upper good recovery**	**GR+**

Respondent details

□ 0 = patient alone

□ 1 = relative

□ 2 = patient plus relative

Date of completion *(dd/mm/yyyy)* : __/__/_____

If relative

Relationship of person with participant (e.g. spouse/brother):

_____

Consciousness

1a) Is the head-injured person able to obey simple commands or say any words?

□ Yes

□ No (VS)

□ Don't know

□ Prefer not to answer

Note: anyone who shows the ability to obey even simple commands or utter any word or communicate specifically in any other way is no longer considered to be in a vegetative state. Eye movements are not reliable evidence of meaningful responsiveness.

Independence at home

2a) Is the assistance of another person at home essential everyday for some activities of daily living?

□ Yes

□ No (VS) **If no: go to 3**

□ Don't know

□ Prefer not to answer

Note: for a NO answer they should be able to look after themselves at home for 24 hours if necessary, though they need not actually look after themselves. Independence includes the ability to plan for and carry out the following activities: getting washed, putting on clean clothes without prompting, preparing food for themselves, dealing with callers and handling minor domestic crises. The person should be able to carry out activities without needing prompting or reminding and should be capable of being left alone overnight.

2b) Do they need frequent help of someone to be around at home most of the time?

□ Yes (lower SD)

□ No (upper SD)

□ Don't know

□ Prefer not to answer

Note for a NO answer they should be able to look after themselves at home up to eight hours during the day if necessary, though they need not actually look after themselves.

2c) Was the patient independent at home before the AES?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Independence outside home

3a) Are they able to shop without assistance?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Note, this includes being able to plan what to buy, take care of money themselves and behave appropriately in public. They need not normally shop, but must be able to do so.

3b) Were they able to shop without assistance before the AES?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

4a) Are they able to travel locally without assistance?

□ Yes

□ No (upper SD)

□ Don't know

□ Prefer not to answer

Note: they may drive or use public transport to get around. Ability to use a taxi is sufficient, provided the person can phone for it themselves, and instruct the driver.

4b) Were they able to travel locally without assistance before the injury?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Work

5a) Are they currently able to work (or look after others at home) to their previous capacity?

□ Yes **if yes, go to 6**

□ No

□ Don't know

□ Prefer not to answer

5b) How restricted are they?

□ Restricted work capacity? (Upper MD)

□ Able to work only in a sheltered workshop or non-competitive job or currently unable to work?

(Lower MD)

□ Don't know

□ Prefer not to answer

5c) Does the level of restriction represent a change in respect to the pre-trauma situation?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Social and leisure activities

6a) Are they able to resume regular social and leisure activities outside home?

□ Yes **if yes, go to 7**

□ No

□ Don't know

□ Prefer not to answer

Note: they need not have resumed all their previous leisure activities, but should not be prevented by physical or mental impairment. If they have stopped the majority of activities because of loss of interest or motivation, then this is also considered a disability.

6b) What is the extent of restriction on their social and leisure activities?

□ Participates a bit less: at least half as often as before injury (lower GR)

□ Participates much less: less than half as often (upper MD)

□ Unable to participate: rarely, if ever, take part (lower MD)

□ Don't know

□ Prefer not to answer

6c) Does the extent of restriction in regular social and leisure activities outside home represent a change compared to before the AES

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Family and friendships

7a) Has there been family or friendship disruption due to psychological problems?

□ Yes

□ No **if no, go to 8**

□ Don't know

□ Prefer not to answer

Note: typical post-traumatic personality changes are: quick temper, irritability, anxiety, insensitivity to others, mood swings, depression and unreasonable or childish behaviour.

7b) What has been the extent of the disruption or strain?

□ Occasional – less than weekly (lower GR)

□ Frequent – once a week or more, but not tolerable (upper MD)

□ Constant – daily and intolerable (lower MD)

□ Don't know

□ Prefer not to answer

7c) Does the level of disruption or strain represent a change in compared to before the AES?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Note: if there were some problems before injury, but these have become markedly worse since the injury then answer yes to the question

Return to normal life

8a) Are there any other current problems relating to the AES which affect daily life?

□ Yes (lower GR)

□ No (upper GR)

□ Don't know

□ Prefer not to answer

Note: for example, memory and concentration problems

8b) If similar problems were present before the AES, have these become markedly worse?

□ Yes (lower GR)

□ No (upper GR)

□ Don’t know

□ Prefer not to answer

9) What is the most important factor in outcome?

□ Effects of head injury

□ Effects of illness or injury to another part of the body

□ A mixture of these

□ Don’t know

□ Prefer not to answer

The overall rating for the Glasgow Outcome Scale is based on the lowest outcome category indicated. Areas in which there has been no change with respect to the pre-AES situation are ignored when the overall rating is made.

Glasgow Outcome Scale completed by:_____

_____

Name

Signature

on *(dd/mm/yyyy)***:** __/__/_____

FOR ADULTS AGED 16 YEARS AND OVER ONLY

EXTENDED GLASGOW OUTCOME SCALE 3 MONTHS AFTER DISCHARGE
(TO BE ANSWERED BY THE PARENT
OR LEGAL GUARDIAN IF THE PATIENT LACKS CAPACITY)

1	**Death**	**D**
2	**Vegetative State**	**VS**
3	**Lower severe disability**	**SD-**
4	**Upper severe disability**	**SD+**
5	**Lower moderate disability**	**MD-**
6	**Upper moderate disability**	**MD+**
7	**Lower good recovery**	**GR-**
8	**Upper good recovery**	**GR+**

Respondent details

□ 0 = patient alone

□ 1 = relative

□ 2 = patient plus relative

If relative

Relationship of person with participant (e.g. spouse/brother):

_____

Date of completion *(dd/mm/yyyy)* : __/__/_____

Consciousness

1a) Is the head-injured person able to obey simple commands or say any words?

□ Yes

□ No (VS)

□ Don't know

□ Prefer not to answer

Note: anyone who shows the ability to obey even simple commands or utter any word or communicate specifically in any other way is no longer considered to be in a vegetative state. Eye movements are not reliable evidence of meaningful responsiveness.

Independence at home

2a) Is the assistance of another person at home essential everyday for some activities of daily living?

□ Yes

□ No (VS) **If no: go to 3**

□ Don't know

□ Prefer not to answer

Note: for a NO answer they should be able to look after themselves at home for 24 hours if necessary, though they need not actually look after themselves. Independence includes the ability to plan for and carry out the following activities: getting washed, putting on clean clothes without prompting, preparing food for themselves, dealing with callers and handling minor domestic crises. The person should be able to carry out activities without needing prompting or reminding and should be capable of being left alone overnight.

2b) Do they need frequent help of someone to be around at home most of the time?

□ Yes (lower SD)

□ No (upper SD)

□ Don't know

□ Prefer not to answer

Note for a NO answer they should be able to look after themselves at home up to eight hours during the day if necessary, though they need not actually look after themselves.

2c) Was the patient independent at home before the AES?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Independence outside home

3a) Are they able to shop without assistance?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Note, this includes being able to plan what to buy, take care of money themselves and behave appropriately in public. They need not normally shop, but must be able to do so.

3b) Were they able to shop without assistance before the AES?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

4a) Are they able to travel locally without assistance?

□ Yes

□ No (upper SD)

□ Don't know

□ Prefer not to answer

Note: they may drive or use public transport to get around. Ability to use a taxi is sufficient, provided the person can phone for it themselves, and instruct the driver.

4b) Were they able to travel locally without assistance before the injury?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Work

5a) Are they currently able to work (or look after others at home) to their previous capacity?

□ Yes **if yes, go to 6**

□ No

□ Don't know

□ Prefer not to answer

5b) How restricted are they?

□ Restricted work capacity? (Upper MD)

□ Able to work only in a sheltered workshop or non-competitive job or currently unable to work?

(Lower MD)

□ Don't know

□ Prefer not to answer

5c) Does the level of restriction represent a change in respect to the pre-trauma situation?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Social and leisure activities

6a) Are they able to resume regular social and leisure activities outside home?

□ Yes **if yes, go to 7**

□ No

□ Don't know

□ Prefer not to answer

Note: they need not have resumed all their previous leisure activities, but should not be prevented by physical or mental impairment. If they have stopped the majority of activities because of loss of interest or motivation, then this is also considered a disability.

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□ Unable to participate: rarely, if ever, take part (lower MD)

□ Don't know

□ Prefer not to answer

6c) Does the extent of restriction in regular social and leisure activities outside home represent a change compared to before the AES

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Family and friendships

7a) Has there been family or friendship disruption due to psychological problems?

□ Yes

□ No **if no, go to 8**

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□ Prefer not to answer

Note: typical post-traumatic personality changes are: quick temper, irritability, anxiety, insensitivity to others, mood swings, depression and unreasonable or childish behaviour.

7b) What has been the extent of the disruption or strain?

□ Occasional – less than weekly (lower GR)

□ Frequent – once a week or more, but not tolerable (upper MD)

□ Constant – daily and intolerable (lower MD)

□ Don't know

□ Prefer not to answer

7c) Does the level of disruption or strain represent a change in compared to before the AES?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

Note: if there were some problems before injury, but these have become markedly worse since the injury then answer yes to the question

Return to normal life

8a) Are there any other current problems relating to the AES which affect daily life?

□ Yes (lower GR)

□ No (upper GR)

□ Don't know

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Note: for example, memory and concentration problems

8b) If similar problems were present before the AES, have these become markedly worse?

□ Yes (lower GR)

□ No (upper GR)

□ Don't know

□ Prefer not to answer

9) What is the most important factor in outcome?

□ Effects of head injury

□ Effects of illness or injury to another part of the body

□ A mixture of these

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□ Prefer not to answer

The overall rating for the Glasgow Outcome Scale is based on the lowest outcome category indicated. Areas in which there has been no change with respect to the pre-AES situation are ignored when the overall rating is made.

Glasgow Outcome Scale completed by:_____

_____

Name

Signature

on *(dd/mm/yyyy)*: __/__/_____

13HN OxTREC 42-15 FGD V4.0 02SEP16

FOCUS GROUP DISCUSSION

1. In the area where you live are there places where you think you would be bitten by mosquitoes? What time of day?

2. What are the ways in which you try to prevent mosquito bites when you are inside your house? What else could you do, even though you currently

don't? Why don't you do these things?

If there is little discussion being generated, prompts including pictures of mosquito nets and insect repellent will be shown.

3. Why do you try to avoid mosquito bites?

4. Do you know of any diseases caused by mosquito bites? Tell me about these diseases and how do you get these? (If not discussed, then ask 'Tell me what you know about Japanese Encephalitis and Dengue Fever")

5. Would you spend time in rice fields e.g. what time of year?

6. What animals are there in your community?

a. Do you have any type of contact with each of these animals? If yes, which animals do you have contact with and what is the type of contact?

- e.g.do they live in the house, do you look after any animals

7. Which animals do you eat? What do other people in your community eat? How often (use counters)? Which of these are eaten raw?

8. How would you feel if we came to your house to perform a questionnaire?

9. How would you feel if we took photographs around your house?

These participatory rural appraisals (PRA) are known techniques used to facilitate community discussion and development (1, 2).

1. Robinson L. Participatory Rural Appraisal: A Brief Introduction. Group Facilitation: A Research and Applications Journal. 2002; 4: 45-51.

2. Kumar, S. (2002). *Methods for community participation : a complete guide for practitioners / Somesh Kumar*. London : ITDG, 2002.

13HN ICF Relatives V4.2 04AUG16

13HN: UNDERSTANDING RISK FACTORS FOR ENCEPHALITIS (BRAIN INFLAMMATION)

Information Sheet For Focus Group Discussion (Hospital Relatives)

One Copy For Study Records And One Copy To Be Given To The Participant

You are being invited to participate in a study looking at risk factors for acute encephalitis syndrome, an inflammation of the brain. Approximately 2000 people will be part of this study. Please read this information sheet carefully or have someone read it to you. Please ask the study staff to explain any information that you are not sure about. It is your choice whether to participate in the study and please do not feel obliged do so. If you would like to take part you will be asked to sign or place a thumbprint on the last page. You will be given a copy of this form for your personal records.

Why is this study being done?

Acute Encephalitis Syndrome (AES) is a syndrome of brain inflammation or swelling. Patients may die or be left disabled. There are around two thousand cases of AES in Viet Nam every year. Although Japanese encephalitis, a virus spread by mosquitoes is a common cause of AES in children and Streptococcus suis, a bacteria associated with eating or preparing raw pork meat is a common cause in adults, for half of the

patients or more, we do not know the cause of their AES. However, we do know that most of the cases occur during the warmer seasons and therefore we think that mosquitoes or other insects are spreading diseases, which cause AES in northern Viet Nam.

This study therefore aims to look at the reasons why people might or might not get AES including where the person lives and their risks in and around their home and work environment and their contact with animals.

Who is doing this study?

This study is being performed by a team from:

The Oxford University Clinical Research Unit in Ha Noi

The University of Liverpool, United Kingdom

The Wellcome Trust Liverpool Glasgow Centre for Global Health Research, United Kingdom

The National Hospital of Tropical Diseases, Ha Noi

What will happen to me in this study and what are the risks?

You have been chosen to take part because we want to get information from members of the community about your daily activities, contact with animals and ways in which you prevent mosquito bites. This will help us to design a questionnaire for a larger study which will help to understand risks associated with acute encephalitis

syndrome. You will then be asked to take part in a group discussion with five other members of the same gender in your community. This will take around one hour.

What will happen to the samples / information taken?

The results of the study will be used to design a questionnaire for a larger study. The results will also be written as a report or thesis for the University of Liverpool and will be published in a journal, which will be made available to the public. The results will also be available in Vietnamese through the OUCRU website and the Hospital for Tropical Diseases website. The results will also be available for the Ministry of Health in Viet Nam. You will not be able to be identified in these results with the exception of pictures of your house and the dot on a map to show the location of your house.

The results will be also be made available to the public through workshops about AES in the community or schools and possibly via the radio or other forms of media.

What is the benefit of participating?

There is no intended personal benefit to taking part however, the benefit will be to the community and people of Viet Nam by help us to understand the risks associated with AES.

You will be reimbursed 100,000 VND for your time spent and you will be offered light refreshments.

What happens if I decline participation or change my mind later on?

You have the right to decline participation or withdraw from the study or any part of the study at any time. Please let us know if you wish to do so. You do not need to give an explanation for this. You can request for your results to be destroyed at any time. There will be no effect to your management.

Can someone else decide to take the patient out of the study?

The research team doctors can withdraw you from the study, if there is a problem with the information collected from you. The Universities and ethics committees can also stop the study.

Confidentiality

All conversations will be recorded using a microphone device and later translated by the research assistant and kept on a secure database. Information about you will be kept confidential and only shared amongst study team members. You will be given an anonymous code, which will be used for the answers. Details linking yourself to this code will be kept in a separate place. All consent forms and recordings will be kept in a locked room. Only those who are involved with the study will have access to these documents which will be kept for 3 years and then put on an electronic system.

The consent forms will be kept in a locked cupboard.

Whom do I contact if I have questions or complaints?

