Multi-centre EuRopean study of MAjor Infectious Disease Syndromes

Arboviral compatible febrile illness

Protocol Version 2.3

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MERMAIDS

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2.0	10-May-2016	• Revised the inclusion criteria to improve clarity. Changed 'rapid onset' to 'recent onset' and removed the symptom of 'backache'. • Removed 'post transfusion temperature' from exclusion criteria • Revised the sections relating to the collection of CSF to indicate that aliquots of CSF collected routinely on any day of hospitalisation are to be collected for the study, if possible. • Added urine sampling at Day 0, 7 and 28 • Extended the window of the Day 28 and 60 visits to -3 / +7 days • Specified that central lab must be notified of all confirmed cases of CCHFV to arrange category A shipment of samples • Removed reference to Modified Rankin outcome score • Specified that participants may be reimbursed for their time and travel expenses up to a maximum of 25 EUR. Receipts will not be required. For full details see: MERMAIDS_Protocol_Arboviruses_V2.0_summary_of_changes.docx
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2.2	22-May-2017	• Removed the list of countries to be included in the study • Removed months that specify recruitment period
2.3	30-Jul-2018	• Extended study duration from 36 to 48 months

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Multi-centre EuRopean study of MAjor Infectious Disease Syndromes (MERMAIDS): Arboviral compatible febrile illness

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Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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1. SYNOPSIS

Study Title	Multi-centre EuRopean study of MAjor Infectious Disease Syndromes (MERMAIDS) : Arboviral compatible febrile illness
Ref. no. / short title	MERMAIDS
Study Design	Prospective, observational study
Study Participants	Adults (≥18 years old) admitted to hospital with a febrile illness compatible with an arbovirus infection
Planned Sample Size	1500 adults
Planned Study Period	48 months

	Objectives	**Outcome measures**
Primary objective	To estimate the proportion of adult hospital admissions with a febrile illness in South East Europe that are attributable to four arbovirus infections: West Nile Virus (WNV), Toscana virus (TOSV), Tick borne encephalitis virus (TBEV) and Crimean Congo haemorrhagic fever virus (CCHFV).	Proportion of adults hospitalised with a clinically compatible illness who have laboratory confirmed or probable TBEV, WNV, TOSV or CCHFV infection.
Secondary objectives	▪ To document treatment, clinical management and outcomes of TOSV, WNV, TBEV and CCHFV infections (in adults ≥ 18 years old) requiring admission to hospital by region ▪ To analyse severity of disease in relation to demographics ▪ To characterise antibody levels	▪ Proportion of patients treated with antivirals, antibiotics and/or steroids ▪ Daily clinical observations (vital signs, neurological and haemorrhagic symptoms) during admission ▪ Level of consciousness (Glasgow Coma Scale in Adults) (Day 0) ▪ Length of stay in hospital ▪ Proportion of cases requiring Intensive Care Unit (ICU)/Critical Care Unit/High Care Unit (HCU) admission ▪ Antibody levels (Day 0, 7, 28, 60)
	▪ To analyze health outcomes and burden of disease in relation to severity of disease and demographics	▪ Neurological recovery and health outcomes at discharge and follow up (day 28 and 60) using short health outcomes questionnaires. Mortality at Day 60

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2. ABBREVIATIONS

CCHFV	Crimean-Congo haemorrhagic fever virus
CI	Chief Investigator
CNS	Central nervous system
CRF	Case Record Form
CSF	Cerebrospinal fluid
CRP	C-reactive Protein
EDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
EQ-5D	European Quality of Life-5 Dimensions
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
ICH	International Conference of Harmonisation
PCR	Polymerase chain reaction
PI	Principal Investigator
REC	Research Ethics Committee
RO	Research Online
SOP	Standard operating procedure
SSC	Study Steering Committee
SMG	Study Management Group
SST	Serum-separating tube
TBEV	Tick-borne encephalitis virus
TOSV	Toscana virus
WNV	West Nile virus

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3. BACKGROUND AND RATIONALE

PREPARE (Platform for European Preparedness Against (Re-)emerging Epidemics) is a European Commission funded network for harmonised large-scale clinical research studies on infectious diseases. The network activities will result in preparedness to rapidly respond to any severe ID outbreak, providing real-time evidence for clinical management of patients and for informing public health responses. For more information on PREPARE please visit our website (www.prepare-europe.eu).

This study, part of the Multi-centre EuRopean study of MAjor Infectious Disease Syndromes (MERMAIDS), comprises work package 3 of the PREPARE platform. The aim of the observational study is to prospectively study the aetiology, clinical management and impact of febrile illnesses that are compatible with arthropod born viral infections (arboviruses) in the South East region of Europe. The study will focus on four arboviruses – West Nile Virus (WNV), tick-borne encephalitis virus (TBEV), Toscana virus (TOSV) and Crimean Congo haemorrhagic fever virus (CCHFV).

