Prevalence, clinical management, and outcomes of adults hospitalised with endemic arbovirus illness in southeast Europe (MERMAIDS-ARBO): a prospective observational study

Louise Sigfrid, Xin Hui S Chan*, Louella M R Kasbergen†, Lauren Hookham†, Jia Wei, Siyu Chen, James L Lee, Reina S Sikkema, Erwin de Bruin, Victor M Corman, Chantal Reusken, Katherine Loens, Corneliu Petru Popescu, Mihaela Lupse, Violeta Briciu, Pellumb Pipero, Arjan Harxhi, Edmond Puca, Albina Ponosheci Biçaku, Maja Travar, Maja Ostojic, Rusmir Baljic, Jurica Arapović, Dragan Ledina, Đurđica Cekinović Grbeša, Ivica Čabraja, Anca Meda Văsieşiu, Ivan-Christian Kurolt, Stela Halichidis, Victoria Birlutiu, Zeljana Sulaver, Irina M Dumitru, Ruxandra Moroti, Aleksandra Barac, Goran Stevanovic, Athina Pyrpasopoulou, Metaxia N Papanikolaou, Vasilios Koulouras, Ljiljana Betica Radić, Emmanuel Roilides, Alemka Markotić, Ushma Galal, Emmanuelle Denis, Lynsey Goodwin, Lance Turtle, Iosif Marincu, Simin Aysel Florescu, Hamdi Ramadani, Remi N Charrel, Herman Goossens, Margareta Ieven, Christian Drosten, Peter W Horby‡, Marion P G Koopmans‡, on behalf of the PREPARE MERMAIDS ARBO investigator group§*

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For the Greek, Albanian, Romanian, Bosnian, Serbian, and Croatian translation of the summary see Online for appendix 1

*Joint first authors

†Joint second authors

‡Joint senior authors

§Members listed at the end of the Article

ERGO, Pandemic Sciences Institute, University of Oxford, Oxford, UK (L Sigfrid PhD, E Denis MSc, Prof P W Horby PhD, J L Lee MSc); **Policy and Practice Research Group, Pandemic Sciences Institute, University of Oxford, Oxford, UK** (L Sigfrid); **Nuffield Department of Medicine, University of Oxford, Oxford, UK** (X H S Chan BMBCh DPhil, S Chen DPhil, L Hookham MBBS, J Wei DPhil); **Department of Viroscience, Erasmus University Medical Center, Rotterdam, Netherlands** (L M R Kasbergen MSc, R S Sikkema PhD, E de Bruin BSc, C Reusken PhD, Prof M P G Koopmans DVM); **High Meadows Environmental Institute, Princeton University, Princeton, NJ, USA** (S Chen);

Summary

Background Arboviruses have expanded into new regions in Europe, yet data indicate gaps in disease notifications and a risk of further spread. We aimed to report on prevalence, clinical management, and outcomes of endemic arbovirus infections in southeast Europe.

Methods In this prospective observational study (MERMAIDS-ARBO), we enrolled adults (age ≥18 years) hospitalised with an arbovirus-compatible disease syndrome within 21 days of symptom onset across 21 hospitals in seven countries in southeast Europe over four arbovirus seasons (May 1–Oct 31, during 2016–19). We obtained data from case report forms completed by site investigators on admission and discharge. Participants were excluded if they had non-infectious CNS disorders, symptoms of another confirmed cause, an identified focal source of infection, or symptoms caused by recurrence of a pre-existing condition. The primary outcome was the proportion of participants with confirmed or probable acute infections with West Nile virus (WNV), tick-borne encephalitis virus (TBEV), Crimean–Congo haemorrhagic fever virus (CCHFV), or Toscana virus (TOSV), per reference laboratory criteria. Secondary outcomes were the proportions of patients treated with antivirals, antibiotics, or corticosteroids; the proportion of patients requiring intensive care; hospital length of stay; and mortality.