If you have questions about the study you may contact: *To be decided*

If you have questions about being in a research study, please contact the Research Ethics Board of the NHTD at (phone number) the Clinical Trials Unit at (phone number)

COMPULSORY FOR STUDY RECRUITMENT	
1. I have read this information sheet or it has been read to me and I understand it.	
2. I have been able to ask questions about the study.	
3. I understand that my participation of behalf of my in the study is voluntary and I do not have to answer questions if I do not wish to do so.	
4. I understand that my information will be recorded using a microphone device.	
5. I understand that I can ask for access to the information I provide or have this information destroyed if I wish.	
6. I understand that I will be given an anonymous code instead of my name.	
7. I agree for the data collected from me to be used in future research publications and presentations without my name.	
8. I agree to take part in the above study.	

- I freely give my permission to join this study.
- I understand that I have the right to withdraw from the study. I will be given a copy of this signed consent form to keep for my reference.
-

Participant ID: 13HN-[__|__]-[__|__|__]

Name of participant (Please Print): _____

By signing/marking my name here, I confirm what is written above.

Signature of Participant:	*Participant Full Name:*	*Date of Signature:*
*x*_____	*x*_____	_____/____/_____

If the person giving consent cannot read the form themselves, a witness must be present and sign here:

I was present throughout the entire informed consent process with the participant. This form was read accurately to the volunteer, all questions from the volunteer were answered and the volunteer has agreed to take part in the research.

Witness Signature	*Witness Full Name*	*Date of Signature*
*x*_____	*x*_____	___/____/_____

Confirmation of study staff:

By signing/marking my name here, I confirm that I have fully explained the relevant information of this study to the person named above and will provide her/him with a copy of this signed and dated informed consent form.

Signature of study staff	Full Name:	Date of Signature:
x_____	x_____	____/____/_____

APPENDIX 3.8

OxTREC 42-15 Questionnaire for relatives V2.1 10JUN16

Risk factors for and spatio-temporal distribution of cases of acute encephalitis syndrome of suspected infectious aetiology in the Red River Delta region, northern Viet Nam: a feasibility study

EPIDEMIOLOGY QUESTIONNAIRE FOR ADULTS (questions can be altered to be answered by parents or relatives)

Please note, all questions will be given the option don't know and prefer not to answer where it is felt applicable.

For all string variables where the participant does not know or prefers not to answer please type this

Participant Code
Date of interview
Start time of interview
Record the province of the participant's house
Record the district of the participant's house
Record the commune of the participant's house

What is your date of birth? Use 77 for don't know and 99 for prefer not to answer
What is your gender? Male Female Transgender
What is your religion? (select all that apply) Buddhist Christian Hindu Muslim Other Prefer not to answer
Please state your other religion
Which ethnic group do you consider yourself to belong to? (select all that apply) Kinh Tay Thai Muong

Hmong Cambodian Khmer Chinese Other Don’t know Prefer not to answer
What is your occupation? If does not work (e.g. retired, student, housewife) document this
Describe where you work (e.g. in the office, in the rice field, in the home)
Describe your typical daily activities at work (e.g. working on the computer, ploughing field, cleaning house/washing)
What is the highest level of education you completed? None Primary Middle Secondary Tertiary Information education

How long have you lived at this address (years)?
Do you have more than one address currently? Yes No
Where else do you live currently?
Do you have any previous addresses? Yes No
Please give the details of previous addresses Details for where the participant lived, when and for how long
Do you or your family own or rent your house? Own Rent Other
Specify the other type of ownership of your house?

How many adults slept in your house last night?
How many children slept in your house last night?
How many people share your bedroom (on average)?
What is your water source? (select all that apply and specify what the water is used for e.g. drinking, bathing, cooking**)** Tap/piped water at home Hand pump Electric pump Public standpipe Bottled/Jar Private protected (covered) well Public protected well Private unprotected well Public unprotected well River/stream/pond/lake Rain water/natural water Tanker water Other (specify) Don't know

Describe the other water source you use for drinking
How often do you use each of the water sources (part of the year, all year, don't know, (select all that apply) Tap/piped water at home Hand pump Public standpipe Bottled/Jar Private protected (covered) well Public protected well Private unprotected well Public unprotected well River/stream/pond/lake Rain water/natural water Tanker water Other (specify)
Do you use any water treatment prior to drinking? Yes No Don't know Prefer not to answer

If yes, what type of water treatment do you use? (select all that apply) Boiling Candle ceramic filters Strained through cloth Water treatment powder Alum SafeWat (liquid hypochlorite solution) Other Don't know
Describe the other type of water treatment you use
What type of fuel does your household use for cooking or heating the house? (select all that apply) LP gas Biogas Agricultural crop residue Kerosene Animal dung Electricity Coal or charcoal Wood Straw/shrubs/grass No food cooked in household

Other
Specify the other type of fuel your household uses for cooking or heating the house
How often do you use each of the types of fuel for cooking or heating the house? (Part of the year; All year; Don't know;) Wood LP gas Biogas Agricultural crop residue Kerosene Animal dung Electricity Coal or charcoal Wood Straw/shrubs/grass Other
What is your household income per month? < 5,000,000 VND 5,000,000 -10,000,000 VND 10,000,000 -20,000,000 VND >20,000,000 VND

Don’t know Prefer not to answer
What are your household sources of income?
Do you or your household own any of the following? (Select all that apply and ask about each individual item**)?** Radio Television Mobile telephone Computer Internet access Bicycle (state number) Motorcycle (state number) Car (state number) Refrigerator Air-conditioning
What are your means of transport? (select all that apply**)** Bicycle Animal-drawn cart Motorcycle/scooter

Car Public transport e.g. bus Taxi Walking Other
Specify the other form(s) of transport you use
Do you use any of the following methods for prevention of mosquito bites? (select all that apply) Simple bednet Insecticide treated bednet Mosquito coils or liquid Mosquito repellent on body or clothes Other
Specify the other type of mosquito prevention you use
How many simple mosquito bed nets are in your house? Use 77 for don't know
How many insecticide treated mosquito bed nets are in your house? Use 77 for don't know

How often do you sleep under a mosquito bed net from June-November? (Include any type of bed net) Never Less frequently that once per week (e.g. one per month) Once per week 2-3 times per week 4-6 times per week Every night
How often do you sleep under a mosquito bed net from December-May? (Include any type of bed net) Never Less frequently that once per week (e.g. one per month) Once per week 2-3 times per week 4-6 times per week Everynight
Did you sleep under a bed net last night? Yes No

Cases: Were you sleeping under a bed net in the month prior to becoming unwell? **Controls: Were you sleeping under a bed net in the last two months?** Yes always Yes sometimes Never
Cases: Were you sleeping under a bed net treated with insecticide in the month prior to becoming unwell? **Controls: Were you sleeping under a bed net treated with insecticide in the last two months?** Yes always Yes sometimes Never Don't know
Cases: Have you been sleeping inside or outside in the month prior to becoming unwell? **Controls: Have you been sleeping inside or outside in the last two months?** (Not include whilst in hospital) Inside Outside Sometimes inside, sometimes, outside

Cases: How often were you using mosquito repellent on your body or clothes in the month before you became unwell? **Controls: How often were you using mosquito repellent on your body or clothes in the last two months?** Never Every day 2-3 times per week Once per week Less than once per week
Are there mosquito screens on the doors or windows of your house? No Yes on all Yes on some Not applicable (e.g. no doors or window in house)
Has your house ever been sprayed with insecticide (Indoor Residual Spraying)? Yes (when) No Don't know
How long ago was your house sprayed with insecticide (e.g. days, months)?

Was the house painted after spraying with insecticide? Yes No Don’t know
How long ago was the house painted after spraying with insecticide (e.g. days, months)?
Are there any pigs living in your house or garden? Yes (number) No
What are the ages of these pigs (select all that apply) **Under 6 months of age** **Over 6 months of age** **Don’t know** **Prefer not to answer**
Where are the pigs kept?

Cases: Did you have any contact with or were close to any pigs in the month prior to becoming unwell? **Controls: Did you have any contact with or were close to any pigs in the last two months?** Yes No Prefer not to answer **If yes, describe this contact (e.g. touch, cleaned, stood next to)**
What were the ages of these pigs (select all that apply) **Under 6 months of age** **6 months of age and over** **Don't know** **Prefer not to answer**
Do you have any ducks in your garden or outside your house? Yes No
Cases: Did you have any contact with ducks in the month prior to becoming unwell? **Controls: Did you have any contact with ducks in the last two months?** Yes No

Prefer not to answer **If yes, describe the contact**
Cases: Did you have any contact with dogs in the month prior to becoming unwell? **Controls: Did you have any contact with dogs in the last two months?** Yes No Prefer not to answer **If yes, describe the contact (e.g. touched, fed, ate)**
Cases: Did you have any contact with cats in the month prior to becoming unwell? **Controls: Did you have any contact with cats in the last two months?** Yes No Prefer not to answer If yes, describe the contact (e.g. touched, fed, ate)

Cases: Have you seen any rats around your house or place of work in the month prior to becoming unwell? **Controls: Have you seen any rats around your house or place of work in the last two months?** Yes No
Cases: Have you had any contact with rats in month prior to becoming unwell? **Controls: Have you had any contact with rats in the last two months?** **Yes** **No** **If yes, describe the contact (e.g. touched, fed, ate)**
Cases: Have you seen any bats around your house in the month prior to becoming unwell? **Controls: Have you seen any bats around your house in the last two months?** Yes No

Cases: Have you had any contact with bats in the month prior to becoming unwell? **Controls: Have you had any contact with bats in the last two months?** **Yes** **No** **If yes, describe the contact (e.g. touched, fed, ate)**
How many buffalo are kept in the house where you are living?
How many cows are kept in the house where you are living?
How many sheep are kept in the house where you are living?
How many goats are kept in the house where you are living?
How many chickens are kept in the house where you are living?

Cases: Have you had any close contact with a sick animal in the month before becoming unwell? **Controls: Have you had any close contact with a sick animal in the last two months?** Yes No Don't know Prefer not to answer
If yes, describe your contact with the sick animal (when (time not date), which animal, what contact did you have)?
Cases: Have you been bitten by an animal (not including insects) within the month before becoming unwell? **Controls: Have you been bitten by an animal (not including insects) within the last two months)?** Yes No Prefer not to answer
Describe when (time e.g. days not date) the bite was and what was the animal

Cases: Did you drink any raw milk in the month before becoming unwell? **Controls: Did you drink any raw milk in the last two months?** Yes No Don't know Prefer not to answer
Cases: Did you milk any animals in the month before becoming unwell? **Controls: Did you drink any raw milk in the last two months?** Yes No
Cases: Have you taken part in the slaughter of an animal in the month before becoming unwell? **Controls: Have you taken part in the slaughter on an animal in the last two months?** Yes No Prefer not to answer

Cases: Did you eat any raw meat, viscera or blood in the month before becoming unwell? **Controls: Did you eat any raw meat, viscera or blood in the last two months?** **Yes** No Prefer not to answer If yes, specify the animals
Cases: Did you prepare or touch any raw meat, viscera or blood in the month before becoming unwell? **Controls: Did you prepare or touch any raw meat, viscera or blood in the last two months?** Yes No Prefer not to answer If yes, specify the animals
Do you work in any rice fields? Yes No Prefer not to answer

When do you work in the rice fields? Spring (January-March) Summer (March-June) Autumn (July-September) Winter (October-December)
Cases: How many times on average per week did you work in the rice fields in the month before you became unwell? **Controls: How many times on average per week did you work in the rice fields during the last two months?** Never Everyday 5-6 days 3-4 days 1-2 days Less than once per week
Cases: Did you walk through any rice fields in the evenings in the month before you became unwell? **Controls: Did you walk through any rice fields in the evenings in the last two months?** Yes No

If yes, how often did you walk through the rice fields on average per week? Everyday On 5-6 days On 3-4 days On 1-2 days Less than once per week Never
Did you visit any forests or woods in the month before you became unwell? Yes No   **If yes, how often did you walk through the forests or woods on average per week?** Everyday On 5-6 days On 3-4 days On 1-2 days Less than once per week Never

Cases: Did you walk through or sit in any grassland in the month before you became unwell? **Controls: Did you walk through or sit in any grassland in the last two months?** Yes No Don’t know
If yes, how often did you walk through or sit any grassland on average per week? Everyday On 5-6 days On 3-4 days On 1-2 days Less than once per week Never Don’t know
Cases: Was there any flooding in your area in the month before you became unwell? **Controls: Was there any flooding in your area in the last two months?** Yes No Don’t know

Cases: Did you swim, wash, walk through or carry out any other activity in any river, stream or lake water in the month before you became unwell? **Controls: Did you swim, wash, walk through or carry out any other activity in any river, stream or lake water in the last two months?** Yes No Prefer not to answer **If yes, describe the activities you carried out, the type of water and how frequently this was in more detail**
How often do you shower or bathe? Daily A few times per week Weekly Less than weekly Prefer not to answer
When (time e.g. in days not date) was the last time you had a shower or bath?

Do you change your clothes after work? Yes always Yes sometimes No Prefer not to answer
When (time e.g. in days not date) did you last wash the clothes you are wearing?
Cases: Did you harvest any fruit in the month before you became unwell? **Controls: Did you harvest any fruit in the last two months?** Yes No Prefer not to answer **If yes, did you harvest any lychees in the month prior to becoming unwell (cases)/previous two months (controls)?** Yes No Prefer not to answer

Cases: Did you ever sit on the floor of your house without a rug in the month before you became unwell? **Controls: Did you ever sit on the floor of your house without a rug in the last two months?** Yes – always Yes - sometimes No Prefer not to answer
Cases; Did you travel outside of your province in the month before you became unwell? **Controls: Did you travel outside of your province in the last two months?** **Yes** **No** **Prefer not to answer** **If yes, which provinces did you travel to and when?**
Cases: Did you travel outside of Viet Nam in the month prior to becoming unwell? **Controls: Did you travel outside of Viet Nam in the last two months?** Yes No Prefer not to answer If yes, where and when did you travel?

Which vaccinations have you had?

Diptheria

Pertussis

Tetanus

Polio

Rubella

Measles

Mumps

Haemophilus influenza

Meningococcal

Japanese encephalitis

Hepatitis A

Hepatitis B

BCG (tuberculosis)

Rotavirus

Typhoid

Other (specify)

Don’t know

Prefer not to answer

If received JE vaccine, how many doses did you have? One Two Three Don’t remember
Do you have your vaccination card against Japanese encephalitis? Yes No
Research assistant to verify JE vaccination card present Yes No Unable to answer (participant’s decision)
End time of interview

VERIFICATION TO BE COMPLETED BY RESEARCH ASSISTANT

What is the device ID?
Please document the date of completion of the form

Document the start time
Please capture the location of the participant's house using the Samsung phone
Record the coordinates of the house using the Garmin GPS
Is the house an individual house, apartment or temporary house Individual house Apartment Temporary house
What is the roof of the house made of? Tile or slate Thatch or straw Cement or concrete Iron Wood and mud Other

Not applicable as no roof present Unable to answer (participant's choice)
Specify the type of roof present
What are the walls of the house made of? Cement Tin Straw or straw and mud Bricks or stones with mud Bricks with cement Wood Bamboo Other (specify) Not applicable (no walls present) Unable to answer (participant's decision)
Specify the type of walls present

What is the floor of the house made of?

Cement/concrete

Earth/sand

Wood/planks

Parquet or polished wood

Cane/bamboo/palm

Vinyl or asphalt strips

Ceramic tiles

Carpet

Other

Unable to answer (participant's decision)

Specify the type of ground present

Where is the place for cooking?