During the last decade a number of arboviruses have emerged in Europe [1-4] and are growing public health challenges. Large Europe-wide outbreaks of novel arbovirus infections in the veterinary world, e.g. blue-tongue in 2006 and Schmallenberg in 2012, have caused huge economic losses. [5] The colonization of Europe by the exotic mosquito *Aedes albopicus,* imported via used tires resulted in autochthonous outbreaks of Dengue in Croatia (2010) and France (2010 and 2014) and Chikungunya in Italy (2007) and France (2010 and 2014). Dengue virus also caused an outbreak with over 1300 cases on Madeira in 2012. [2, 6-12] An increase in global travel, trade, mass migration, socio-economic change, and climate change poses a risk for the introduction of emerging and re-emerging infections with epidemic potential in Europe. [13]

Despite the known presence of significant arbovirus related disease in Southeast Europe (as outlined below), there are gaps in data and no systematic study of arboviral infections across the sub-region. This uncertainty about the current extent and burden of arboviral illness represents a European vulnerability to the detection of the emergence and spread of arboviruses. This study aims to address the knowledge gaps and provide the baseline for detecting and assessing arboviral emergence in Europe.

3.1 West Nile virus

West Nile virus (WNV), present in Europe since the 1960's, has caused outbreaks with clusters of neurological illness in parts of Europe and re-emerged with an outbreak in Romania 1996, and subsequent outbreaks in France, Greece, Italy and Hungary in the last decade. [14] Transmission to humans occurs primarily through mosquito bites and can cause neuro-invasive disease (meningitis, encephalitis) in some (<1%). About 20% of people who become infected will develop West Nile fever, whereas 80% of cases are thought to be asymptomatic. [15]

WNV is endemic in the south-east of Europe and EU level notification rates have increased steadily in later years in all countries but Greece, which already shows high rates. The EU notified rate of autochthonous cases was 0.07 per 100 000 population in 2012, with the highest autochthonous rate observed in Greece (1.46 per 100 000) and the lowest in Italy (0.05 per 100 000). In Bulgaria, Hungary and Romania the rates were 0.06, 0.17 and 0.08 per 100 000 population, respectively.

The overall EU reported case-fatality rate in 2012 was 9.4 % (n=22). [14] WNV infections have also been picked up in new blood donor screening programmes in Italy and Greece, and transfusion transmission has been reported causing concerns for blood bank donations and supplies during epidemic seasons. [16] The geographic distribution of recognized WNV cases shows gaps, suggesting that the disease is present but unrecognized in some areas and hospitals.

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3.2 Tick-borne encephalitis virus

TBEV, a viral infectious disease, transmitted by *Ixodus* spp. ticks and unpasteurized milk, is another growing challenge. TBEV affects the central nervous system and can in some result in long-term neurological symptoms requiring long hospitalisations and recovery time. The European subtype is associated with mortality rates of 0.5–2%. [14] Two thirds of infections are thought to be asymptomatic. The number of cases in all endemic regions of Europe has increased by almost 400% in the last 30 years; the risk areas have increased and new foci have been discovered. [14]

There were 2106 EU notified confirmed cases in 2012. However, 10 countries, including Bulgaria and Italy, did not report. Countries with reported increased risk of TBEV includes; Austria, the Czech Republic, Estonia, Finland, Germany, Hungary, Latvia, Lithuania, Poland, Slovakia, Slovenia, and Sweden. There is a clear need to strengthen reporting in susceptible countries (e.g. in the Balkan region), to improve diagnostics, and to monitor areas of higher risk of TBEV at the EU level. [14]

3.3 Toscana virus

Sand flies, widespread geographically in many parts of southern Europe, frequently transmit Toscana virus (TOSV), one of the leading causes of aseptic meningitis in the summer months in Italy, Spain and France. [17] Since the 80s, TOSV circulation has been increasingly reported in the Mediterranean basin, in Portugal, France, Spain, Greece, Bosnia-Herzegovina, Kosovo, Malta, Cyprus, Turkey, Croatia, Morocco and Tunisia. [17, 18] Seroprevalence studies indicate that a significant proportion of TOSV infections are asymptomatic or present with only mild symptoms. Some, pre-dominantly older people, develop neuro-invasive disease requiring hospitalisation. [18] A study from regions in Italy showed an incidence rate of 17% (n=61 confirmed cases) in hospital patients tested in 2010-12 and seroprevalence of 77% in forestry workers, and 22% in urban populations in one region. [18] A seroprevalence study from Portugal (n=538) targeting patients suspect of vector-borne virus infections in 2004 -08, found that 4.2% of those with neurological signs, and 1.3% of those without neurological signs were found to contain IgG reactive against TOSV. [18] An epidemiological study in Granada, Spain found a 24.9% seroprevalence of TOSV, similar to studies in Italy, while seroprevalence studies from northern Europe report lower rates, showing that TOSV is endemic in the Mediterranean area. [19] In recent years there have been reports of more severe cases with unusual clinical pathologies, e.g. facial paralysis and coma. This could be due to improved diagnostics and/or indicate modification in host, vector, or viral agents, responsible for the unexpected neuro-virulence. [20] There have been limited studies on Toscana virus, even though it is one of the three most common causes of aseptic meningitis in Italy, France and Portugal during the summer months. The extent of TOSV transmission in neighbouring countries in the South East Mediterranean areas is not well characterised.