Findings Of 2896 adults screened for eligibility, 929 were recruited and 913 met protocol-defined eligibility criteria (median age 43·1 years [IQR 29·5–59·7]; 550 [60%] men, 361 [40%] women, and two [<1%] with missing data). 530 (58%) participants presented with suspected meningitis, encephalitis, or both, and 318 (35%) with fever plus myalgia, fever plus arthralgia, or both. 820 (90%) reported no international travel within 21 days before symptom onset. 727 (80%) were administered antibiotics, 379 (42%) corticosteroids, and 222 (24%) antivirals. The median length of hospital stay was 9 days (IQR 6–14), and 113 (12%) required intensive care. Of 847 participants with a reference laboratory sample who met full eligibility criteria for analysis, 110 (13%) were diagnosed with 114 confirmed or probable acute arbovirus infections (four had coinfections or cross-reactivity): one (<1%) with CCHFV, 16 (2%) with TBEV, 44 (5%) with TOSV, and 53 (6%) with WNV. There was one death (<1%) of an individual with WNV. Of the 110 participants, 49 (45%) had a local clinician-attributed arbovirus discharge diagnosis.

Interpretation Our data highlight the need to strengthen arbovirus surveillance systems for the early detection of emerging and re-emerging outbreaks, including investments to increase awareness of arbovirus infections among clinicians, to improve access to specialist diagnostics, and to develop effective and accessible vaccines and treatments to protect populations and health systems in southeast Europe.

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Introduction

Arboviruses are important emerging pathogens and major contributors to the global burden of infectious diseases. They are sustained in a transmission cycle between arthropod vectors (predominantly mosquitoes, ticks, midges, and sandflies) and vertebrate animal

Research in context

Evidence before this study

Arbovirus infections are considered an emerging threat for Europe and globally. At the conception of this study, we did a rapid evidence review of published (PubMed) and grey literature (European Centre for Disease Prevention and Control, WHO, and Google) up to Jan 31, 2015, to inform the study protocol. In PubMed, we used the search terms "arthropod borne" OR "arbovirus*" AND "Europe" AND "human*" AND "infection*". We searched for peer-reviewed publications and reports in English presenting data on arboviruses endemic to southeast Europe that caused outbreaks in humans, together with consultations with European arbovirus experts. We found that endemic arboviruses, including West Nile virus (WNV), Crimean–Congo haemorrhagic fever virus (CCHFV), tick-borne encephalitis virus (TBEV), and Toscana virus, and their vectors have increased their geographical range in Europe since the 1990s. Models of the predicted distributions of *Aedes albopictus* and *Aedes aegypti* mosquitoes indicate a risk of further geographical expansion across south and southeast Europe. Cases of CCHFV detected in humans in southwest Europe, together with modelling of suitability of CCHFV occurrences, also highlight a risk of geographical expansion. Southeast Europe is an area affected by regular outbreaks of CCHFV, TBEV, WNV, and Toscana virus; however, surveillance data reported to the European surveillance system indicate that although the number of countries providing reports has increased over time since the system was introduced, there are gaps and heterogeneity in reporting. The identification of emerging outbreaks of arboviruses is challenging, and the data indicate that the true burden of arbovirus infections in the region is largely unknown.

Added value of this study

In this prospective, observational study in 21 hospitals in seven countries across southeast Europe during the summer months of 2016–19, we identified that arbovirus infections contributed more to adult hospital admissions than were diagnosed by local clinicians. Most participants admitted to hospital with arbovirus-compatible symptoms had meningoencephalitis and lengthy hospital stays. Most were administered antibiotics, nearly half were administered corticosteroids, and 24% were administered antivirals. 110 (13%) of 847 participants with a reference laboratory sample were diagnosed with a confirmed or probable acute infection with one of the endemic arboviruses of interest (CCHFV, TBEV, Toscana virus, and WNV). 5% of participants were diagnosed with confirmed or probable Toscana virus, yet only one local laboratory tested for the virus. We showed the feasibility of implementing a collaborative, multisite observational study across southeast Europe and, with a syndromic approach, the capacity to include any emerging arboviruses presenting in the participating sites. We also report the first human infections with Toscana virus in Albania.