In the house

In a separate building

Outdoors

Other No food cooked in household Unable to answer (participant's decision)
Specify the location where cooking takes place
What is the drinking water source? (select all that apply**)** Tap/piped water at home Hand pump Public standpipe Bottled/Jar Protected (covered) well – specify if drilled or dug/public or private River/stream/pond/lake Rain water/natural water Unprotected (uncovered) well – specify if drilled or dug/public or private Tanker water Other (specify)

Take a photo of the drinking water sources
What type of toilet is present (circle all that apply)? No toilet (use bush or field) Bucket toilet Public/shared pit toilet (state if traditional or ventilation improved pit toilet) Own pit toilet (state if traditional or ventilation improved pit toilet) Public/shared flush toilet Own flush toilet Private pit latrine without flush (bucket used) Private pit latrine with flush Private western style toilet Other Unable to answer (participant's decision)

Is there a bed net over the participant's bed? Yes No Unable to answer (participant's decision)
Are there any abnormal holes in the participant's bed net? Yes No Unable to answer (participant's decision)
Take a photo of the bedne Unable to answer (participant's decision)
Are there door or window screens present? Yes on all doors and windows Yes on some doors and windows No Unable to answer (participant's decision)

Where is the bed of the participant located?
Are there any pigs living in or next to the house Yes No Unable to answer (participant's decision)
Record the GPS coordinates for the nearest pig pen/house Unable to answer (participant's decision)
Is there any evidence of the following animals next to the house and their number? Ducks Wild birds Cats

Dogs Buffalo Milk cows Horses Sheep Goats Chickens Unable to answer (participant's decision)
Specify the other animals present and their number
Is there a kitchen garden or garden next to the house? Yes No Unable to answer (participant's decision)
Are there any water storage containers outside the house? Including tyres and trenches Yes

No Unable to answer (participant's decision)
Describe the water containers and their number
Are all of the water storage containers covered? Yes No Unable to answer (participant's decision)
Take a photo of the water storage containers
Are there any bat droppings within a 10m radius of the house? Yes No Unable to answer (participant's decision)
Take a photo of the bat droppings

Is there any evidence of rodent activity within a 10m radius of the house?

(all that apply)

Droppings

Burrows

Chewed Holes

Unable to answer (participant's decision)

Take a photo of the rodent activity

Are there any weeds in the house or yards?

Yes, house

Yes yard

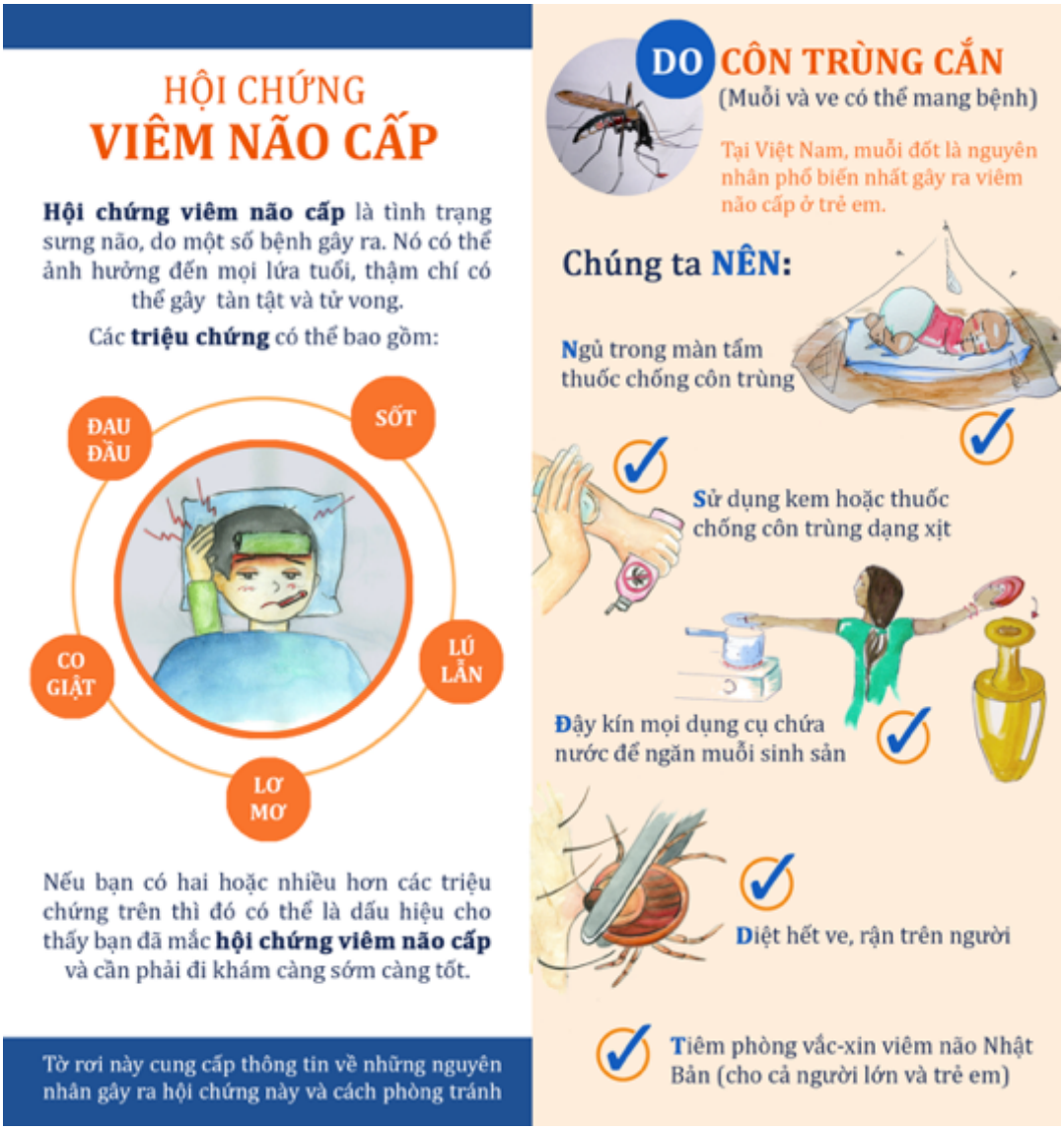
Neither

Unable to answer (participant's decision)

Take a photo of the weeds
Document the end time

13HN leaflet EN V1.0 10JUN16

Drawings by artists



ĐỘNG VẬT

Rửa tay bằng xà phòng sau khi chạm vào thịt sống

Nấu chín thịt trước khi ăn

Không ăn thịt tái hoặc tiết canh vì có khả năng nhiễm liên cầu khuẩn lợn Streptococcus suis – nguyên nhân chính gây viêm màng não cấp ở người lớn tại Việt Nam

Không ăn thịt của động vật ốm hoặc chết

ĐỘNG VẬT

Nếu có động vật chết bệnh trong nhà - hãy thiêu hoặc chôn với vôi bột

Không ném xuống mương nước, ao, hồ để tránh lây bệnh cho người và con vật khác

Nếu bị chó hoặc mèo cắn, rửa sạch vết cắn, đi khám và tiêm vắc-xin phòng bệnh dại ngay lập tức

Nếu bạn làm việc liên quan đến chó, bạn và gia đình cần tiêm phòng dại để bảo vệ sức khỏe bản thân.

Nếu không được tiêm phòng, bệnh nhân bệnh dại chắc chắn **tử vong** 100%.

Không nên chạm hoặc ăn thịt dơi vì dơi mang theo rất nhiều loại vi-rút

Tránh ăn hoa quả trên cây có dơi ăn hoặc có phân dơi xung quanh.

Nguồn tham khảo:
Hiệp hội Nghiên cứu Bệnh Viêm não Anh Quốc
Trung tâm Kiểm soát Dịch bệnh Hoa Kì

Hình ảnh minh họa bởi: ***Sian Aggett*** và ***Trần Tô Hương Mai***

APPENDIX 3.10

The FTD multiplex PCRs were performed in accordance with the manufacturers' guidelines

1. The pathogen RNA was extracted.

2. The RNA was transcribed into cDNA by reverse transcription (RT-PCR) using sequence-specific primers which bind to the RNA.

3. The cDNA was amplified in a thermocycler using a programme as follows:

    50 ºC for 15 minutes hold; 94 ºC for 1 minute hold;

    40 cycles of 94 ºC for 8 seconds;

    60ºC for one minute.

    The viral and bacterial meningitis also required a 2-minute hold at 4ºC at the end of the process.

4. If the specific pathogen sequences were present, these were seen as an increase in fluorescence from a probe.

5. The number of cycles of PCR required to see the increase in fluorescence was reported as the cycle-threshold (Ct value). The lower the Ct value, the greater the amount of nucleic acid present. To validate the assay, the positive and internal controls were required to be above a Ct of 33 and the negative control, below the threshold.

6. Brome mosaic virus (BMV) was used as the internal control for the viral meningitis PCR and *Streptococcus equi* (Sequi) as the extraction control (internal control) for the bacterial meningitis PCR and tropical fever core.

This information is taken from the following SOPs “FTlyo 28-32_64-MANUAL-v3-2016_10 EN; FTlyo 13-32_64-MANUAL-v1-2017_02 EN and FTD 36-32_64-MANUAL-v5-2016_12 EN”.

APPENDIX 3.11

The in-house PCR for *S. suis*, measles virus, rubella virus and *O. tsutsugamushi* were tested using the generic Real Time-PCR TaqMan assay. Following RT-PCR, the cDNA is amplified using different cycles as shown below and the TaqMan probe emits fluorescence. The criteria for defining positive *S. suis* required a cut-off Ct value of <40; for and *O. tsutugamushi* ≤40; for rubella <38 and measles there was no defined cut-off. This information was taken from the following SOPs.

"Realtime PCR for Detection of *Streptococcus Suis* Serotype 2, *Streptococcus Pneumoniae*, *Neisseria Meningitidis*, *Haemophilus Influenzae* Type b; OUCRU-VN; TP-TC-EVI-002; Version 02; Effective 7 April 2014"

"Measles realtime RTPCR; OUCRU-VN; HN-TP-TF-VI-009; Version 001; Effective 12 November 2013"

"Rubella Real-time PCR; OUCRU-VN; HN-TP-TF-VI-013; Version 001; Effective 12 November 2016"

"Orientia Tsutsugamushi 47kda Gene-Based Realtime PCR assay using Taqman probe; OUCRU-VN; TP-TC-ETR-006; Version 02; Effective 02/06/2014"

S. suis	Cycle 1 (1X): Pre-incubation
	95 ºC for 10 min
	Cycle 2 (40-45x): PCR amplification
	Step 1: 95 ºC for 10 seconds

	Step 2: 58 or 60 ºC (60 ºC in case of VZV) for 30 seconds Step 3: 72 ºC/10 sec, read Cycle 3: cool at 40 ºC/30 seconds
Measles virus	1x 15 minutes at 95 ºC 45x (30 seconds at 94 ºC, 30 seconds at 57ºC, 30 seconds at 72 ºC with plate read).
Rubella virus	1x 30 minutes at 50 ºC 1 x 2 minutes at 90 ºC 45x (15 seconds at 95 ºC, 30 seconds at 57ºC, 30 seconds at 72 ºC with plate read).
	45x (30 seconds at 94 ºC, 1 minute at 60 ºC) Plate read for collecting fluorescence data FAM.
O. tsutsugamushi	* Hold at 50 ºC for 2 minutes * Hold at 95 ºC for 2 minutes * 45 cycles of the following conditions: 95 ºC for 15 seconds

60 ºC for 30 seconds, acquiring with all channel at the end of each cycle

* Start the run using the 47kDa *O. tsutsugamushi* template and fill the name of the samples in the computer table.

Extraction for PCR used the MagNA pure 96 extraction machine (SOP “NA Extraction using MagNA Pure 96 DNA and Viral NA Small Volume Kit, OUCRU-VN; HN-TP-TF-AL-004; Version 001; Effective 15 August 2015”).

oucru

JE Detect IgM Antibody capture ELISA

HN-TP-TF-IM-011 Version 001 Effective:

1. Purpose

The JE Detect MAC-ELISA test for exposure to Japanese Encephalitis Virus (JEV) is an ELISA assay system for the detection of IgM antibodies in human serum to JEV derived recombinant antigen (JERA). This test is to aid in the diagnosis of recent human exposure to or infection with the Japanese Encephalitis Virus (JEV). It is not intended to screen blood or blood components, and is for professional in vitro diagnostic use only.

2. Principle:

The JE Detect MAC-ELISA consists of one enzymatically amplified "two-step" sandwich type immunoassay. In this assay, NTC, PC, and unknown serum samples are diluted with Sample Dilution Buffer, then incubated in microtitration wells coated with anti-human IgM antibodies. This is followed by incubation with both JEV derived recombinant JERA and Normal Cell Antigen (NCA) separately. After incubation and washing, the wells are treated with a JERA-specific antibody labeled with the enzyme horseradish peroxidase (HRP). After a third incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbencies of the JERA and the control wells accurately determines whether antibodies to JEVare present.

3. Scope and responsibilities:

Lab technician have responsibility for performing testing following this defined procedure

4. Reference

Package insert of JE Detect IgM Antibody Capture ELISA.Cat. No. JEMS-1, Brand: InBios

5. Definition

JEV: Japanese Encephalitis Virus

JERA: JEV derived recombinant antigen

NCA: normal cell antigen

HRP: Horseradish Peroxidase

mAb: monoclonal antibody

TMB: tetramethylbenzidine

NTC: Negative Control

PC: Positive control

ISR: Immune Status Ratio

6. Specimen requirement

Serum

As the manufacturers have not optimised the kit for testing of CSF, the Laos-Oxford-Mahosot Wellcome Trust Research Unit (LOMWRU) Standard Operating Procedure “Guidelines for DENV diagnosis” will be used.

CSF should be diluted to 1/10.