3.4 Crimean Congo haemorrhagic fever virus

Crimean Congo haemorrhagic fever (CCHFV) is a tick-borne viral disease endemic in the Balkans, which can cause severe haemorrhagic and neurological symptoms in humans, with a reported mortality rate of 2 – 6% in the Balkans. [21] Notifications have increased in endemic areas in South East Europe, with the highest reported number of cases in 2008 in Turkey (n=1154), followed by Bulgaria (n=7) and Greece (n=1). [21] Cases have also been reported in Albania, Kosovo and Serbia. [21] CCHFV can also spread by human – human transmission and nosocomial infections have been reported. With the potential for human-to-human transmission, early detection of cases is essential for the implementation of timely instigation of infection control measures and treatment. [14]

3.5 Treatment and long term outcomes

There are no specific treatments for these viral infections, besides standard supportive care. Ribavirin and cortisone have been used, and for CCHFV convalescent plasma and transfusions, but there is a lack of controlled trials or reviews establishing effectiveness of treatments. Ribavirin is also used as

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prophylaxis for health care workers in contact with CCHFV infected fluids or tissues. There is a vaccine against TBEV and prophylactic vaccinations against CCHFV are used on high risk groups in some countries.

The acute symptoms have been well documented for several arbovirus diseases, but far less is known about the potential long-term clinical and functional sequelae, and risk factors for developing more severe disease. Some people hospitalised with neuro-invasive WNV infection have been shown to develop long-lasting functional sequelae [22, 23] and vaccination programmes against TBEV have been shown to be cost effective[24]. Further studies are needed to assess impact on long term outcomes and long term economic and public health impact of arbovirus disease infections in Europe to inform future treatment and prevention programmes.

3.6 Demographics and risk factors

Risk factors for infections are living in or visiting and spending time outdoors in endemic areas. Other risk factors are working with animals, livestock or slaughter in endemic areas. TBEV has also been linked to consuming unpasteurized milk products in some regions. The highest numbers of cases are seen in the warmer months, ranging from April/May through to Oct/Nov with peaks of reported cases for all four infections seen in July – September. The reported infection rate is slightly higher in men, especially for TBEV. The overall EU notification for TBEV in 2012 was 1.58:1 (0.67 per 100 000 in males and 0.42 per 100 000 in females) and for WNV it was 1.2:1 (0.08 and 0.06 per 100 000). [14]

The highest reported rates are seen in adults, with highest EU notified rates in 2012 for WNV in the ≥ 65 year-old age group (0.22 cases per 100 000), followed by 45–64 year-olds (0.07 cases per 100 000). Only eight WNV cases (3.5 %) were reported among children under the age of 15. For TBEV the highest confirmed case notification rate was in the 45 to 64 year-old age group (0.75 cases per 100 000), followed by the over 65 year-olds (0.58 cases per 100 000). [14] Individual TOSV studies have shown highest number of confirmed cases in adults > 25 years old, with an increase with age seen in seroprevalence studies. [20] There is a need for data on demographics of CCHFV infections.

The data show the increasing pressure and costs of arboviruses on European health and public health systems and the increased geographical spread of arbovirus infections with epidemic potential within Europe over the last decades. It also shows wide variation in reporting and notification of infections within endemic regions. Only TBEV and WNV have EU case definitions. Available surveillance data are patchy in many areas, with some countries in endemic areas not reporting or reporting very few cases.

This shows a clear need to increase awareness of emerging arbovirus infections in endemic regions, and to strengthen surveillance and notification systems and networks across EU to increase early detection, diagnosis and reporting. While observational studies are being undertaken in individual countries, there are limited data on the geographical distribution of arboviruses across the region and especially from some endemic countries in the South East region of Europe.

The objective of this observational study is to identify regional variation and burden of TOSV, WNV, TBEV and CCHFV community acquired infections (in adults ≥ 18 years old) requiring admission to hospital care facilities in South East Europe. Moreover, to document treatment and clinical management of arboviral compatible febrile illnesses across the region. This study will provide a baseline inventory that will support the identification of the extension or intensification of arbovirus transmission.

3.7 Clinical syndromes included in the study:

1. CNS infections
2. Haemorrhagic symptoms
3. Myalgia/arthralgia
4. Non-specific febrile illness

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3.8 Regions

Sites to be included in the study were selected from countries that are part of the Balkan Peninsula in South East Europe, where the viruses and vectors are present in at least part of the country.

Fig 1: CCHFV cases or seroprevalence reported

Fig 2: TBEV cases or seroprevalence reported

Fig 3: TSOV cases or seroprevalence reported

Fig 4: WNV cases or seroprevalence reported

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Fig 5: Countries with two or more of the viruses (CCHFV, TBEV, TSOV or WNV) reported in case or seroprevalence studies

3.9 Primary objective

The primary objective is to estimate the proportion of adult hospital admissions with a febrile illness that are attributable to four arbovirus infections: West Nile Virus, Toscana virus, tick-borne encephalitis virus and Crimean Congo haemorrhagic fever virus.

3.10 Secondary objectives

- To document treatment and clinical management, severity of disease and outcomes by pathogen and demographics (age, sex, ethnicity, region).
- To analyse antibody levels in relation to disease severity and demographics up to day 60 post-admission.
- To review health outcomes and burden of disease (≤60 days) in relation to disease severity and demographics.

3.11 Study design

A prospective, observational study in South East Europe.