Implications of all the available evidence

Our data show a risk of endemic arbovirus infections in southeast Europe during the summer months and indicate underdiagnosis of cases locally. There is a clear need to strengthen awareness and surveillance of arbovirus infections in the region to strengthen its capacity for early identification of emerging, re-emerging, and travel-imported arboviruses of epidemic potential. Improving access to multiplex serology and real-time quantitative PCR and investing in the development of rapid diagnostics accessible to differently resourced settings should be a priority. The risk of hospitalisation from arbovirus infections and length of hospital stay show that arbovirus infections place an appreciable burden on individuals and health systems and indicate a need to invest in multi-site trials to identify effective treatment and prevention strategies. Our study shows the need and capability of research centres in southeast Europe to be part of this work.

reservoirs and hosts, including humans.[1] Since the 1990s, arbovirus diseases that were once limited to tropical and subtropical regions have been increasingly identified in temperate areas in Europe, driven by socioeconomic, ecological, and environmental factors.[2,3] A large and increasing proportion of the global population is at risk from arboviruses, with mosquito-borne diseases being the largest contributor to the arbovirus disease burden.[3] In Europe, health systems are dealing with the growing challenge of arbovirus infections, with an increase in notifications and geographical distribution of vectors and viruses.[2,4] However, studies identifying viruses in vectors, wildlife, and humans indicate gaps in reporting of arbovirus infections in some regions.[4] A range of arboviruses are endemic in Europe and pose a risk to human health and health-care systems. West Nile virus (WNV), tick-borne encephalitis virus (TBEV), Toscana virus (TOSV), and Crimean–Congo haemorrhagic fever virus (CCHFV) are some of the most commonly detected.[2,5–7]

WNV (family Flaviviridae, genus *Orthoflavivirus*) is transmitted from birds (the primary reservoir host) to humans by *Culex* spp mosquitoes. WNV has caused sporadic outbreaks in Europe since 1962 and re-emerged in the 1990s to cause regular outbreaks in southeast Europe.[2] An estimated 80% of infections are asymptomatic, with less than 1% of patients having severe complications, such as neuroinvasive disease (eg, meningoencephalitis and acute flaccid paralysis), myocarditis, pancreatitis, and fulminant hepatitis.[8] 707 human infections were reported to the European surveillance system in 2023, with a 9·5% fatality rate.[9] Most infections were detected in Italy (47·5%), Greece (22·9%), and Romania (14·6%).[9]

Institute of Virology, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health, Berlin, Germany (V M Corman MD, Prof C Drosten MD); **German Center for Infection Research (DZIF), Berlin, Germany** (V M Corman); **Centre for Infectious Disease Control, National Institute for Public Health and the Environment, RIVML, Bilthoven, Netherlands** (C Reusken); **Department of Medical Microbiology, University of Antwerp UIA, Antwerp, Belgium** (K Loens PhD, Prof M Ieven PhD); **Carol Davila University of Medicine and Pharmacy, Bucharest, Romania** (C P Popescu MD PhD, R Moroti PhD, Prof S A Florescu MD PhD); **Dr Victor Babes Clinical Hospital of Infectious and Tropical Diseases, Bucharest, Romania** (C P Popescu, Prof S A Florescu); **Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania** (Prof M Lupse MD PhD, V Briciu MD PhD); **The Teaching Hospital for Infectious Diseases, Cluj-Napoca, Romania** (Prof M Lupse, V Briciu); **Department of Infectious Diseases, Mother Teresa University Hospital Center, Tirana, Albania** (Prof P Pipero PhD, E Puca PhD); **Faculty of Medicine, Medical University of Tirana, Tirana, Albania** (Prof A Harxhi MD PhD); **Clinic of Infectious diseases, University Clinical Center of Kosovo, Prishtina, Kosovo** (A Ponosheci Biçaku PhD, Prof H Ramadani MD PhD); **Department of Microbiology, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina** (Prof M Travar MD PhD); **School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina** (M Ostojic MD PhD, Prof J Arapović MD PhD); **Unit for Infectious Disease, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina** (Prof R Baljic MD PhD); **Department of Infectious Diseases, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina**