7. Materials/Equipments used

7.1 Kit content:

No	Components	Quantity/Volume	Store condition
1	Anti-Human IgM Coated Microtiter Strips	12X8strips	2-8°C
2	Sample Dilution Buffer for JE Detect IgM	1 bottle of 25ml	2-8°C
3	Ready-to-use JE Antigen (JERA) for IgM	1 tube of 3 ml	2-8°C
4	Ready-to-use normal cell antigen (NCA) for IgM	1 tube of 3 ml	2-8°C
5	JE Detect Negative Control	1 vial of 30 µL	Store at 2-8°C until ready to use for up to 7 days. For long-term storage, serum can be further aliquoted in a smaller volume and stored at –70°C.
6	JE Detect IgM Positive Control	1 vial of 30 µL	Store at 2-8 °C until ready to use for up to 7 days. For long-term storage, serum can be further aliquoted in a smaller volume and stored at –70°C.
7	10X Wash Buffer	1 bottle of 120ml	2-8°C

8	Ready to Use Enzyme Conjugate-HRP for IgM	1 bottle of 6ml	2-8°C
9	EnWash	1 bottle of 20 ml	2-8°C
10	Liquid TMB Substrate	1 bottle of 9 ml	2-8°C, should be kept in a light -protected bottle at all times as provided
11	Stop Solution	1 bottle of 6 ml	2-8°C

7.2 Other materials and Equipments required

1. 1-10 µL Single-Channel Pipetters, 50-200 µL Single and Multi-Channel Pipetters.
2. ELISA Spectrophotometer capable of absorbance measurement at 450 nm (ELx808-Biotek)
3. Plate Washer and Vacuum Pump (in case of using Automated Plate Washer)
4. 37°C Incubator
5. Biological or High-Grade Water
6. Polypropylene tubes (falcon)
7. Parafilm or similar plate cover
8. Timer
9. Vortex

8. Procedures

Step	Activities
1	Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion
2	Preparation of 1X Wash Buffer: Mix 120 ml 10X wash buffer with 1080 ml distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved. After diluting to 1X, store at room temperature for up to 4 months. Check for contamination prior to use.
3	Select the number of coated wells required for the assay. Positive, negative controls should be assayed in duplicate for both JERA and NCA portions of assay. Unknown serum samples can be assayed singly or in duplicate but must be assayed for both JERA and NCA portions of assay. Up to 44 or 22 test samples can be tested on one 96 well plate depending on being assayed singly or in duplicate. Place remaining unused wells back quickly into the pouch and stored at 2-8°C. Mark the microtitration strips to be used.
4	Dilute NTC, PC,CAL and sera to 1/100. Using U-bottom plate: To 10 µL serum add 90 µL of Sample Diluent. Take 20 µL of the diluted serum and add 180 µL Sample Diluent. Mix well. When using cluster tube: add to 5ul serum add 495 ul of Sample Diluent. Mix well. For CSF: Dilute CSF to 1/10. To 20ul of CSF add 180ul Sample Diluent. Mix well.
5	Apply the 50 µL/well of 1/100 diluted test sera, PC, NTC to the plate. An exemplary arrangement for 44 test serum samples singly is shown in 16.1. Cover the plate with parafilm.

6	Incubate the plate at 37 °C for 1hour in an incubator as a single layer for even temperature distribution. Do not stack plates on top of each other and not use CO_2 or other gases.
7	After the incubation, wash the plate 6 times with automatic plate washer or manually using 1x Wash buffer following the washing procedure in 16.2
8	Add 50µl /well of JERA into row A-D and 50µl /well of NCA into row E-H. An exemplary application for JERA and NCA is shown in 16.3.
9	Cover the plate with parafilm

10	Incubate the plate at 37 °C for 1 hour in the incubator
11	After the incubation, wash the plate 6 times
12	Add 50µl /well of ready to use Enzyme-HRP conjugate into all wells by multi-channel pipetter.
13	Cover the plate with parafilm
14	Incubate the plate at 37 °C for 1 hour in the incubator in darkness.
15	After the incubation, wash the plate 6 times
16	Add 150µl /well of EnWash into all wells
17	Incubate the plate at room temperature for 5 minutes without any cover on the plate.
18	After the incubation, wash the plate 6 times
19	Add 75µl /well of Liquid TMB substrate into all wells
20	Incubate the plate at room temperature in a dark place (or container) for 10 minutes without any cover on the plate.
21	After the incubation, add 50µl /well of Stop solution into all wells

22	Within 5 minutes, read the RAW OD 450 value with a Microplate reader (ELx808-Biotek).

Step by step of testing instruction.

9. Quality Control

Each kit contains positive and negative control sera. Acceptable Immune Status Ratio (ISR) values for these controls are found on specification table below. The test is invalid and must be repeated if the ISR value of either the controls do not meet the specifications. If the test is invalid, patient results cannot be reported.

10. Calculations

Calculation of the Negative Control	Calculate the mean JE Detect Negative Control values with JERA and with the Control antigen, then calculate the ISR. Example: JE Detect Negative Control OD JERA NCA No 1 0.188 0.129 No 2 0.192 0.125 Total 0.380 0.254 Averages (JERA)=0.380/2=0.190 (NCA)=0.254/2=0.127

	ISR= JERA/NCA ratio: 0.190/0.127=1.50
Calculation of the Positive Control	Calculate the mean JE Detect Positive Control values with JERA and with the Control antigen, then calculate the ISR.
Obtain following Factors and compare to the criteria in specific sheet for an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated	Factors include: 1.Mean JE Detect Negative Control OD in JERA 2.Mean JE Detect IgM Positive Control OD in JERA 3.JE Detect IgM Positive Control Immune Status Ratio (ISR) 4.JE Detect Negative Control Immune Status Ratio (ISR)
Calculation for unknown samples analysis	Calculate the average JERA and NCA OD450 of sera, for test samples run in singlet, obtain the individual JERA and NCA values ISR = JERA OD450nm/NCA OD450nm

11. Interpretation of results and report

ISR	Results	Interpretation
<4.0	Negative	No detectable IgM antibody by the ELISA test
4-6	Equivocal	Need confirmatory test
>6	Positive	Indicates presence of detectable IgM antibody. Recommend supplemental confirmatory testing

If a sample is positive for JEV and DENV using both InBios JEV IgM ELISA and Panbio dengue IgM capture ELISA, both pathogens should be re-tested using eight twofold dilutions (1/20 to 1/2560). The last positive dilution gives the titre for the corresponding test. The actual IgM corresponds to the kit that gives the highest titre.

12. Reporting of results

Describe the reporting units

13. Reference range and critical value:

Describe the reference range for the test and the critical value for the rest

14. Limitations:

1. Since this is an indirect screening method, the presence of false positive and negative results must be considered.
2. All reactive samples must be evaluated by a confirmatory test.
3. The reagents supplied in this kit are optimized to measure JERA reactive antibody levels in serum

4. Serological cross-reactivity across the flavivirus group is common. Certain sera from patients infected with Dengue, West Nile, and Saint Louis virus may give false positive results. Therefore any JE positive sera must be confirmed with other tests.
5. The assay performance characteristics have not been established for visual result determination.
6. Results from immunosuppressed patients must be interpreted with caution.
7. Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.

15. Safety

1. All human controls and antigen should be handled as potentially infectious material at BSL2.

State safety issue related testing procedure.

2. Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.

3. MSDS of entire kit can be found here**:** **Z:\Group Folders\Laboratory\Chemical classification\MSDS\Inbios\SDS 4009-01 EU, JE Detect IgM Capture ELISA Kit SDS.pdf**

16. Supplementary notes

16.1 Example for Serum Sample Application.

	1	2	3	4	5	6	7	8	9	10	11	12
A	JE Negative Control	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21
B	JE Negative Control	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
C	JE IgM Positive Control	S#23	S#25	S#27	S#29	S#31	S#33	S#35	S#37	S#39	S#41	S#43
D	JE IgM Positive Control	S#24	S#26	S#28	S#30	S#32	S#34	S#36	S#38	S#40	S#42	S#44
E	JE IgM Positive Control	S#24	S#26	S#28	S#30	S#32	S#34	S#36	S#38	S#40	S#42	S#44
F	JE IgM Positive Control	S#23	S#25	S#27	S#29	S#31	S#33	S#35	S#37	S#39	S#41	S#43
G	JE Negative Control	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
H	JE Negative Control	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21

16.2 Washing procedure

A. Automated Plate Washer

(1) Completely aspirate all wells.

(2) Fill all wells to rim (300 µL) during wash cycle.

(3) On completion of six (6) washes, invert plate and tap firmly on absorbent paper towel to ensure all Wash Buffer is removed.

(4) Automated plate washers must be well maintained to ensure

efficient washing. Manufacturer's cleaning instructions should be followed at all times.

B. Manual Washing

(1) Discard contents of plate in appropriate waste container.

(2) Fill wells with Wash Buffer using a suitable squeeze bottle. Avoid bubbling of Wash Buffer as this may reduce wash efficiency. Discard Wash Buffer from wells immediately.

(3) Refill wells with Wash Buffer and discard immediately.

(4) Repeat (3) another four times. This will make a total of six (6) washes with Wash Buffer.

(5) After the final wash, discard contents of wells and tap the plate on absorbent paper towel to ensure all Wash Buffer is removed.

16.3 Example for JE Antigens Application

	1	2	3	4	5	6	7	8	9	10	11	12
A	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
B	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
C	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
D	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
E	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
F	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
G	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA

17. Risk assessment and risk management:

Describe the risk associated with the test and mitigation plan

The list of subheading is not exhaustive, additional sub heading can be added as required for different technical SOPs and some subheadings can be deleted as necessary.

18. Related Documents:

Document name	Document code
Panbio Dengue IgM Capture ELISA	HN-TP-TF-IM-010 Version 001
LOMWRU SOP Guidelines for Dengue diagnosis	SER 020-01
Package insert of Panbio Dengue IgM Capture ELISA.	No. 01PE20/01PE21
Package insert of JE Detect IgM Antibody capture ELISA. Brand InBios	No : JEMS-1
Safety data sheet (SDS) of JE Detect™ IgM Antibody Capture Elisa kit	SDS 4009-01

19. Document History:

Revision no.	Effective date	Details of amendment

Panbio Dengue IgM Capture ELISA

HN-TP-TF-IM-010 Version 001 Effective:

1. Purpose

The Panbio Dengue IgM Capture ELISA is for the qualitative detection of IgM antibodies to dengue virus antigen in serum, as an aid in the clinical laboratory diagnosis of patients with clinical symptoms consistent with dengue fever. The kit should be used in conjunction with other dengue serology.

2. Principle:

Serum antibodies of the IgM class, when present, combine with anti-human IgM antibodies attached to the polystyrene surface of the microwell test strips. A concentrated pool of Dengue virus 1-4 Antigens is diluted to the correct working volume with Antigen Diluent. The antigens are produced using an insect cell expression system and immunopurified utilising a specific monoclonal antibody. An equal volume of the HRP Conjugated Monoclonal Antibody (MAb) is added to the diluted antigen, which allows the formation of antigen-MAb complexes. Residual serum is removed from the assay plate by washing, and complexed antigen-MAb is added to the assay plate. After incubation, the microwells are washed and a colourless substrate system, tetramethylbenzidine / hydrogen peroxide (TMB Chromogen) is added. The substrate is hydrolysed by the enzyme and the chromogen changes to a blue colour. After stopping the reaction with acid, the TMB becomes yellow. Colour development is indicative of the presence of anti-dengue IgM antibodies in the test sample.

3. Scope and responsibilities:

Lab technician have responsibility for performing testing following this defined procedure

Reference

Package insert of Panbio Dengue IgM Capture ELISA.Cat. No. 01PE20/01PE21

Guideline for DENV diagnosis- LOMWRU Standard Operating Procedure. Document No: SER 020-01. Created by Audrey Dubot-Peres. Date of original: 18th September 2015.

4. Definition

HRP: Horseradish Peroxidase

MAb: Monoclonal Antibody

TMB: tetramethylbenzidine

CSF: Cerebrospinal fluid

NTC: Negative Control

PC: Positive control

CAL: Calibrator

5. Specimen requirement

Serum(*)

CSF(**)

(*):Icteric or lipaemic sera, or sera exhibiting haemolysis or microbial growth should not be used.

(**): The package insert states “This test should be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been established”. We will use the Laos-Oxford-Mahosot Wellcome Trust Research Unit (LOMWRU) Standard Operating Procedure “Guidelines for DENV diagnosis” in which CSF is diluted to 1/10.

6. Materials/Equipments used

6.1 Kit content:

No	Component	Quantity/ Volume
1	Anti-human IgM Coated Microwells	12x8wells
2	Dengue virus 1-4 Antigens (Recombinant)	1 vial of 150 ul
3	Wash Buffer (20x)	1 bottle of 60 mL
4	Sample Diluent	2 bottles of 50 mL
5	Antigen Diluent	1 bottle of 50 mL
6	HRP Conjugated Monoclonal Antibody Tracer	1 bottle of 7 mL
7	TMB Chromogen (TMB)	1 bottle of 15 mL
8	Positive Control	1 vial of 200 ul
9	Calibrator	1 vial of 400 ul
10	Negative Control	1 vial of 200 ul
11	Stop Solution	1 bottle of 15 mL

6.2 Additional materials and Equipment

1. Pipette and pipette tips of 5-1000 µl

2. Deionised water

3. ELISA reader (ELx808-Biotek)

4. Timer

5. Microplate to dilute serum (U-bottom plate, Cat: 3797, Brand: Corning) or Cluster tube to dilute serum (Cat: 4418, Brand: Corning).

7. Glass or plastic tubes or vials for diluting antigen (falcon)

7. Procedures

Step	Activity
1	Equilibrate all reagents to room temperature (20-25 °C)
2	Remove the required number of microwells from the foil sachet and insert into strip holder. Five microwells are required for NTC, PC and CAL in triplicate. Seal unused microwells in the foil sachet tightly.
3	Dilute NTC, PC, CAL and sera to 1/100. Using U-bottom plate: To 10 µL serum add 90 µL of Sample Diluent. Take 20 µL of the diluted serum and add 180 µL Sample Diluent. Mix well. When using cluster tube: add to 5ul serum add 495 ul of Sample Diluent. Mix well. For CSF: Dilute CSF to 1/10. Example: To 20ul of CSF add 180ul Sample Diluent. Mix well.
4	Determine the required number of wells for the assay. Dilute the antigen 1/250 using the Antigen Diluent and the mixed solution will become a pale blue colour (recommended a minimum of 10 µL of antigen into 2.5 mL of Antigen Diluent

	and this is sufficient for up to five strips (40 wells). The remaining unused concentrated antigen is stored back at 2-8ºC.
5	Remove the required volume of diluted antigen and mix with an equal volume of MAb Tracer in a clean 15ml falcon tube. Gently mix the antigen-MAb Tracer solution and leave at room temperature (20-25 ºC) until required. Discard the unused diluted antigen.
6	Within 10 minutes after mixing the MAb Tracer and diluted antigen, pipette 100 µL diluted sera, CSF and Controls into their respective microwells of the assay plate
7	Cover plate and incubate for 1 hour at 37 ºC ±1 ºC.
8	Wash six (6) times with diluted Wash Buffer manually or using automated plate washer as in washing procedure in 15.1.
9	Mix the antigen-MAb tracer solution before transfer. Pipette 100 µL of antigen-MAb complexes from the antigen vial to the appropriate wells of the assay plate
10	Cover plate and incubate for 1 hour at 37 ºC ±1 ºC.
11	Wash six (6) times with diluted Wash Buffer manually or using automated plate washer as in washing procedure in 15.1.
12	Pipette 100 µL TMB into each well
13	Incubate for 10 minutes at room temperature (20-25 ºC), timing from the first addition. A blue colour will develop.
14	Pipette 100 µL of Stop Solution into all wells in the same sequence and timing as the TMB addition. Mix well. The blue colour will change to yellow.

15	Within 30 minutes read the absorbance of each well at a wavelength of 450 nm with a reference filter of 600-650 nm

8. Quality Control

Each kit contains Calibrator, Positive and Negative Controls. Acceptable values for these are found on the accompanying specification sheet. The Negative and Positive Controls are intended to monitor for substantial reagent failure.

The test is invalid and should be repeated if the absorbance readings of either the Controls or the Calibrator do not meet the specifications. If the test is invalid, patient results cannot be reported.

9. Calculations

Obtain the calibration factor value in the specification sheet before commencing calculations.