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4. PARTICIPANT IDENTIFICATION

4.1 Study Participants

Adults (≥ 18 years old) admitted to hospital facilities at selected sites in South East Europe, with recent onset of symptoms suggestive of possible infection with West Nile virus, Toscana virus, tick-borne encephalitis virus or Crimean Congo haemorrhagic fever virus.

4.2 Inclusion Criteria

Adults (≥ 18 years old) admitted to hospital with recent onset (<21 days) of symptoms of suspected:

- Encephalitis or
- Meningitis

OR

Recent onset of temp.≥ 38˚C of unknown etiology within the past 21 days AND onset of at least ONE of the signs or symptoms below within the past 21 days:

- A neurological symptom (such as: neck stiffness, photophobia, partial paralysis, polyradiculitis, periorbital pain, confusion, altered mental state)
- Severe headache
- Myalgia
- Arthralgia
- Maculopapular rash
- Haemorrhagic symptom
- Thrombocytopenia (<150 000 cells per microliter of blood)

4.3 Exclusion criteria

- Patients with non-infectious central nervous system (CNS) disorders due to hypoxic, vascular, toxic or metabolic causes
- Patients where the symptoms are due to another confirmed cause, such as bacterial infection, malaria, malignancy, immune disorders or trauma
- Patients with a focal source of infection identified, such as pneumonia, viral respiratory tract infection, acute infectious diarrhea, urinary tract infection (positive urine cultures), or skin or soft-tissue infection
- Patients where the symptoms are caused by recurrence of a pre-existing condition

4.4 Definitions of encephalitis and meningitis[1]

4.4.1 Suspected encephalitis

Acute or sub-acute (<21 days) alteration in consciousness, cognition, personality or behaviour*persisting for more than 24 hours AND any TWO of:

a. Fever (≥ 38ºC)
b. Seizures: New onset
c. One or more Focal Neurological Signs (Acute or Sub-acute onset), including:
    - Focal weakness;
    - Oromotor dysfunction;

[1] Adapted from the ENCEPH UK Study, University of Liverpool, The Walton Centre for Neurology and Neurosurgery NHS Trust . www.encephuk.org/index.aspx

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- Movement disorders**including Parkinsonism***;
- Amnesia

d. Pleocytosis: Cerebrospinal fluid white cell count >4 cells/µl
e. Neuroimaging: Compatible with encephalitis
f. Electroencephalogram (EEG): compatible with encephalitis

OR

Clinical suspicion of encephalitis but above investigations have not yet been completed

OR

Clinical suspicion of encephalitis and the patient died before investigations completed

* personality / behaviour change includes: agitation, psychosis, somnolence , insomnia, catatonia, mood lability, altered sleep pattern and (in children): new onset enuresis, or irritability, ** Movement disorder includes: chorea, athetosis, dystonia, hemiballismus, stereotypies, orolingual dyskinesia and tics, ***bradykinesia, tremor, rigidity and postural instability

4.4.2 Suspected meningitis

Illness ≤21 days with:
Headache one or both of:

a. Neck stiffness
b. Photophobia

Excluding those with very sudden onset of severe headache defined as no pain to maximal pain within 1 minute.

OR

Patients with symptoms in whom the clinician feels a lumbar puncture is needed to diagnose/exclude meningitis.

4.5 Screening and Eligibility Assessment

Potential patients for the study will be identified by study staff and assessed against the eligibility criteria listed above and their eligibility recorded in the site study protocol. A 'Screening Log' will be maintained of all the patients who undergo screening regardless of whether they decide to participate in the study. If there is a delay between introduction of the study/initial eligibility assessment and consent, then the recruiter must confirm all eligibility criteria are still met.

If a patient is recruited in the emergency setting it may be necessary to defer consent (section 8.2) for the extra serum sample and CSF sample (when available) in order to get them taken in a timely manner and with routine blood samples. Written consent will be obtained from the patient as soon as is feasible, and within 48 hours. If a patient does not give written consent at this time, the samples will be destroyed.

4.6 Withdrawal from Study

Patients are free to withdraw consent at any time without providing a reason. Patients who wish to withdraw consent for the study will have anonymised data collected up to the point of that withdrawal of consent included in the analyses. The patient will not contribute further data to the study. Data up to the time of withdrawal will be included in the analyses unless the patient explicitly states that this is not their wish.

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4.7 Co-enrolment

Patients can enrol into other non-interventional studies if the study co-coordinating team has been informed and has given their approval.

4.8 Potential risks and benefits

Since this is a non-interventional, observational study, we do not foresee any risks involved in participating in the study, and during the informed consent process the opportunity will be given to understand the objectives and any inconveniences of the study. Participating in the study will not directly benefit the participant, but can provide additional understanding of the aetiology of their illness and provide information about the impact, medical management and outcomes of the disease, which can inform future prevention strategies and clinical management of arboviral infections and research responses to emerging and (re-)emerging infections with epidemic potential.

5. ASSESSMENTS

Following provision of consent, baseline data will be recorded into the patient's Case Record Form (CRF). The information retrieved will include demographics, co-morbidities, clinical observations, findings from physical examinations, investigations and acute management. Vaccination status and risk factors will be recorded in the Case Record Form. Data including results from biological samples, observations, medical management and treatments will be collected throughout the patient's hospital episode and at two follow up appointments using the CRF, according to Table 5.