(Prof J Arapović, Z Sulaver MD PhD); **Department of Infectious Diseases, University Hospital Split, Split, Croatia** (D Ledina PhD); **Clinic for infectious diseases, Clinical hospital Center Rijeka, Rijeka, Croatia** (Đ Cekinović Grbeša PhD); **Department of Infectious Diseases, Dr Josip Benčević General Hospital, Slavonski Brod, Croatia** (I Čabraja MD); **Department of Infectious Diseases, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Târgu Mureș, Romania** (A M Văsieșiu PhD); **Dr Fran Mihaljević University Hospital for Infectious Diseases, Zagreb, Croatia** (I-C Kurolt PhD, Prof A Markotić MD PhD); **Clinical Infectious Diseases Hospital, Constanța, Romania** (S Halichidis PhD); **Faculty of Medicine, Ovidius University, Constanța, Romania** (S Halichidis); **Faculty of Medicine, Lucian Blaga University of Sibiu, Sibiu, Romania** (Prof V Birlutiu MD PhD); **County Clinical Emergency Hospital, Sibiu, Romania** (Prof V Birlutiu); **Ovidius University of Constanța, Clinical Hospital of Infectious Diseases, Academy of Romanian Scientists, Bucharest, Romania** (Prof I M Dumitru MD PhD); **National Institute for Infectious Diseases Matei Bals, Bucharest, Romania** (R Moroti); **Clinic for Infectious and Tropical Diseases, University Clinical Center of Serbia, Belgrade, Serbia** (A Barac MD PhD, Prof G Stevanovic MD PhD); **Faculty of Medicine, University of Belgrade, Belgrade, Serbia** (A Barac, Prof G Stevanovic); **Infectious Diseases Unit, Hippokration General Hospital, Thessaloniki, Greece** (A Pyrpasopoulou PhD, Prof E Roilides MD PhD); **Intensive Care Unit, University Hospital of Ioannina, University of Ioannina, Ioannina, Greece** (M N Papanikolaou MD, Prof V Koulouras MD PhD); **University Hospital of Ioannina, Ioannina, Greece** (Prof V Koulouras); **Department of Infectious Diseases, General Hospital Dubrovnik,**

TBEV (family Flaviviridae, genus *Orthoflavivirus*) causes symptomatic, neuroinvasive disease in a minority of infections.[10] TBEV is predominantly transmitted by *Ixodes* spp ticks, which have extended their range into higher latitudes and altitudes than previously reported.[11] TBEV can also be acquired from the consumption of unpasteurised milk from infected animals.[5] There are three main subtypes: European, Siberian, and Far Eastern.[5] European TBEV is the predominant subtype in Europe, with patients typically presenting with a biphasic disease, initially as a non-specific febrile illness with a short period of recovery before onset of neurological manifestations (eg, headache, meningoencephalitis, and paralysis) with long-term sequelae. Fatality rates vary by subtype, with the European subtype associated with mortality rates of 0·5–2% and severe neurological sequelae in up to 10% of patients.[4,12]

TOSV (family Phenuiviridae, genus *Phlebovirus*), transmitted by sandflies, is a common cause of aseptic meningoencephalitis in the Mediterranean region during the summer months.[6] The sandfly vectors have expanded their range within the Mediterranean region in the past two decades.[6,13] TOSV disease predominantly affects younger adults (median age 44·4 years), with symptomatic infections (estimated to be around 20% of infections) characterised by fever, headache, neck stiffness, nausea and vomiting, and ocular manifestations.[6] TOSV is not a notifiable disease in Europe. Access to diagnostics and epidemiological data is scarce.