Step		
1	Calculate Cutoff Value	Cut-off Value = [(Absorbance of the triplicates of the Calibrator)/3] x (Calibration factor)
2	Calculate index value	Index Value = Sample Absorbance/ Cut-off Value
3	Calculate Panbio Units.	Panbio Units = Index Value X 10

10. Interpretation of results and report

INDEX	Panbio Units	Result
<0.9	<9	Negative
0.9-1.1	9-11	Equivocal
>1.1	>11	Positive

Result	Interpretation
Negative	No detectable IgM antibody. The result does not rule out dengue virus infection. An additional sample should be tested in 7-14 days if early infection is suspected. Other dengue assays should be performed to rule out acute infection.
Equivocal	Equivocal samples should be repeated. Samples that remain equivocal after repeat testing should be repeated by an alternative method or another sample should be collected.
Positive	Presence of detectable IgM antibody. Other dengue serology assays should be performed to confirm dengue infection. If differentiation between primary and secondary infection is required, the Dengue Duo (07PE10) ELISA should be used.

11. Reporting of results

Reporting unit is Panbio Unit.

If a sample is positive for JEV and DENV using both InBios JEV IgM ELISA and Panbio dengue IgM capture ELISA, both pathogens should be re-tested using eight twofold dilutions (from 1/20 to 1/2560). The last positive dilution gives the titre for the corresponding test. The actual IgM corresponds to the kit that gives the highest titre.

12. Reference range and critical value:

13. Limitations:

1. The clinical diagnosis must be interpreted with clinical signs and symptoms of the patient. The results from this kit are not by themselves diagnostic and should be considered in association with other clinical data and patient symptoms.

2. Serological cross-reactivity across the flavivirus group is common (i.e. between dengue 1, 2, 3 & 4, Murray Valley encephalitis, Japanese encephalitis, Yellow fever and West Nile viruses). These diseases must be excluded before confirmation of diagnosis.

3. Heterophilic antibodies are a well-recognised cause of interference in immunoassays. These antibodies to animal IgG may cross-react with reagent antibodies and generate a false positive signal. This must be excluded before confirmation of diagnosis.

4. The performance characteristics have not been established for visual result determination.

5. This assay employs insect expressed proteins. The cross-reactivity or interference of human anti-insect antibodies is unknown with the assay's results.

14. Safety

1. All human controls and antigen should be handled as potentially infectious material at BSL2

2. Hazard information for the components under applicable European Community (EC) Directives is as per the table below.
3. The MSDS sheet of the kit can be found on Z:\Group Folders\Laboratory\Chemical classification\MSDS\Panbio\msds_panbio_dengue_igm_capture_elisa_01pe20_2013.pdf

Components	**Hazard Nature**
Wash buffer 20x Concentrate	Irritant R36/38, R43
TMB Chromogen (ready for use)	Irritant R36/37/38
Stop Solution (ready for use)	Irritant R36/38
Sample Diluent	Irritant R36/38, R43
Antigen Diluent	Irritant R36/38, R43

Dengue 1-4 Antigen	Irritant R36/38, R43
Dengue IgM Capture MAb Tracer HRP	Irritant R36/38, R43
Anti-Human IgM Antibody Coated Microwells	Not Considered Hazardous
Dengue IgM Capture Positive Control	Harmful R22, R32, R43
Dengue IgM Capture Calibrator	Harmful R22, R32, R43
Dengue IgM Capture Negative Control	Harmful R22, R32, R43

15. Supplementary notes

15.1Washing procedure

A. Automated Plate Washer

(1) Completely aspirate all wells.

(2) Fill all wells to rim (350 µL) during wash cycle.

(3) On completion of six (6) washes, invert plate and tap firmly on absorbent paper towel to ensure all Wash Buffer is removed.

(4) Automated plate washers must be well maintained to ensure efficient washing. Manufacturer's cleaning instructions should be followed at all times.

B. Manual Washing

(1) Discard contents of plate in appropriate waste container.

(2) Fill wells with Wash Buffer using a suitable squeeze bottle. Avoid bubbling of Wash Buffer as this may reduce wash efficiency. Discard Wash Buffer from wells immediately.

(3) Refill wells with Wash Buffer and discard immediately.

(4) Repeat (3) another four times. This will make a total of six (6) washes with Wash Buffer.

(5) After the final wash, discard contents of wells and tap the plate on absorbent paper towel to ensure all Wash Buffer is removed.

16. Risk assessment and risk management:

Some kit components (PC, NTC, CAL) contain sodium azide, which may react with lead or copper plumbing to form highly explosive metal azide compounds. When disposing of these reagents through plumbing fixtures, flush with a large volume of water to prevent azide build-up in drains.

17. Related Documents:

Document name	Document code
Package insert of Panbio Dengue IgM Capture ELISA. Brand PanBio.	No. 01PE20/01PE21
Package insert of JE Detect IgM Antibody capture ELISA. Brand InBios	No : JEMS-1
JE Detect IgM Antibody capture ELISA	HN-TP-TF-IM-011 version 001
LOMWRU SOP Guidelines for Dengue diagnosis	SER 020-01
MSDS sheet for entire Panbio Dengue IgM Capture ELISA kit, Cat : 01PE20	No.:SD-MSDS-01PE2

18. Document History:

Revision no.	Effective date	Details of amendment

GROUP DISCUSSION FOR STUDY STAFF (HOSPITAL)

How did you find the recruitment of cases?

Were there any problems with the recruitment of cases?

If yes, what were these?

How did you find recruitment of controls?

Were there any problems with the recruitment of controls?

If yes, what were there?

How did you find the completion of the CRF?

Did you find any problems with it?

What were the reasons for unsuccessful recruitment of cases?

13HN FeedbackField EN V2.0 15MAR16

To be completed for each participant recruited. Please return to CTU, OUCRU at the end of every two weeks

1. Participant code 13HN-[__|__]-[__|__|__|__]

2. Date you made contact with the participant by phone

(dd/mm/yyyy) : __/__/____

Not applicable as not able to contact the participant ☐

3. Date home visited *(dd/mm/yyyy)* : __/__/____

4. Were any of the following problems encountered during the house visit?

House difficult to find

Participant not at home on arrival ☐ If yes, go to 4a

Participant/relative did not allow you to enter the house ☐ If yes, go to 4a

Other (specify)_____

4a) When were you next able to return to the house

[__|__] hours

[__|__] days

Not applicable as unable to return to the house ☐

5. How long did it take you to travel to the home of the participant

[__|__] minutes

[__|__] hours

6. Did you have to obtain consent from the participant?

☐ Yes ☐ No

7. How long did the questionnaire take to complete?

[__|__] minutes

8. Were there any problems with the ODK system?

Yes ☐ If yes, go to 8a

No ☐

8a) Please specify what these problems were

9. Were there any problems with the Samsung Android?

Yes ☐ If yes, go to 9a

No ☐

9a) Please specify what these problems were

GROUP DISCUSSION FOR STUDY STAFF (FIELD)

How did you find the travel to the homes of the participants in terms of time taken and road conditions?

If there were any problems, what were these?

Were you often able to see cases and controls in one day?

How easy was it to contact participants to arrange a time to visit their homes?

If there were any problems what were these?

Were most participants at home during the time you arranged?

How did participants find the questionnaire?

How easy was it for you to perform the questionnaire? Were there any problems (including with the mobile technology and question structures)?

How did you find recruitment of controls?

Were there any problems with the recruitment of controls?

How did participants feel about you looking around their houses and taking photos?

How easy was it for you to perform the verification form? Were there any problems?

APPENDIX 3.17

Codebook for FGDs

Question 1	Rural women	Rural men	Urban women
House/household	11	5	12
Lake(s)	7		2
Water	6	3	6
Tyres	5		
Chicken(s)	4		3
Plant(s)	4		1
Pig(s)/pigsty	3		5
Standing	3		
Banana	2		
Birds	2		
Garden	2	2	3
Rubber	2		
Spray	2		1
Swimming	2		
Trees	4		
Cattle	1		5
Stools	1		2
Vegetable(s)	2		5
Work/workshop	4		
Drainage		3	1
Tank		3	
Countryside		2	
Pond(s)		2	4
Rice		2	
Morning	6		
Afternoon	5	2	1
Evening		1	4

Question 2	Rural women	Rural men	Urban women
Spray(s)	6	3	5
Season	3		
House/home	2		4
Net	8	5	10
Bednet		5	
Cream	2	2	3
Tank	8		
Water	8		
Bed	6		10
Children	4		
Family	4	3	1
Evening	3		1
Treated	3	2	
Chemical	2		1
Door/s	4	3	3
Dung	2		
Racket	1	1	4
Machine			3
Electric	1	1	2
Incense	1	1	2

Question 3	Rural women	Rural men	Urban women
Disease(s)	4	1	
Dengue	2		1

Question 4	Rural women	Rural men	Urban women
Dengue	8	1	6
Japanese encephalitis	1		
Malaria		8	2
Meningitis			2

Question 5	Rural women	Rural men	Urban women
December	2		
June	2	1	
October	2		
January	1	3	1

Question 6	Rural women	Rural men	Urban women
Dog	7	2	6
Chicken	3	6	3
Mouse/mice	2	10	6
Goats		4	
Cat(s)	10	3	6
Cattle		3	2
Slaughtering		5	
Poultry		2	1
Pigeons			4

Question 7	Rural women	Rural men	Urban women
Pork/pig	27	7	7
Blood	10		2
Chicken	8	1	6
Pudding	8		2
Beef	7	2	8
Fish	9	3	9
Mice	5	1	
Duck	3		
Hedgehog	2		
Horse	2		
Rat	2		
Goat		7	
Cat		1	
Dog		1	
Pigeon			8

Question 8	Rural women	Rural men	Urban women
Poor	6		
Good	4		3
Ugly	4		
Clean	3		
Dust	3		
Laugh	3		
Mountainous	3		
City	2		
Hygiene	2		
Uncomfortable	2		
Problem		2	
No		4	

Information from the CRF of the cases, the potential numbers of controls available matched on different criteria and information from the questionnaire conducted during the home visit of the cases and the controls.

Table A3.1 The number and percentage of cases living in each province.

	Total (N=38)
Province	
Bac Ninh	2 (5.3%)
Ha Nam	3 (7.9%)
Hai Duong	4 (10.5%)
Hai Phong	2 (5.3%)
Hanoi	10 (26.3%)
Hung Yen	1 (2.6%)
Nam Dinh	5 (13.2%)
Ninh Binh	6 (15.8%)
Thai Binh	5 (13.2%)
Van Phuc	0

Table A3.2 The neurological inclusion criteria.

	Total (N=38)
Change in consciousness level	38 (100%)
Seizures	10 (26.3%)
Limb paresis	2 (5.3%)
Facial paresis	1 (2.6%)
Speech abnormality	1 (2.6%)
Hearing abnormality	2 (5.3%)
Visual impairment	0
Imbalance	0

Table A3.3 The age and sex of the cases.

	Total (N=38)
Age (years) (median (range))	60 (9 - 85, N=29)
Sex	
Female	11 (28.9%)
Male	27 (71.1%)

Table A3.4 Markers and proxy markers of progression of symptoms and severity of illness of the cases.

	Total (N=38)
Time between first symptoms and first seeking medical care (days) (median (range))	2 (0 - 7)*
Ward admitted to	
ICU	9 (23.7%)
Non-ICU ward	29 (76.3%)
Intubated on admission to NHTD	
Yes	11 (28.9%)
No	27 (71.1%)

* N=3

Table A3.5 The GCS on admission of the cases.

	To initial hospital	**To NHTD**
Eyes (median (range))	4 (2 - 4, N=7)	3 (2 - 4, N=14)
Verbal (median (range))	3 (2 - 4, N=7)	3 (1 - 5, N=14)
Motor (median (range)	4 (4 - 6, N=7)	5 (3 - 6, N=14)
Total (median (range))	11 (6 - 15, N=19)	12 (6 - 15, N=33)

Table A3.6 The medication taken by the cases prior to admission.

	Total (N=38)
Aciclovir	1 (2.6%)
Ampicillin	5 (13.2%)
Ceftriaxone	10 (26.3%)
Colistin	1 (2.6%)
Levofloxacin	0
Linezolid	1 (2.6%)
Meropenem	2 (5.3%)
Vancomycin	1 (2.6%)
Steroids	3 (7.9%)

Table A3.7 The location of the first lumbar puncture (LP).

	Total (N=38)
Location of first lumbar puncture	
NHTD	18 (48.6%)
Site other than NHTD	19 (51.4%)

Table A3.8 The CSF results of first LP.

	Total (N=38)
White cell count (cells/μl) (median (range))	310 (3 - 15634, N=30)
Neutrophils (%) (median (range))	70 (2 - 97)
Lymphocytes (%) (median (range))	10 (1 - 96)
Eosinophils (%) (median (range))	6 (2 -18)
Neutrophil to lymphocyte ratio (median (range))	9 (0 - 89)
CSF protein (g/L) (median (range))	1.7 (0.2, 7.3)

Table A3.9 The length of the hospital stay and the outcome of the cases.

	Total (N=38)
Length of hospital stay (from initial hospital to discharge at NHTD) (days) (median (range))	21 (2 - 64, N=31)
Outcome	
Discharge to home	20 (52.6%)
Discharged to die	3 (7.9%)
Transferred to another hospital	15 (39.5%)

Table A3.10 The Glasgow Outcome Scale at discharge of the adult cases.

	Total (N=12)
Time between discharge from NHTD and follow-up (days)	
Median (Range)	27.0 (4.0-144.0)
Final score	
Lower severe disability	5 (41.7%)

Upper severe disability	4 (33.3%)
Lower moderate disability	0 (0.0%)
Lower good recovery	2 (16.7%)
Upper good recovery	1 (8.3%)

Table A3.11 The Glasgow outcome score at three months after discharge of the adult cases.

	Total (N=13)
Time between discharge from NHTD and follow-up (days)	
Median (Range)	108.0 (35.0 - 258.0)
Final score	
Lower severe disability	4 (30.8%)
Upper severe disability	3 (23.1%)
Lower moderate disability	3 (23.1%)
Lower good recovery	3 (23.1%)
Upper good recovery	0 (0.0%)

Table A3.12 The number of potential controls matched on different criteria.

Province	Ratio of potential controls to number required based on province and gender/number		Ratio of potential controls to number required based on province, gender, age/number	Ratio of potential controls to number required based on province, gender, age/number and living with the patient
	Male	Female		
Bac Ninh	0.44	1.33	0.33	0.17
Ha Nam	0.42	NA	0	0
Ha Noi	2	10	1.78	1.72
Hai Duong	0.25	NA	0.33	0.25
Hai Phong	1.67	2.33	0.33	0.17
Hung Yen	1.67	5.67	0	0
Nam Dinh	0.28	1.5	0.13	0.13
Ninh Binh	0.33	1.33	0.33	0.17
Thai Binh	0.33	0.44	0.25	0.25
Vinh Phuc	NA	NA	NA	NA

Table A3.13 A comparison of the cases with controls by occupation, socio-demographics and water source.