	Day	0	1	2	3	4	5	6	7 ^	8	9	10	11	12	13	14	28†	60†
										If still hospitalised							Follow up	
	Eligibility screen	X																
	Informed consent	X																
	Baseline data	X																
	Physical examination	X																
Research samples **	CSF sample*	X*																
	EDTA blood	X							X									
	SST (serum)	X							X								X	X
	Urine	X							X								X	
	Clinical observations	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
	Clinical management	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
	Glasgow coma scale	X																
	Health outcome scores								At discharge								X	X

Table 5. Schedule of procedures and samples for the arbovirus study

*CSF samples taken as part of routine care on any day(s) from admission to discharge

^or day before or on day of discharge if discharged before day 7

** research samples sent to the study reference laboratory

† - 3/+7 days

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5.1 Blood, serum and urine samples

The research samples collected for this study will not be used to inform clinical management. The results will not be reported back to the treating clinician in a time frame that will inform patient care, but final results will be reported back to each site by the end of the study. All samples will be stored at the local site according to guidelines for later transport to the study laboratory in Antwerp, where samples will then be distributed to all study laboratories (in Antwerp, Rotterdam and Bonn) for reference testing as below.

5.1.1 Baseline

The research blood, serum and urine samples will be collected at baseline (Day 0). Day 0 is the date of study enrolment. Wherever possible, Day 0 should be the same day as hospital admission. Patients may be enrolled up to three days after admission to hospital. Samples will be prepared and stored according to guidelines for later transport to the study laboratory in Antwerp, where samples will be distributed for reference testing in Antwerp and Rotterdam for WNV, TOSV, TBEV and CCHFV infections using serology and RT-PCR.

5.1.2 During admission

One additional blood, serum and urine sample will be collected during the hospital admission episode. These samples will be collected on day 7, unless the patient is discharged before day 7. Then they will be collected on the day before or the day of discharge. The samples will be prepared and stored for later transport to the study laboratory in Antwerp (as above), for serology and RT-PCR.

5.1.3 Follow up

The patient will be invited back for an additional follow up urine and serum sample on day 28. A final follow up visit will take place on day 60 and serum sample will be collected. In order to facilitate scheduling we are allowing a visit window of three days prior to and up to seven days post day 28 and day 60 respectively for follow-ups. Follow up urine and serum samples will be stored for later transport to the study reference laboratories in Antwerp and Rotterdam for analysis. Short clinical observations and health outcome tests will also be carried out at both follow up visits.

5.2 CSF sample

If CSF is collected as part of standard care, then a CSF sample will also be labelled with the unique patient code allocated at enrolment, and stored according to guidelines for later transport to the reference laboratories in Antwerp and Rotterdam for serology and RT-PCR. If more than one CSF sample is taken, then an aliquot of each sample will be collected for the study if enough sample is available.

5.3 Other routine samples

Results from any other samples taken as part of routine diagnostics and care (blood culture, blood/serum, CSF, nasopharyngeal, stool, urine) by the local hospital site will be analysed locally as per standard care and diagnostic work up. The results of these local tests will also be recorded in the CRF.

5.4 Clinical observations, treatment and interventions

Clinical observations, treatments and medical interventions will be recorded in the CRF on Day 0 (day of study enrolment).

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Specimen	Collection container	Assay	Timing	Archive sample	Centralised/ decentralized testing	Result reported to clinician
Blood	EDTA vacutainer	RT-PCR (TOSV, WNV, TBEC, CCHFV)	Day 0, 7	Y	C	At the end of study
Serum	SST vacutainer	Antibody assays (TOSV, WNV, TBEV, CCHFV)	Day 0, 7, 28, 60	Y	C	At the end of study
Urine	Universal specimen container	PCR (TOSV, WNV, TBEV, CCHFV)	Day 0, 7 and 28	Y	C	At the end of study
CSF	Universal sterile	RT-PCR (TOSV, WNV, TBEV, CCHFV) Antibody assay	Day 0 and at any additional day from Day 0 until discharge	Y	C	At the end of study

Table 6. Study reference laboratory sampling and analysis

5.5 Research reference laboratory assessments

Research samples will be collected at baseline, and on day 7, and additional serum and urine sample on day 28 and serum on day 60. If the patient is discharged before day 7, samples will be collected either the day before or on day of discharge (Table 5). Samples will be collected as part of standard care when possible to minimise patient discomfort.

Samples of blood and serum collected on Day 0 (day of study enrolment) and follow up samples on Day 7, and serum and urine on day 28 and serum on day 60 and, when available, CSF samples will be stored according to guidelines and sent to the reference laboratory in Antwerp, according to guidelines for transport of clinical samples, for diagnostics and serology of WNV, TBEV, CCHFV and TOSV infections. With patient consent, samples will also be stored for future study-related analysis of immunopathogenesis and host genetic factor studies. The samples will be stored in the PREPARE biobank in Antwerp, Belgium, in accordance with Belgian law. These samples may be sent for analysis in other parts of the world if appropriate ethics approvals are in place. **If a patient has a confirmed diagnosis of CCHFV the site must contact the PREPARE lab team to discuss shipment as category A for those samples. All other samples** will be sent as category B. The central lab is responsible for arranging all sample shipping to Antwerp.