CCHFV (family Nairoviridae, genus *Orthonairovirus*) is a viral haemorrhagic fever transmitted via ticks or close contact with bodily fluids of infected people and animals.[14] *Hyalomma marginatum* ticks, the main vector, have expanded into new regions,[7] with the first two human infections with CCHFV detected in Spain in 2016,[15] and infected ticks detected in France in 2023.[16] Predictive modelling indicates a risk of further geographical expansion.[17] Autochthonous cases have been reported from Albania, Greece, Kosovo, Spain, and Türkiye, with most cases detected in Bulgaria.[17] Fatality rates range from 10% to 40%.[18]

The identification of emerging outbreaks of arboviruses is challenging. A large proportion of infections are asymptomatic, are subclinical, or have non-specific presentations. Together with a variability in access to diagnostic testing[4] and antibody cross-reactivity between related arboviruses, many cases might be undiagnosed and thus the true burden of arbovirus infections is largely unknown. In this study, we aimed to estimate the proportion of adult hospital admissions with an arbovirus-compatible illness that is attributable to endemic arboviruses in southeast Europe and to document the clinical management and outcomes of these patients.

Methods

Study design and participants

The MERMAIDS-ARBO study was a prospective, seasonal, observational study in Albania, Bosnia and Herzegovina, Croatia, Greece, Kosovo, Romania, and Serbia, conducted within the EU-funded Platform for European Preparedness against (re-)emerging epidemics (PREPARE). The study recruited adults (≥18 years) admitted to 21 hospitals across the seven countries (appendix 2 pp 2, 5) from May 1 to Oct 31, in each year between 2016 and 2019, to coincide with the main arbovirus season, with the final follow-up of patients completed in Jan 31, 2020. Participating hospital sites were identified by convenience inclusion via clinical networks (CLIN-Net and regional networks). Sites gained national or local research ethics and regulatory approval, or both, as per the requirements of each country and site. The study protocol (appendix 3) was approved by the Oxford Tropical Research Ethics Committee (ISRCTN74074706).

Potential participants were identified when presenting to participating secondary care sites with arbovirus-compatible symptoms requiring hospital admission. Arbovirus-compatible symptoms were defined as suspected encephalitis or meningitis, or fever (ie, ≥38°C, self-reported or measured) and neurological symptoms (eg, neck stiffness, partial paralysis, or altered mental state), severe headache, myalgia, arthralgia, maculopapular rash, haemorrhagic symptoms, or thrombocytopenia (ie, <150 000 platelets per µL of blood). The inclusion criteria were designed using a syndromic approach to enable inclusion of patients presenting with any arbovirus illness, to be ready to respond in the event of an emerging (eg, travel-imported) outbreak of public health concern. Patients were excluded if they had a non-infectious CNS disorder due to hypoxic, vascular, toxic, or metabolic causes; symptoms due to another confirmed cause (eg, bacterial infection, malaria, malignancy, immune disorder, or trauma); a focal, localised source of infection identified (eg, pneumonia, viral respiratory tract infection, acute infectious diarrhoea, urinary tract infection, or skin or soft tissue infection); or symptoms caused by recurrence of a pre-existing condition. Participants provided written informed consent.

Procedures

An electronic case report form (appendix 4) was completed by the site investigators on admission and discharge, documenting data on demographics (ie, self-reported age, sex at birth, and ethnicity), comorbidities (from medical records or self-reported), exposures (eg, travel history, previous arbovirus infection, and vaccination status; self-reported), symptoms at presentation, severity of disease, medical management, treatments, discharge diagnosis, and discharge outcomes, all either self-reported or taken from medical records. Research samples (ie, blood and serum) were taken on admission (day 0) and day 7 (or discharge if earlier). Additional serum samples were taken on day 28 and day 60 for antibody analysis.[19] Samples were

stored at –70°C or –80°C and shipped at the end of each season to the study team in Antwerp, Belgium, where aliquots were distributed to the study reference laboratory in Germany for real-time quantitative PCR (RT-qPCR) and to the one in the Netherlands for serology.