Variable	Cases (n=27)	Controls (n=6)	Odds ratio (95% confidence interval)	p value
Works as a farmer (%)				
Yes	8 (29.6%)	2 (33.3%)	0.79 (0.14-5.23)	
No	19 (70.4%)	4 (66.7%)		0.793
Highest level of education (%)				
None	5 (18.5%)	0		
Primary	6 (22.2%)	0	1.18 (0.01-236.85)	0.936
Middle	8 (29.6%)	3 (50%)	0.22 (0.002-3)	0.286
Secondary	5 (18.5%)	2 (33.3%)	0.20 (0.001-3.27)	0.279
Tertiary	3 (11.1%)	1 (16.7%)	0.21 (0.001-5.21)	0.348
Monthly household income (%)				
<5 million VND				
5-10 million VND	7 (25.9%)	2 (33.3%)	2.07 (0.27-16.07)	0.469
10-20 million VND	15 (55.6%)	2 (33.3%)	1 (0.1-13.61)	1
>20 million VND	4 (14.8%)	1 (16.7%)	1 (0.04-165.65)	1
Unknown	1 (3.7%)	0	0.11 (0.001-2.79)	0.182
	0	1 (16.7%)		
Number of people sharing the bedroom				
0	5 (18.5%)	2 (33.3%)	2.40 (0.33-16.18)	
1	18 (66.7%)	3 (50.0%)	1.36 (0.13-19.21)	0.369
2	4 (14.8%)	1 (16.7%)		0.797
Water source includes an unprotected well or river/stream/pond/lake				
Yes				
No	3 (11.1%)	1 (16.7%)	0.52 (0.07-6.19)	0.563
	24 (88.9%)	5 (83.3%)		

Table A3.14 A comparison of cases with controls by mosquito prevention measures and animal contact.

Variable	Cases (n=27)	Controls (n=6)	Odds ratio (95% confidence interval)	p value
Use of mosquito prevention measures				
Simple bed net				
Yes	27 (100%)	6 (100%)	NA	NA
No	0	0		
Insecticide treated bed net (ITN)				
Yes	1 (3.7%)	0		
No	26 (96.3%)	6 (100%)	0.74 (0.04-112.49)	0.896
Mosquito coils or liquid				
Yes	2 (7.4%)	1 (16.7%)	0.36 (0.04-4.49)	0.385
No	25 (92.6%)	5 (83.3%)		
Mosquito repellent on body or clothes				
Yes	2 (7.4%)	1 (16.7%)	0.36 (0.04-4.49)	0.385
No	25 (92.6%)	5 (83.3%)		
Slept under a bed net in the month prior to becoming unwell (cases) **Slept under a bed net in the last two months (controls)**				
Yes always	20 (74.1%)	6 (100%)	0.63 (0.004-9.19)	0.766
Yes sometimes	5 (18.5%)	0	2.20 (0.01-471.11)	0.714
Never	2 (7.4%	0		
Indoor residual spraying of the house had previously been carried out				
Yes	8 (29.6%)	6 (100%)	0.03 (0.002-0.37)	0.002
No	19 (70.4%)	0		

Table A3.15 A comparison of the cases with controls by animal contact.

Variable	Cases (n=27)	Controls (n=6)	Odds ratio (95% confidence interval)	p value
Pig contact in the month prior to becoming unwell (cases)/last two months (controls)				
Yes	5 (18.5%)	0	3.18 (0.29-437.80)	0.395
No	22 (81.5%)	6 (100%)		
Saw rats around the house/place of work in the month prior to becoming unwell (cases)/last two months (controls)				
Yes	20 (74.1%)	5 (83.3%)	0.75 (0.07-4.70)	0.768
No	7 (25.9%)	1 (16.7%)		
Saw bats around the house/place of work in the month prior to becoming unwell (cases)/last two months (controls)				
Yes	4 (14.8%)	0	2.60 (0.23-361.58)	0.497
No	22 (81.5%)	6 (100%)		
Unknown	1 (3.7%)	0	0.87 (0.04-132.72)	0.933
Had close contact with a sick animal in the month prior to becoming unwell (cases)/last two months (controls)				
Yes	2 (7.4%)	0	1.33 (0.09-191.83)	0.857
No	24 (88.9%)	6 (100%)		
Unknown	1 (3.7%)	0	0.80 (0.04-121.77)	0.894
Slaughtered an animal in the month before becoming unwell (cases)/last two months (controls)				
Yes	4 (14.8%)	1 (16.7%)	0.70 (0.1-8.03)	0.742
No	23 (85.2%)	5 (83.3%)		
Ate or prepared raw meat, viscera or blood in the month before becoming unwell (cases)/last two months (controls)				
Yes	18 (66.7%)	3 (50%)	1.95 (0.35-11)	0.437
No	9 (33.3%)	3 (50%)		

Table A3.16 A comparison of the cases with controls by environmental exposures and travel.

Variable	Cases (n=27)	Controls (n=6)	Odds ratio (95% confidence interval)	p value
Works in rice fields				
Yes	8 (29.6%)	3 (50%)	0.44 (0.08-2.45)	0.337
No	19 (70.4%)	3 (50%)		
Walked through grassland in the month before becoming unwell (cases)/last two months (controls)				
Yes	13 (48.1%)	2 (33.3%)	1.67 (0.31-10.96)	0.55
No	14 (51.9%)	4 (66.7%)		
Flooding in the area in the month before becoming unwell (cases)/last two months (controls)				
Yes	2 (7.4%)	0	1.32 (0.09-191.83)	0.857
No	24 (88.9%)	6 (100%)		
Unknown	1 (3.7%)	0	0.80 (0.04-121.77)	0.894
Swum/washed/walked through or carried out any other activity in any river/stream/lake in the month before becoming unwell (cases)/last two months (controls)				
Yes	6 (22.2%)	0	3.93 (0.37-538.20)	0.298
No	21 (77.8%)	6 (100%)		
Travelled outside of the province in the month before becoming unwell (cases)/last two months (controls)				
Yes	6 (22.2%)	2 (33.3%)	0.54 (0.09-3.71)	0.51
No	21 (77.8%)	4 (66.7%)		

Table A3.17 A comparison of the cases with controls by vaccinations received.

Variable	Cases (n=27)	Controls (n=6)	Odds ratio (95% confidence interval)	p value
Vaccinations received				
JE				
Yes	2 (7.4%)		0.39 (0.02-62.39)	0.612
No	19 (70.4%)	1 (16.7%)		
Unknown	6 (22.2%)	5 (83.3%)	0.09 (0.01-0.57)	0.009
Meningococcal				
Yes	0	0		
No	20 (74.1%)	1 (16.7%)		
Unknown	7 (25.9%)	5 (83.3%)	0.1 (0.01-0.61)	0.012
H. influenzae				
Yes	0	0		
No	19 (70.4%)	1 (16.7%)		
Unknown	8 (29.6%)	5 (83.3%)	0.12 (0.01-0.72)	0.02
Measles				
Yes	3 (11.1%)	1 (16.7%)	0.19 (0.01-2.83)	0.21
No	18 (66.7%)	1 (16.7%)		
Unknown	6 (22.2%)	4 (66.7%)	0.03 (0.01-0.79)	0.03

13HNA FGD V2.0 04AUG16

13HNA: Evaluation of the daily activities and contact patterns of healthy adults and children in selected rural and urban settings in Ha Nam province, Viet Nam to explore potential risk factors for acute encephalitis syndrome

Focus Group Discussion

1. What do you know about GPS devices?

Participants will then be given an introduction to the iGotU GPS device to be used for the study including the purpose of the research, how the device works, an example of the data collected from the device and a description of the contact pattern diary.

2. How would you feel if we asked you to wear one of the GPS devices we will use for the study?

What would your concerns be?

If there is poor discussion generated surrounding this question, the following questions will be used as prompts:

2a) Would you be concerned about how to work the device? Why?

2b) Would you be concerned about losing or breaking the device?

2c) Would you be concerned about your privacy e.g. what is recorded from the device and people from the research team knowing about your activities?

3. How would you feel about a research assistant asking you questions about your daily activities? Is there anything you would not like to be asked?

13HNA ICF FGD V2.0 04AUG16

13HNA: Evaluation of daily activities to understand how these relate to encephalitis

(brain inflammation)

INFORMED CONSENT - FOCUS GROUP DISCUSSION

We are conducting a study looking at behavior in relation to risk factors for acute encephalitis syndrome, an inflammation of the brain. However, before we perform this study we would like to talk about some aspects of it with yourselves as part of a group discussion. Please read this information sheet carefully or have someone read it to you. Please ask the study staff to explain any information that you are not sure about. It is your choice whether to participate in the study and please do not feel obliged do so. If you would like to take part you will be asked to sign or place a thumbprint on the last page. You will be given a copy of this form for your personal records.

Why is this study being done?

Acute Encephalitis Syndrome (AES) is a syndrome of brain inflammation or swelling. Patients may die or be left disabled. There are around two thousand cases of AES in Viet Nam every year. Although Japanese encephalitis, a virus spread by mosquitoes is a common cause of AES in children and Streptococcus suis, a bacteria associated with eating or preparing raw pork meat is a common cause in adults, for half of the patients or more, we do not know the cause of their AES. However, we do know that most of the cases occur during the warmer seasons and therefore we think that

mosquitoes or other insects are spreading diseases, which cause AES in northern Viet Nam.

This study therefore aims to look at the reasons why people might or might not get AES including where the person lives and their risks in and around their home and work environment and their contact with animals. To collect this information, part of this study therefore involves attaching global positioning system (GPS) devices to people to look at their movements throughout the day and to ask people to complete a diary about their contact with people and animals, where they went and food they ate.

Who is doing this study?

This study is being performed by a team from:

The Oxford University Clinical Research Unit in Ha Noi

The University of Liverpool, United Kingdom

The Wellcome Trust Liverpool Glasgow Centre for Global Health Research, United Kingdom

The National Institute of Hygiene and Epidemiology, Ha Noi

What will happen to me in this study and what are the risks?

You have been chosen to take part because we want to get information from members of the community about your views on wearing the GPS and completing a diary. You will be asked to take part in a group discussion with seven other members of the same gender in your community. This will take around one hour.

What will happen to the samples / information taken?

The results of the study will be used to design a questionnaire for a larger study. The results will also be written as a report or thesis for the University of Liverpool and will be published in a journal, which will be made available to the public. The results will also be available in Vietnamese through the OUCRU website and the National Institute of Hygiene and Epidemiology website. The results will also be available for the Ministry of Health in Viet Nam. You will not be able to be identified in these results as you will have a unique code and only your code will appear in the study data, results, and publications/reports.

The results will be also be made available to the public through workshops about AES in the community or schools and possibly via the radio or other forms of media.

What is the benefit of participating?

There is no intended personal benefit to taking part however, the benefit will be to the community and people of Viet Nam by helping us to understand the risks associated with AES.

You will be reimbursed 100,000 VND for your time spent and you will be offered light refreshments.

What happens if I decline participation or change my mind later on?

You have the right to decline participation or withdraw from the study at any time. Please let us know if you wish to do so. You do not need to give an explanation for this. You can request for your results to be destroyed at any time. There will be no effect to your management.

Confidentiality

All conversations will be recorded using a microphone device and later translated by the research assistant and kept on a secure database. Information about you will be kept confidential and only shared amongst study team members. You will be given an anonymous code, which will be used for the answers. Details linking yourself to this code will be kept in a separate place. All consent forms and recordings will be kept in a locked room. Only those who are involved with the study will have access to these documents which will be kept for 3 years and then put on an electronic system. The consent forms will be kept in a locked cupboard.

Whom do I contact if I have questions or complaints?

If you have questions about the study you may contact: *To be decided*

If you have questions about being in a research study, please contact the Research Ethics Board of the NHTD at (phone number) the Clinical Trials Unit at (phone number).

Thank you for reading this and thinking about joining this project. Please ask the study staff if you have any questions. If you agree to be in the study please sign below (one page per person).

- O I have read this information sheet or it has been read to me and I understand it.
- O I have been able to ask questions about the study.
- O I understand that my participation in the study is voluntary and I do not have to answer questions if I do not wish to do so
- O I understand that my information will be recorded using a microphone device.

- O I understand that I can ask for access to the information I provide or have this information destroyed if I wish.
- O I agree for the data collected from me to be used in future research publications and presentations without my name.
- O I understand that I have the right to withdraw from the study at any time.
- O I agree to take part in the above study.

Participant ID: 13HN-[__|__]-[__|__|__]

Name of participant (Please Print): _____

By signing/marking my name here, I confirm what is written above (aged 16 years and above)

x_____	*x_____*	*___/____/_____*
Signature of Participant	*Full Name*	*Date of Signature*

I, the undersigned, have fully explained the relevant information of this program to the person named above and will provide her/him with a copy of this signed and dated informed consent form.

x_____	*x_____*	*___/____/_____*
Investigator/Designee Signature	*Full Name*	*Date of Signature*

If the person giving consent cannot read the form themselves, a witness must be present and sign here:

I was present throughout the entire informed consent process with the participant. This form was read accurately to the volunteer, all questions from the volunteer were answered and the volunteer has agreed to take part in the research.

x_____	*x_____*	*___/____/_____*
Witness Signature	*Full Name*	*Date of Signature*

13HNA ICF FGD V2.0 04AUG16

13HNA: Evaluation of daily activities to understand how these relate to encephalitis

(brain inflammation)

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If you have questions about the study you may contact: *To be decided*

If you have questions about being in a research study, please contact the Research Ethics Board of the NHTD at (phone number) the Clinical Trials Unit at (phone number).

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- O I agree to take part in the above study.

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Name of participant (Please Print): _____

By signing/marking my name here, I confirm what is written above (aged 16 years and above)

x_____	*x_____*	*___/____/_____*
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I, the undersigned, have fully explained the relevant information of this program to the person named above and will provide her/him with a copy of this signed and dated informed consent form.

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If the person giving consent cannot read the form themselves, a witness must be present and sign here:

I was present throughout the entire informed consent process with the participant. This form was read accurately to the volunteer, all questions from the volunteer were answered and the volunteer has agreed to take part in the research.

x_____	*x_____*	*___/____/_____*
Witness Signature	*Full Name*	*Date of Signature*

13HNA CPD V2.1 017OCT16

Please complete this diary for the 3 days the participants wear the GPS. If the participants feel uncomfortable with anything they do not have to include this.