Any results from future studies will not be linked back to patient identifiable information. Samples sent to the reference laboratory will be analysed in batches and results reported back to the study site at the end of the study. Samples will be labelled with the unique study participant code given to subjects at enrolment in to the study, and this number will be used when reporting back the final results. Patient identifiable information will not be shared with the reference laboratory.

6. CASE DEFINITIONS

Probable TOSV, TBEV, WNV or CCHFV disease

A case that meets the clinical study inclusion criteria and the following laboratory criteria:

- Virus-specific IgM antibodies in a single clinical specimen.

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Confirmed TOSV, TBEV, WNV or CCHFV disease

A case that meets the clinical study inclusion criteria AND at least ONE of the following laboratory criteria for a confirmed case:

- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in clinical specimen
  OR
- Four-fold or greater change in virus-specific quantitative antibody (IgM and/or IgG) titres in paired sera (acute and convalescent phase)
  OR
- Virus-specific IgM antibodies in serum or CSF with confirmatory virus-specific neutralizing antibodies in the same or a later specimen

  *Serological results will be interpreted according to vaccination status and previous exposure to arboviral infections.

7. OUTCOME MEASURES:

7.1 Primary outcome measures:

- Proportion of enrolled patients with confirmed and probable WNV, TOSV, TBEV and CCHFV infection

7.2 Secondary outcome measures:

- Proportion of patients treated with antivirals, antibiotics and/or steroids
- Daily clinical observations (vital signs, neurological and haemorrhagic symptoms) during admission
- Level of consciousness (Glasgow Coma Scale in adults) (Day 0)
- Length of stay from admission (Day 0)
- Proportion of patients receiving intensive care treatment and duration
- Antibody levels (Day 0, 7, 28, 60)
- Urine antigen levels (Day 0, 7 and 28)
- Health outcomes at discharge and follow up
- Mortality rate at Day 60

8. GENERAL STUDY PROCEDURES

8.1 Recruitment

Local PREPARE study coordinators will identify potential participants from patient presentation at the recruitment site. Participants will be allocated a code at recruitment.

8.2 Informed Consent

The participant must sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed. If, due to their current illness, the participant lacks the capacity to provide consent an appropriate substitute decision maker (a close family member or carer) may act as a proxy. In cases where proxy consent is obtained the participant will be asked to give consent as soon as they regain capacity.

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We will obtain samples as close to admission as possible, and preferably with the admission bloods and/ or lumbar puncture. Due to the emergency nature of suspected CNS infections, blood samples may be taken for study purposes at the same time as the routine blood (BUT NOT PROCESSED) without written informed consent. If a patient is recruited in the emergency setting it may be necessary to defer consent for the extra study blood samples in order to obtain them in a timely manner and with the rest of the routine blood samples. If a patient has had a lumbar puncture as part of routine care any remaining CSF will be saved for study purposes. Written consent will be obtained from the patient or their substitute decision maker as soon as is feasible. If written consent is not obtained the study samples will be DESTROYED.

Written and verbal versions of the Participant Information Sheet and Informed Consent will be presented to the participants or their substitute decision maker and will detail: the exact nature of the study, the implications and constraints of the protocol and any risks or benefits involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for no reason without prejudice to future care, and with no obligation to give the reason for withdrawal. Participant Information Sheets will be available in the common local language. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator, and will ensure that the participant or their substitute decision maker has understood the information given before signing the consent form. A copy of the signed Informed Consent will be given to the participant or their substitute decision maker. The original signed form will be retained at the study site.

8.3 Sample Handling

Sample handling will adhere to the PREPARE Multi-centre EuRopean study of MAjor Infectious Disease Syndromes (MERMAIDS) guidelines for sample handling.

8.4 Standard of care for Sampling from Patients

Samples taken for the purpose of medical management will, at all times, have priority over samples requested for research reasons. Research samples will, wherever possible, be collected at a time coinciding with blood draw for routine care. The maximum amount of CSF and blood taken in total, will not exceed recommendations.

8.5 Future Use of Samples

Samples collected will be used for the purpose of this study as stated in the protocol and stored for future use with consent. The standard consent form will request consent from subjects for sample storage and/or export of samples to collaborating institutions for investigations as outlined in this protocol. Any proposed plans to use samples other than for those relevant to the study question will be submitted to the relevant ethics committees prior to any testing.

Any database will only identify participants by a participant number. Participant names or any other identifying details will NOT be included. Data may be used alone or in combination with data from related studies in secondary analyses.

8.6 Medical Management and Safety Reporting

Medical management will adhere to standard of care at the treating site and is not a part of this research protocol. Research interventions include only collection of clinical information and specimens and therefore adverse event reporting is not applicable as there is no intervention.

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8.7 Discontinuation/Withdrawal of participants from study

Subjects can leave the study at any time for any or no reason, if they wish to do so, without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.8 Definition of end of study

The end of the study is the date when the last assay has been processed and all follow up of patients completed.

9. STATISTICS AND ANALYSES

9.1 Description of Statistical Methods

Statistical methods will be mainly descriptive estimates of rates and proportions of confirmed cases in each country, region and according to demographic characteristics. Monthly rates of confirmed cases will also be estimated where numbers are adequate to estimate proportions.