Patient samples were tested by RT-qPCR at Charité University Hospital, Berlin, Germany, with commercially available assays for TOSV RNA (TIB Molbiol, Berlin, Germany) and WNV and CCHFV RNA (Altona, Hamburg, Germany), according to the manufacturers' instructions. For the detection of TBEV, an in-house assay was used for which primers and probes were previously published.[2] Serum samples were tested at Viroscience, Erasmus University Medical Center, Rotterdam, the Netherlands, for the presence of IgG and IgM antibodies specific to WNV, TBEV, and TOSV by use of protein microarray, as described elsewhere.[21,22] For CCHFV, samples were tested at the reference laboratory in the Netherlands with the commercially available VectoCrimean-CHF-IgG and VectoCrimean-CHF-IgM ELISAs (Vector Best, Novosibirsk, Russia[23]), according to the manufacturer's instructions. Samples with protein microarray signals above the cutoff (≥20 for IgG and a median fluorescence signal ≥10 000 for IgM) were tested with virus neutralisation test (VNT) assays; for the detection of antibodies to CCHFV, a commercial indirect immunofluorescence test (IIFT; Mosaic 2, EUROIMMUN, Lubeck, Germany) was used if the ELISA signal was positive.[24,25] Patients with a positive RT-qPCR, or with IgM, IgG, or both at any timepoint followed by seroconversion or a four-fold or greater increase in consecutive samples in confirmation assays (VNT or IIFT), were considered to be confirmed acute infections. Patients were characterised as having probable acute infections when in consecutive samples IgM detection was followed by IgG seroconversion, there was at least a four-fold increase in IgM or IgG signals, IgM was detected with IgG at plateau (maximum titre signal), or there was a four-fold or greater increase in IgG but no IgM (only detected on screening assays: protein microarray or ELISA).[19]

Outcomes

The primary objective was to estimate the proportion of adult hospital admissions with an arbovirus-compatible illness in southeast Europe attributable to one of the main endemic arboviruses of public health significance in the region: WNV, TBEV, TOSV, and CCHFV. Acute arbovirus infections were defined as patients diagnosed with a confirmed or probable acute infection by the study reference laboratories. Secondary objectives were to document medical management, treatments, and clinical outcomes. Secondary outcomes were the proportions of patients treated with antivirals, antibiotics, or corticosteroids; the proportion of patients requiring intensive care; hospital length of stay; and mortality on discharge. Further secondary objectives listed in the protocol will be reported in separate manuscripts.

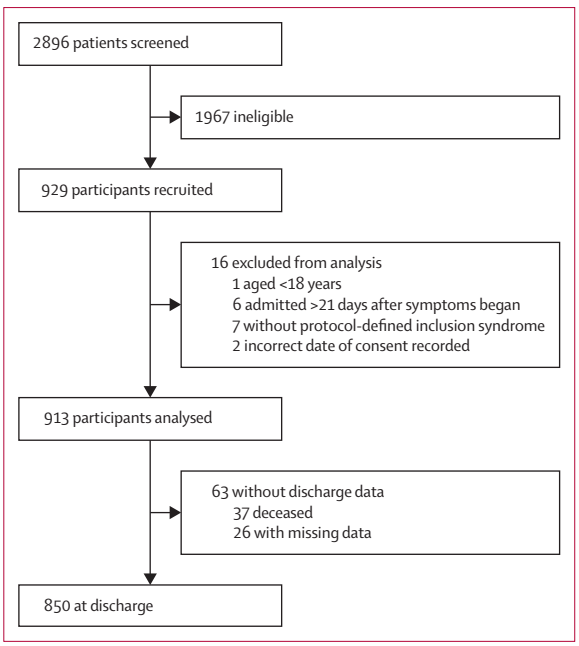

Figure 1: **Study profile**

Statistical analysis

Existing knowledge and scarce data on the determinants of arbovirus disease in other settings indicate 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 patients with community-acquired infection per country should provide more than a 90% chance, and a subgroup of 100 patients should provide more than an 85% chance, to observe at least one patient with a particular determinant, as long as the true prevalence is more than or equal to 2% in the region. Numbers were monitored as the study progressed, and additional sites were added as needed to recruit sufficient patients.