GENERAL INFORMATION:

District of Participant's house	
Commune of Participant's house	
GPS coordinates of participant's house (using Garmin eTrex GPS)	
4. Date of birth of the participant (in dd/mm/yyyy) – if not known state 'not known' or put in mm/yyyy etc.	
5. Gender of the participant	

DIARIES: (Please refer the coding numbers underneath to complete the form. If there are multiple answers please list all)

		Who did you/your child have contact with? (list each person in the boxes below)[1]	What was the type of contact?[2]	What was the age of the person (years)?[3]	Where did you/your child meet the person?[4]	List the locations where you went	What were you/your child doing at those locations?	List what you/your child ate	Was this raw or cooked?
Day 1 Date of interview: __ __/__ __/__ __	Morning (wake up until 12 pm)								

		Who did you/your child have contact with? (list each person in the boxes below)[1]	What was the type of contact?[2]	What was the age of the person (years)?[3]	Where did you/your child meet the person?[4]	List the locations where you went	What were you/your child doing at those locations?	List what you/your child ate	Was this raw or cooked?
Start time of interview: __ __:__ __ End time of interview:	Afternoon (after 12pm until 6 pm)								
	Evening (after 6								

		Who did you/your child have contact with? (list each person in the boxes below)[1]	What was the type of contact?[2]	What was the age of the person (years)?[3]	Where did you/your child meet the person?[4]	List the locations where you went	What were you/your child doing at those locations?	List what you/your child ate	Was this raw or cooked?
__ __:__ __	pm until bedtime)								
Day 2: Date of interview: __ __/__ __/__ __	Morning (wake up								

		Who did you/your child have contact with? (list each person in the boxes below)[1]	What was the type of contact?[2]	What was the age of the person (years)?[3]	Where did you/your child meet the person?[4]	List the locations where you went	What were you/your child doing at those locations?	List what you/your child ate	Was this raw or cooked?
Start time of interview: __ __:__ __	until 12 pm)								
	Afternoon (after 12pm until 6 pm)								

		Who did you/your child have contact with? (list each person in the boxes below)[1]	What was the type of contact?[2]	What was the age of the person (years)?[3]	Where did you/your child meet the person?[4]	List the locations where you went	What were you/your child doing at those locations?	List what you/your child ate	Was this raw or cooked?
End time of interview: __ __:__ __									
	Evening (after 6 pm until bedtime)								

		Who did you/your child have contact with? (list each person in the boxes below)[1]	What was the type of contact?[2]	What was the age of the person (years)?[3]	Where did you/your child meet the person?[4]	List the locations where you went	What were you/your child doing at those locations?	List what you/your child ate	Was this raw or cooked?
Day 3: Date of interview: __ __/__ __/__ __ Start time of interview:	Morning (wake up until 12 pm)								

		Who did you/your child have contact with? (list each person in the boxes below)[1]	What was the type of contact?[2]	What was the age of the person (years)?[3]	Where did you/your child meet the person?[4]	List the locations where you went	What were you/your child doing at those locations?	List what you/your child ate	Was this raw or cooked?
__ __:__ __ End time of interview: __ __:__ __	Afternoon (after 12pm until 6 pm								

		Who did you/your child have contact with? (list each person in the boxes below)[1]	What was the type of contact?[2]	What was the age of the person (years)?[3]	Where did you/your child meet the person?[4]	List the locations where you went	What were you/your child doing at those locations?	List what you/your child ate	Was this raw or cooked?
	Evening (after 6 pm until bedtime)								

1. (1) Family members (2) Friend (3) Other (777) Don't know (999) Prefer not to answer

2. (1) Face to face conversation (2) Skin to skin touch (777) Don't know (999) Prefer not to answer

3. (777) Don't know (999) Prefer not to answer

4. (1) Home (2) Work (3) School (4) Other (777) Don't know (999) Prefer not to answe

	Day 1	**Day 2**	**Day 3**
1. Did you/your child have any problems with the GPS device?	☐ Yes ☐ No ☐ Prefer not to answer **If yes, what were these problems?**	☐ Yes ☐ No ☐ Prefer not to answer **If yes, what were these problems?**	☐ Yes ☐ No ☐ Prefer not to answer **If yes, what were these problems?**
2. During the day did you/your child forger to wear the device?	☐ Yes ☐ No ☐ Prefer not to answer	☐ Yes ☐ No ☐ Prefer not to answer	☐ Yes ☐ No ☐ Prefer not to answer

2a. If yes, for how long did you/your child forget to wear it?	☐ Less than one hour ☐ Between one and six hours ☐ Between 7 and 24 hours ☐ More than 24 hours ☐ Don't know ☐ Prefer not to answer	☐ Less than one hour ☐ Between one and six hours ☐ Between 7 and 24 hours ☐ More than 24 hours ☐ Don't know ☐ Prefer not to answer	☐ Less than one hour ☐ Between one and six hours ☐ Between 7 and 24 hours ☐ More than 24 hours ☐ Don't know ☐ Prefer not to answer
3. Did you/your child remove it purposefully for a period of time?	☐ Yes ☐ No ☐ Prefer not to answer	☐ Yes ☐ No ☐ Prefer not to answer	☐ Yes ☐ No ☐ Prefer not to answer
3a. If yes, for how long did you/your child remove it?	☐ Less than one hour ☐ Between one and six hours ☐ Between 7 and 24 hours ☐ More than 24 hours	☐ Less than one hour ☐ Between one and six hours ☐ Between 7 and 24 hours ☐ More than 24 hours	☐ Less than one hour ☐ Between one and six hours ☐ Between 7 and 24 hours ☐ More than 24 hours

	☐ Don't know ☐ Prefer not to answer	☐ Don't know ☐ Prefer not to answer	☐ Don't know ☐ Prefer not to answer
Why did you/your child remove it?			
Do you have any other comments you would like to make?			

Tables from chapter four.

Table A4.1 Time spent in each of the NDVI quartiles per participant comparing urban to rural participants

Group	Lowest (0-0.25) (median (range))	Lowest-Medium (0.26-0.5) (median (range))	Medium-Highest (0.51-0.75) (median (range))	Highest (0.76-1.0) (median (range))
Urban	1.5 days (4.8 minutes – 7.2 days)	3.4 hours (19.1 minutes – 4.0 days)	6.28 hours (4.1 minutes – 10.9 hours)	12 days (3.0 - 23.5 days)
Rural	5.1 minutes (2.7 minutes – 11.1 minutes)	4.9 days (1.1 – 7.8 days)	6.0 days (2.3 – 10.2 days)	9.0 days (3.0 – 13.0 days)

Table A4.2 Time spent in each of the NDVI quartiles comparing adults to children

Group	Lowest (0-0.25) (median (range))	Lowest-Medium (0.26-0.5) (median (range))	Medium-Highest (0.51-0.75) (median (range))	Highest (0.76-1.0) (median (range))
Adults	1.4 hours (2.7 minutes – 7.2 days)	3.0 days (19.1 hours – 7.8 days)	5.4 days (41.0 minutes – 10.2 days)	10.5 days (3.0 – 23.5 days)
Children	2.4 days (4.8 minutes – 2.9 days)	3.6 days (51.5 minutes – 6.5 days)	5.8 days (4.1 minutes – 9.7 days)	9.2 days (3.0 hours – 17.6 days)

Table A4.3 The time spent in each of the NDVI quartiles comparing males to females.

Group	Lowest (0-0.25) (median (range))	Lowest-Medium (0.26-0.5) (median (range))	Medium-Highest (0.51-0.75) (median (range))	Highest (0.76-1.0) (median (range))
Males	48.9 minutes (2.7 minutes – 2.9 days)	3.6 days (1.3 hours - 7.4 days)	5.5 days (4.1 minutes-10.2 days)	10.1 days (3.0 hours-23.5 days)
Females	2.5 hours (8.1 minutes – 7.2 days)	3.2 days (19.1 minutes – 7.8 days)	5.8 days (41.0 minutes – 9.7 days)	9.0 days (3.4 hours – 21.1 days)

Table A4.4 The time spent in each of the NDVI quartiles between seasons.

Group	Lowest (0-0.25) (median (range) per participant)	Lowest-Medium (0.26-0.5) (median (range))	Medium-Highest (0.51-0.75) (median (range))	Highest (0.76-1.0) (median (range))
Spring	3.9 hours (1.4 minutes – 4.1 days)	2.1 hours (2.4 minutes – 3.8 days)	5.8 days (2.8 hours - 8.1 days)	5.1 days (1.4 hours - 6.0 days)
Summer	5.4 minutes (5.4 - 5.4 minutes)	2.3 minutes (21 seconds – 4.9 minutes)	4.4 minutes (4.1 minutes – 4.8 minutes)	6.0 days (24.1 hours – 6.7 days)
Autumn	2.5 minutes (6 seconds – 8.0 minutes)	13.3 hours (51.5 minutes – 4.0 days)	2.1 days (27.9 minutes -3.7 days)	3.2 days (2.0 minutes – 7.0 days)
Winter	16.7 hours (3.1 minutes – 3.3 days)	2.7 days (54 seconds – 4.9 days)	36.1 minutes (20.8 – 51.3 minutes)	3.0 days (17.5 hours – 8.7 days)

Table A4.5 Time spent in each of the NDVI quartiles comparing times of the day.

Group	Lowest (0-0.25) (median (range) per participant)	Lowest-Medium (0.26-0.5) (median (range))	Medium-Highest (0.51-0.75) (median (range))	Highest (0.76-1.0) (median (range))
Morning	10.9 hours (2.3 minutes – 32.8 hours)	21.0 hours (18 seconds – 53.7 hours)	1.7 days (15.7 minutes – 2.9 days)	3.1 days (14.0 hours – 8.2 days)
Afternoon	7.1 hours (1.7 minutes – 2.5 days)	18.0 hours (4.5 minutes – 1.8 days)	1.3 days (43.5 minutes – 2.6 days)	2.3 days (9.8 minutes -5.9 days)
Evening	5.0 hours (2.3 minutes – 1.7 hours)	11.4 hours (24.6 minutes– 22.5 hours)	15.0 hours (1.9 minutes – 26.9 hours)	1.1 days (6.0 hours – 4.5 days)

Table A4.6 The number of non-physical contacts by age category.

Non-physical contact (face to face)						
Age group (years)	**Number of participants**	**Total number of contacts**	**Number of contacts per participant over the study period**		**Number of contacts per participant per day**	
			Median (range)	**p value**	**Median (range)**	**p value**
0-5	6	1306	205 (188 – 269)	-	8 (1-21)	-
6-12	14	3917	279.5 (91 -398)	0.139	13 (1-30)	<0.001
13-17	4	897	217.5 (129 -333)	0.895	10 (1-22)	0.686
18-35	8	2474	302 (127-451)	0.061	14 (1-30)	<0.001
36-60	12	3257	270.5 (85-514)	0.205	11 (1-28)	<0.001
61 and over	4	953	215 (187-336)	0.688	9 (2-24)	0.303

Table A4.7 The number of physical contacts by age category.

Physical contact (touch)						
Age group (years)	**Number of participants**	**Total number of contacts**	**Number of contacts per participant over the study period**		**Number of contacts per participant per day**	
			Median (range)	**p value**	**Median (range)**	**p value**
0-5	6	311	42.5 (2-127)	-	5.5 (1-17)	-
6-12	14	1173	84 (3-229)	0.234	4 (1-24)	0.086
13-17	4	253	51 (42-109)	0.709	3 (1-13)	<0.001
18-35	8	465	70 (3-147)	0.590	5 (1-30)	0.075
36-60	12	629	59 (14-132)	0.651	4 (1-28)	<0.001
61 and over	4	80	18.5 (3-40)	0.079	2 (1-6)	<0.001

Table A4.8 Number of non-physical contacts by gender.

Non-physical contact (face to face)						
Gender	**Number of participants**	**Total number of contacts**	**Number of contacts per participant over the study period**		**Number of contacts per participant per day**	
			Median (range)	**p value**	**Median (range)**	**p value**
Female	24	6283	239 (85-451)	-	10 (1-30)	-
Male	24	6521	273 (91-514)	0.246	12 (1-28)	<0.001

Table A4.9 Number of physical contacts by gender.

Physical contact (touch)						
Gender	**Number of participants**	**Total number of contacts**	**Number of contacts per participant over the study period**		**Number of contacts per participant per day**	
			Median (range)	**p value**	**Median (range)**	**p value**
Female	24	1633	63 (2-179)	-	4 (1-24)	-
Male	24	1278	51 (3-229)	0.934	3 (1-20)	0.019

Table A4.10 The number of non-physical contacts by urban and rural participants.

Non-physical contact (face to face)						
	Number of participants	Total number of contacts	Number of contacts per participant over the study period		Number of contacts per participant per day	
			Median (range)	p value	Median (range)	p value
Urban	24	6974	273 (156-514)	-	13 (1-30)	-
Rural	24	5830	237.5 (85-410)	0.080	10 (1-30)	<0.001

Table A4.11 The number of physical contacts by urban and rural participants.

Physical contact (touch)						
	Number of participants	Total number of contacts	Number of contacts per participant over the study period		Number of contacts per participant per day	
			Median (range)	p value	Median (range)	p value
Urban	24	1553	65 (3-127)	-	3 (1-15)	-
Rural	24	1358	34 (2-229)	0.733	5 (1-24)	<0.001

Table A4.12 The number of non-physical contacts by urban and rural participants.

Non-physical contact (face to face)						
	Number of participants	Total number of contacts	Number of contacts per participant over the study period		Number of contacts per participant per day	
			Median (range)	p value	Median (range)	p value
Weekdays	48	8757	182 (58-360)	-	11.5 (1-30)	-
Weekends	48	4047	79 (20- 154)	<0.001	11 (1-28)	0.122

Table A4.13 The number of physical contacts by urban and rural participants.

Physical contact (touch)						
	Number of participants	Total number of contacts	Number of contacts per participant over the study period		Number of contacts per participant per day	
			Median (range)	p value	Median (range)	p value
Weekdays	48	1961	37 (2-166)	-	4 (1-24)	-
Weekends	48	950	25.5 (4-63)	0.003	4 (1-23)	0.887

Table A4.14 The number of non-physical contacts by season.

Non-physical contact (face to face)						
	Number of participants	**Total number of contacts**	**Number of contacts per participant over the study period**		**Number of contacts per participant per day**	
			Median (range)	**p value**	**Median (range)**	**p value**
Spring	48	3713	75 (19-170)	-	12 (1-30)	-
Summer	45	2622	56 (3-125)	0.009	11 (1-24)	<0.001
Autumn	48	3397	69.5 (7-130)	0.405	12 (1-24)	0.056
Winter	48	3072	63.5 (2-119)	0.077	9 (1-30)	<0.001

Table A4.15 The number of physical contacts by season.

Physical contact (touch)						
	Number of participants	**Total number of contacts**	**Number of contacts per participant over the study period**		**Number of contacts per participant per day**	
			Median (range)	**p value**	**Median (range)**	**p value**
Spring	48	346	14 (2-31)	-	3 (1-9)	-
Summer	45	1044	22.5 (1-74)	<0.001	5 (1-20)	<0.001
Autumn	48	592	13 (1-88)	0.139	3 (1-24)	<0.001
Winter	48	929	18 (2-90)	0.029	4 (1-23)	<0.001

Table A4.16 The number of non-physical contacts by time of the day.

Non-physical contact (face to face)						
	Number of participants	**Total number of contacts**	**Number of contacts per participant over the study period**		**Number of contacts per participant per day**	
			Median (range)	**p value**	**Median (range)**	**p value**
Morning	48	4917	96.5 (31-207)	-	4 (1-10)	-
Afternoon	48	4653	92.5 (32-196)	0.499	4 (1-10)	0.006
Evening	48	3234	64.5 (180125)	<0.001	3 (1-10)	<0.001

Table A4.17 Number of physical contacts by time of the day.

Physical contact (touch)						
	Number of participants	**Total number of contacts**	**Number of contacts per participant over the study period**		**Number of contacts per participant per day**	
			Median (range)	**p value**	**Median (range)**	**p value**
Morning	48	963	17.5 (1-99)	-	2 (1-10)	-
Afternoon	48	920	21 (2-77)	0.476	2 (1-10)	0.399
Evening	48	1028	22 (1-71)	<0.001	2 (1-10)	0.330

APPENDIX 5.1

A summary of the JEV cELISA Methods from CSIRO

1. The ELISA plate is coated with JEV antigen.

2. The test and control sera are diluted to 1/10.

3. The plate is washed.

2. The diluted sera are added to each well.

3. JEV 989 monoclonal antibody is added to the wells.

4. The anti-mouse horseradish peroxidase (HRPO) conjugate is diluted to 1/400.

5. The plate is washed.

6. The diluted anti-mouse HRPO conjugate is added to the wells.

7. The plate is washed.

8. The tetramethylbenzidine (TMB) substrate is added to the wells followed by sulphuric acid.

9. Sera with percentage inhibition of greater than 50% are classified as positive, less than 40% as negative and 40-50%, suspected positive.