A missing at random assumption will be made for missing data however this will be investigated to determine whether reasons for missingness can be obtained and to determine if such an assumption is realistic. Patterns of missing data across regions, countries and demographic characteristics will be reported.

9.2 Number of Participants

The primary goal of this study is to estimate the proportion of adult hospital admissions with a febrile illness in South East Europe that are attributable to WNV, TBEV, TOSV and CCHFV and to document the medical management, treatments, antibody responses and clinical outcomes. The rate and cause of infection in these patients will vary between age groups and regions. We estimate that the total bacterial/viral causes of the majority of CNS syndromes could be due to approximately 30 organisms in European countries, but the comparative data are limited.

Existing knowledge and limited data on the determinants of disease in other settings indicates that detecting a 2% prevalence of any determinant would generate data that would have public health policy and clinical implications. With this in mind, a sample of 125 community-acquired patients per country should provide greater than 90% chance to observe at least one case of a particular determinant, so long as the true prevalence is 2% or greater in that region. The study also has greater than 85% chance to detect a particular determinant of each syndrome in subgroups of 100 patients (e.g., in a seasonal strata) so long as the prevalence is at least 2% (see Table 10).

Hence we will aim to recruit a minimum of 125 patients per country or region. The number might vary depending on rates of symptomatic patients presenting and that agree to participate. Number will be monitored as the study progresses and enrolment targets per country adjusted as necessary. Additional countries or sites can be added to the study if needed in order to recruit sufficient numbers.

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	True prevalence				
N (strata size)	**0.5%**	**1%**	**2%**	**5%**	**10%**
50	22%	39%	64%	92%	99%
100	39%	63%	87%	99%	>99%
125	47%	72%	92%	>99%	>99%
250	71%	92%	99%	>99%	>99%
500	92%	99%	>99%	>99%	>99%

Table 10. Chance of observing at least one case of a particular determinant of each syndrome, as a function of the strata size and underlying true prevalence

The expected precision of simultaneous confidence intervals for multinomial proportions depends on the unknown patterns of determinants of each syndrome within any particular region or age strata. However, reasonable precision for event-level confidence intervals should be achieved for the planned study size, as outlined in Table 11.

	Observed prevalence		
N (strata size)	**1%**	**10%**	**50%**
50	*	(3.3, 21.8)	(35.5, 64.5)
100[†]	(0.0, 5.4)	(4.9, 17.6)	(39.8, 60.2)
200	(0.1, 3.6)	(6.2, 15.0)	(42.9, 57.1)
300	(0.2, 2.9)	(6.8, 14.0)	(44.2, 55.8)
400	(0.3, 2.5)	(7.2, 13.4)	(45.0, 55.0)
500	(0.3, 2.3)	(7.5, 13.0)	(45.5, 54.5)

* Not possible to observe a 1% rate with N = 50.
[†] Precision for N=125 will be somewhat better than for N=100

Table 11. Expected precision of exact, 95% event-level confidence intervals as a function of strata size and observed prevalence values

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Number subjects enrolled	Proportion of arboviral infections detected (with 95% two-sided clopper-pearson exact confidence interval)							
	0% Upper Limit 95% CI	2%	5%	10%	20%	30%	40%	50%
500	0.006	0.02 (0.0096, 0.0365)	0.05 (0.0326, 0.0729)	0.1 (0.0751, 0.1297)	0.2 (0.1658, 0.2378)	0.3 (0.2601, 0.3423)	0.4 (0.3568, 0.4444)	0.5 (0.4553, 0.5447)
750	0.004	0.02 (0.0112, 0.0328)	0.05 (0.0355, 0.0681)	0.1 (0.0795, 0.1237)	0.2 (0.1719, 0.2304)	0.3 (0.2674, 0.3342)	0.4 (0.3647, 0.4361)	0.5 (0.4636, 0.5364)
1000	0.003	0.02 (0.0123, 0.0307)	0.05 (0.0373, 0.0654)	0.1 (0.0821, 0.1203)	0.2 (0.1756, 0.2262)	0.3 (0.2717, 0.3295)	0.4 (0.3695, 0.4311)	0.5 (0.4685, 0.5315)
1250	0.0024	0.02 (0.013, 0.0294)	0.05 (0.0386, 0.0636)	0.1 (0.0839, 0.118)	0.2 (0.1782, 0.2233)	0.3 (0.2747, 0.3263)	0.4 (0.3727, 0.4278)	0.5 (0.4719, 0.5281)
1500	0.002	0.02 (0.0135, 0.0284)	0.05 (0.0395, 0.0623)	0.1 (0.0853, 0.1163)	0.2 (0.18, 0.2212)	0.3 (0.2769, 0.3239)	0.4 (0.3751, 0.4253)	0.5 (0.4744, 0.5256)

Table 12. Proportion of arboviral infections detected by sample size

9.3 Analysis of Outcome Measures

Analysis for the primary outcome will be conducted on all participants enrolled into the study who have provided samples for laboratory testing. An estimate of the proportion of confirmed cases for each disease will be calculated as the number of central laboratory confirmed cases divided by the number of enrolled participants with samples available for testing. As a sensitivity/secondary analysis, the proportion of confirmed cases by any method will also be estimated.