APPENDIX 5.2

Standard Operating Procedure **SOP**	Center for Vaccine Development Mahidol University	SOP No. : 63 Effective Date: 11 Jul 2012
Title: Plaque Reduction Neutralization Test for JE Viruses in LLC-MK2 Cells		

Printed Name	Title	**Signature**	**Date**
Preparer: Mr.Kamolchanok Tubthong	Medical Research Technologist		
Reviewer: Sutee Yoksan, M.D.,Ph.D.	Director, CVD		
Approved by: Sutee Yoksan, M.D.Ph.D.	Director, CVD		

1. PRINCIPLE

These procedures describe the method for determination of neutralizing antibodies against Japanese Encephalitis viruses in test sera, including sera from vaccination and infections.

Neutralization tests conducted in cell culture may be applied in two ways to the laboratory diagnosis of viral infections. First, virus strains isolated from the patient may be identified by demonstrating the ability of known, specific immune serum to neutralize the infective capacity of the virus, i.e., to prevent a CPE, plaque formation, or other manifestations of infection in cell cultures. Secondly, infections, and vaccinations may be diagnosed by demonstrating a significant increase in neutralizing antibody for a given virus between acute- and convalescent- phase serum specimens collected from the patient.

In cell culture systems, serum specimens are generally assayed for neutralizing antibody content by testing serial dilutions of serum against a standard dose (usually 100 $TCID_{50}$) of the virus. This is referred to as the constant virus-varying serum technique, and it is more useful for demonstrating significant increases in neutralizing antibody than is the constant serum-varying virus procedure in which undiluted serum is tested against serial dilutions of virus. In

the constant virus-varying serum system the antibody titer is expressed as the highest serum dilution, which neutralizes the test dose of virus.

2. SPECIMEN COLLECTION / TREATMENT

3. EQUIPMENT / REAGENTS

a. Equipment

i. Pipettes, sterile, multi-PK, 1 ml

ii. Pipettes, sterile, 5 ml

iii. Pipettes, sterile, 10 ml

iv. 6-well Cluster Plates

v. Biosafety Cabinet (Laminar flow hood)

vi. 37°C, 5% CO_2 Incubator

vii. 13 × 100 mm Sterile Glass Tubes, capped Pyrex

viii. 12 × 75 mm Sterile Polyethylene tube, capped. Falcon/Cat. 2063

ix. 37°C water bath

x. 56°C water bath

xi. Light Box

xii. Vortex mixer

xiii. Rocker platform, Bellco glass, inc./7740-20000

b. Reagents

i. CELLS
LLC-MK2 cells (ATCC)

ii. Virus Diluent : MEM + 10% heat-inactivate Fetal Bovine Serum (FBS) + Pen-Step 300 units, adjust pH to 7.4-7.8 with 7.5% $NaHCO_3$
PBS, pH 7.5 with 20% heat-inactivated Fetal Bovine Serum

iii. Reference viruses: (Vaccine strain)
Japanese encephalitis JEV (Beij)

iv. Standard positive sera :

Note: Standard positive sera $PRNT_{50}$ VS JE as follows:

Virus	$PRNT_{50}$
JE (Beij)	830

Normal Human serum control (Donor) PRNT titer < 1:10

v. Test sera, heat-inactivated 56°C [54°C to 58°C], 30 minutes
vi. Growth medium for LLC-MK2 cells:
Fetal Bovine Serum
100 ml

(heat-inactivated at 56°C [54°C to 58°C], 30 minutes)

L-glutamine
5.0 ml

Penicillin-Streptomycin mixture
5.0 ml

(10,000 units/ml of Penicillin G, and 10,000 ug/ml

of streptomycin sulfate in 0.85% saline)

M-199 to
500 ml

Adjust pH to 7.4 ± 0.1 with 7.5% Sodium bicarbonate

vii. Overlayer medium

100	ml	MEM (2 X)
10	ml	∇ FCS
1	ml	L-glutamine
2	ml	P & S
50	ml	3.0% CMC
3	ml	Neutral Red

4. CALIBRATION

5. QUALITY CONTROL

The number of plaques in each assay plate is critical to avoid the "plaque overlap phenomenon." Generally, between 40-60 plaques in a 6 well plate.

In each assay, checked titration of virus control must be done. This helps to check or confirm the number of plaques in serum/plasma-virus mixtures. Each assay has duplicate virus control in each plate.

At least two replication wells should be included for all test serum/plasma for reliability.

6. PROCEDURES

a. Assay in LLC-MK2 Cells

i. Preparation of LLC-MK2 cells: seed in a 6 well plate, 4 ml of 1.0×10^5 cells per well, cells will form monolayer in 6-8 days at 35°C [33°C to 37°C] in 5% CO_2 incubator.

ii. Preparation of serum dilution (prepare in advance)

1. In an ice-bath, prepare ten fold dilutions of the test sera with the virus diluent as follow.

	Tube 1		Tube 2		Tube 3
Diluent ml	0.4		0.9		0.9
Test Serum ml	0.1 mix	→	0.1 mix	→	0.1 mix
Dilution	1:5		1:50		1:500

Start with 1:5 and continue on the dilution required to encompass the expected 50% plaques reduction end point (3dilutions are standard)

2. Transfer 0.3 ml each of each dilution to new tube. For virus control, place 0.3 ml each of virus diluent into each extra empty tubes (for JE). Store at -20°C (-25°C to -15°C). The diluted samples should be used within 7-10 days.

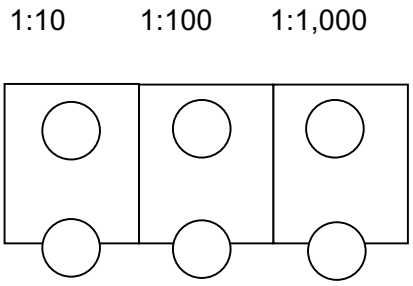
iii. Serum-virus mixtures:

1. Bring the diluted sera from storage (6.1.2.2), place in an ice-bath then add an equal volume (0.3 ml) of the virus suspension, diluted to contain approximately 50 pfu/0.2 ml (for 6 well plate) to each tube. For virus control add 0.3 ml each.
2. Shake rack of tubes thoroughly then incubate in 37°C water bath for one hour.
3. Place rack of tubes in an ice-bath, the virus-serum mixture and virus control are ready to be inoculated on cells.

iv. Inoculation of serum-virus mixture onto LLC-MK2 cells

1. Discard all growth medium of LLC-ML2 in 6 well plates (or Flask)
2. Inoculate 0.2 ml of each virus-serum mixture on to a well of cell sheet of prepared LLC-MK2 monolayer, 2 wells per dilution. 2 wells each for virus control.
3. Let absorption for 90 min at 37°C with continuous rocking to distribute the fluid.
4. Discard excess of serum-virus mixture.

5. Add 4.0 ml of overlay medium into each well. Incubate plates 37°C [33°C to 37°C] in 5% CO_2 atmosphere incubator, 7 days JE
6. Plaque counting: count numbers of plaque in each well after 7days.



7. RESULTS REPORTING AND INTERPRETATION

Determination of plaque neutralizing titer ($PRNT_{50}$): The neutralizing activity of a test serum is determined by its ability to reduce the number of virus plaques as to compare to the number seen in control cultures. A reduction of plaque count of 50% is taken as the neutralizing end point. This can be done by plotting the percentage of plaques observed at each dilution of test sera on probability x 2 cycle logarithmic paper or using SPSS Program. The 50% end point is that dilution at which the line of best fit (by visual inspection) crosses the 50% probit line.

8. PROCEDURAL NOTES

Any changes, suggestions, or recommendations for the SOP should be sent to Director, CVD.

9. LIMITATIONS/INTERFERENCES

If the number of control plaques do not match with the check titration of control, or the number of control plaques is not in the range, the test should not be accepted.

10. REFERENCES

Russell, P.K. Nisalak, A., Sukhavachana, P., and Vivona, S., 1967. A plaque reduction test for dengue neutralizing antibodies. J. Immunol. 99: 285-290.

APPENDIX 5.3

Table A5.1 Univariate logistic regression using a GLM to compare the type of farm from which the pig originated with the seroprevalence of JEV.

Variable	**Random effects**	**Seropositive (N (%, 95%CI))**	**Seronegative (N (%, 95%CI)**	**Odds ratio (95%CI)**	**p value**	**Monte-Carlo simulation of Moran I for spatial autocorrelation of residuals**
Industrial farm	Nil	150 (61.7%, 55.3-67.8%)	54 (30.5%, 23.9-37.9%)	0.71 (0.47-1.07)	0.1	statistic = -0.006 p=0.724
Familial farm* (baseline)		123 (69.5%, 62.1-76.1%)	93 (38.3%, 32.2-44.7%)	NA		

Table A5.2 Univariate logistic regression using an INLA model comparing the type of farm from which the pig originated with the seroprevalence of JEV with month fitted as a random effect.

Variable	**Mean posterior estimate**	**SD**	**2.5 percentile**	**97.5 percentile**
Industrial farm	-0.139	0.043	-0.224	-0.054

Table A5.3 Univariate logistic regression using GLM to compare the environmental suitability for *Cx. tritaeniorhynchus* with the seroprevalence of JEV in pigs.

Variable	Random effects	Seropositive (mean)	Seronegative (mean)	Odds ratio (95%CI)	p value	Monte-Carlo simulation of Moran I for spatial autocorrelation of residuals
Environmental suitability for *Cx. triataeniorhynchus*	Nil	0.49	0.47	7.15 (2.53-20.51)	<0.001	statistic = 0.006 p=0.007

Table A5.4 Univariate logistic regression using as an INLA model to compare the environmental suitability for *Cx. tritaeniorhynchus* with seroprevalence with province and month fitted as random effects.

Variable	Mean posterior estimate	SD	2.5 percentile	97.5 percentile
Suitability	0.425	0.313	-0.185	1.063

Table A5.5 Univariate logistic regression using GLM to compare the incidence of human AES with the seroprevalence of JEV in pigs.

Variable	Random effects	Seropositive (mean (SD))	Seronegative (mean (SD))	Odds ratio (95%CI)	p value	Monte-Carlo simulation of Moran I for spatial autocorrelation of residuals
Incidence of human AES (per 100,000 population)	Nil	0.09 (0.23)	0.06 (0.13)	3.23 (1.61-7.29)	0.002	Statistic = 0.012 p = 0.001

Table A5.6 Univariate logistic regression using an INLA model to compare the incidence of AES with the seroprevalence of JEV in pigs with province and month fitted as random effects.

Variable	Mean posterior estimate	SD	2.5 percentile	97.5 percentile
Incidence of AES	0.01	0.00	0.00	0.01

Table A5.7 Univariate logistic regression using GLM to compare the incidence of human AES with the seroprevalence of JEV in pigs.

Variable	Random effects	Seropositive (mean (SD))	Seronegative (mean (SD))	Odds ratio (95%CI)	p value	Monte-Carlo simulation of Moran I for spatial autocorrelation of residuals
Incidence of human JE (per 100,000 population)	Nil	0.03 (0.07)	0.03 (0.07)	1.53 (0.34-7.40)	0.59	Statistic = 0.012 p=0.001

Table A5.8 Univariate logistic regression using an INLA model to compare the incidence of human JE with the seroprevalence of JEV in pigs with province and month fitted as random effects.

Variable	Mean posterior estimate	SD	2.5 percentile	97.5 percentile
Incidence of JE	-0.26	0.24	-0.72	0.20

Table A5.9 Univariate logistic regression using GLM to compare vaccination coverage against JE with the seroprevalence of JEV in pigs.

Variable	Random effects	Seropositive (mean (SD))	Seronegative (mean (SD))	Odds ratio (95%CI)	p value	Monte-Carlo simulation of Moran I for spatial autocorrelation of residuals
JE vaccination coverage (%)	Nil	95.49 (8.45)	96.69 (2.54)	1.00 (1.00-1.01)	0.164	Statistic = 0.012 p=0.001

Table 5.10 Univariate logistic regression using INLA to compare vaccination coverage against JE with the seroprevalence of JEV in pigs including province and month fitted as random effects.

Variable	Mean posterior estimate	SD	2.5 percentile	97.5 percentile
JE vaccination coverage (%)	0.00	0.00	-0.00	0.00

Table A5.11 The output of multivariate model 1 with an outcome of seropositivity of JEV in pigs.

	Mean	SD	0.025quant	0.975quant

(Intercept)	1.927	0.195	1.541	2.309
FarmIndustrial	-0.16	0.048	-0.256	-0.067
Environmental.suitability	0.135	0.269	-0.347	0.711
JE	-0.571	0.397	-1.354	0.204
JE.vaccination	-0.003	0.002	-0.007	0.001
Precision for the logistic observations	2.31	0.099	2.12	2.51
Precision for idregion (iid component)	1361.19	1560.28	44.64	5506.76
Precision for the logistic observations	1808.83	1821.115	117.95	6642.56
Precision for month	17.57	8.43	6.16	38.41

Table A5.11 The output of multivariate model 2 with an outcome of seropositivity of JEV in pigs.

	Mean	**SD**	**0.025quant**	**0.975quant**
(Intercept)	1.927	0.195	1.539	2.309
FarmIndustrial	-0.144	0.048	-0.241	-0.051

Environmental.suitability	0.013	0.284	-0.482	0.677
AES	-0.015	0.007	-0.028	-0.002
JE.vaccination	-0.002	0.002	-0.006	0.001
Precision for the logistic observations	2.33	0.103	2.12	2.52
Precision for idregion (iid component)	18.28	9.61	6.5	43.04
Precision for the logistic observations	898.01	1088.969	13.75	3878.47
Precision for month	1702.86	1763.961	102.11	6411.65

Table A5.12 The output of multivariate model 3 with an outcome of seropositivity of JEV in pigs.

	Mean	**SD**	**0.025quant**	**0.975quant**
(Intercept)	1.48	0.153	1.168	1.778
Environmental.suitability	0.395	0.307	-0.205	1.019

JE	-0.215	0.237	-0.685	0.247
JE.vaccination	0	0	-0.001	0
Precision for the logistic observations	2.13	0.049	2.04	2.23
Precision for idregion (iid component)	124.75	80.286	29.79	330.78
Precision for the logistic observations	1962.2	1888.564	127.07	6950.66
Precision for month	50.76	26.59	17.66	118.88

Table A5.13 The output of multivariate model 3 with an outcome of seropositivity of JEV in pigs.

	Mean	**SD**	**0.025quant**	**0.975quant**
(Intercept)	1.436	0.148	1.132	1.723
Environmental.suitability	0.47	0.299	-0.112	1.083
AES	0.008	0.003	0.003	0.013
JE.vaccination	0	0	-0.001	0
Precision for the logistic observations	2.14	0.049	2.04	2.23
Precision for idregion (iid component)	140.57	102.703	34.6	411.27
Precision for the logistic observations	1828.13	1825.579	128.81	6634.56
Precision for month	53.56	27.122	18.81	122.34