Statistical comparison of prevalence across countries, regions and demographic characteristics will be conducted using generalised linear models for binary data. Models will combine data from all countries and estimate the relative differences in rates between subgroups or between countries by modelling these variables as fixed effects. Additionally, separate exploratory models which adjust for a number of risk factors such as gender, previous arbovirus infections and TBEV vaccination status will be used to assess the impact of these variables on period prevalence, disease severity and time to hospital discharge and mortality.

The length of stay in hospital will be explored by summarising the number of days between hospital admission and discharge using the median and range. Additionally time to event variables, such as time to hospital discharge will be presented using the Kaplan-Meier method with comparisons between countries/regions or according to baseline characteristics conducted using log-rank tests. Antibody levels will be summarised by presenting the geometric mean titres and 95% confidence intervals at each time point. Linear models using log-transformed antibody data will be used to explore regional variation in immunological parameters if data conform to model assumptions for normality. Mortality rates at day 60 after admission will be expressed as the number of deaths per 1000 cases. Health outcomes and neurological sequelae at day 60 will be stratified by demographic variables and disease severity and will be represented descriptively using counts and percentages.

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10. DATA MANAGEMENT

10.1 Source Data

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, general practice and hospital medical records. CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). In this study the CRF will be used as the source document for the direct documentation of inclusion and exclusion criteria, and baseline assessment information which will include, but not be limited to, demographics, exposure, comorbidities, vaccination status, and severity of symptoms. Records of clinical observations, medical management and tests taken will be recorded directly into the CRF by clinical staff members. Patients will complete the health outcome questionnaires at discharge and the specified intervals. If a patient does not turn up for follow up appointments the questionnaires will be posted to the patients. All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent and contact details form, the participant will be referred to by the study participant ID, not by name, including on all reference laboratory results.

10.2 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, the host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

10.3 Data Recording and Record Keeping

Research Online (RO) is an electronic data capture (EDC) system that will be used for data collection. Web-based case report forms (eCRF) are implemented into the system to facilitate the study specific data collection. These forms can easily be accessed by all standard web browsers.

Multiple validation and range checks will be programmed in the eCRF to assure complete and high quality data. Data that does not comply with these rules or ranges will generate a query that must be resolved immediately or at a later stage. Electronic workflows as multiple skip and jump rules will ensure that only information that is applicable to the patient will appear. After the data of last subject is entered, the database can rapidly be closed and all data collected made available to the Prepare study team for further analysis and publication purposes. This data will not contain any patient identifiable information.

RO meets all requirements according to ICH-GCP standards for electronic data entry with respect to safeguarding data integrity and data security regulations. Users will have role based access to the system logging in using their personal username and password. The system will log all data entry steps with timestamps and user information. The role based access to the system will avoid unauthorised data access and prevents users from performing actions that they do not have authorisation for.

Project management of the study is facilitated by the integrated real live study progress reports. RO data traffic over the internet is encrypted using secured data communication protocols. Dedicated databases and web servers are hosted in a secure data centre, and the database (PostgreSQL) is backed up on a daily basis.

The participants will be identified by a unique study specific code in the database. The name and any other personal identifiable information will not be included in any study data electronic files.

10.4 Standard procedures

To assure high quality, the data management (DM) department of the Julius Centre (JC), Utrecht, who will be responsible for the data management within the study, works according to a Quality

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Management System. All work will be carried out in accordance with our written Standard Operating Procedures (SOP) and work instructions (WI). Where necessary, new Project Specific Procedures (PSP) will be written and followed.

11. QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, GCP, relevant regulations and MERMAIDS Standard Operating Procedures. The monitoring will be performed by the MERMAIDS Trial Manager or delegate; these tasks may be delegated to a qualified Contract Research Organisation (CRO). The level of monitoring required will be informed by a risk assessment. All investigators and trial related site staff will receive training in trial procedures and GCP. Regular study monitoring will be conducted as below to ensure that the rights and wellbeing of human participants are protected during the course of the study and that the data collected is credible and accurate.

A Study Management Group (SMG) and Study Steering Committee (SSC) will be appointed. The responsibilities of each group are as follows:

- SMG- responsible for the day-to-day running of the trial, including monitoring all aspects of the trial and ensuring that the protocol is being adhered to.

- SSC- to provide overall supervision of the trial on behalf of the Sponsor and the Funder to ensure that it is being conducted in accordance with GCP. The SSC will review the trial regularly, agree on any amendments and provide advice on all aspects of the trial.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

12.2 Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

12.3 Approvals

The protocol, informed consent form, participant information sheet will be submitted to an appropriate Research Ethics Committee (REC), and host institution(s) for written approval. This will be done in collaboration with Work Package 1 of the PREPARE Programme to ensure that the correct regulatory approvals are gained in each participating country.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

12.4 Reporting

The Principal Investigator shall submit once a year throughout the study or on request, an Annual Progress report to the REC Committee, host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

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12.5 Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

12.6 Expenses and Benefits

Participants will be reimbursed for their time and travel expenses for all study visits in addition to normal care.

13. FINANCE AND INSURANCE

13.1 Funding

The trial is funded by the European Commission FP7 Programme.

13.2 Insurance

The University of Oxford has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

14. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the European Commission FP7 Programme. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

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