Participant demographics, comorbidities, and exposures were summarised descriptively and compared across countries. Categorical variables (ie, sex, ethnicity, comorbidities) were compared with the χ^2 test or Fisher's exact test for small sample sizes (expected cell count fewer than two patients or >20% of expected cell counts fewer than five patients), and continuous variables (ie, age) were compared with the Kruskal–Wallis test. The primary outcome was assessed in participants with samples available for testing and with sufficient data completeness to be eligible for inclusion in the clinical data analysis. Some participant with samples but incomplete data records were not included in this analysis but are included in our linked serology analysis.[19] We

Dubrovnik, Croatia (L Betica Radić PhD); **Nuffield Department of Primary Care Health Sciences, Clinical Trials Unit, University of Oxford, Oxford, UK** (U Galal MSc); **NIHR Health Protection Research Unit for Emerging Zoonotic Infections, University of Liverpool, Liverpool, UK** (L Goodwin MD, Prof L Turtle FRCP); **Victor Babes University of Medicine and Pharmacy of Timisoara, Timisoara, Romania** (Prof I Marincu MD PhD); **Unite des Virus Emergents, Aix-Marseille Université, Universita di Corsica, IRD 190, Inserm 1207, IRBA, Marseille, France** (Prof R N Charrel MD PhD); **Laboratory of Medical Microbiology, Vaccine & Infectious Disease Institute, University of Antwerp, Antwerp, Belgium** (Prof H Goossens PhD, Prof M Ieven)

Correspondence to:
Dr Louise Sigfrid, ERGO, Pandemic Sciences Institute, University of Oxford, Oxford OX3 7LF, UK
louise.sigfrid@ndm.ox.ac.uk

See **Online** for appendix 2

See **Online** for appendix 3

See **Online** for appendix 4

	Albania (n=282)	Bosnia and Herzegovina (n=65)	Croatia (n=119)	Greece (n=31)	Kosovo (n=91)	Romania (n=293)	Serbia (n=32)	Total (N=913)	p value
Median age, years (IQR)	48·6 (28·5–61·0)	40·4 (26·8–66·1)	43·6 (32·4–55·6)	52·6 (34·9–67·4)	36·8 (28·2–53·9)	41·7 (29·9–57·2)	40·3 (29·5–67·5)	43·1 (29·5–59·7)	0·069
Sex at birth									0·26
Female	109 (39%)	25 (38%)	41 (34%)	14 (45%)	45 (49%)	113 (39%)	14 (44%)	361 (40%)	..
Male	173 (61%)	39 (60%)	77 (65%)	17 (55%)	46 (51%)	180 (61%)	18 (56%)	550 (60%)	..
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)	..
Ethnicity									0·37
Asian	1 (<1%)	0	0	0	0	0	0	1 (<1%)	..
Black	4 (1%)	0	0	0	0	0	0	4 (<1%)	..
Other*	2 (1%)	0	0	1 (3%)	1 (1%)	1 (<1%)	0	5 (1%)	..
White	274 (97%)	64 (98%)	118 (99%)	30 (97%)	90 (99%)	292 (100%)	32 (100%)	900 (99%)	..
Missing	1 (<1%)	1 (2%)	1 (1%)	0	0	0	0	3 (<1%)	..
Chronic cardiovascular disease									<0·0001
No	213 (76%)	43 (66%)	105 (88%)	26 (84%)	68 (75%)	219 (75%)	15 (47%)	689 (75%)	..
Unknown	1 (<1%)	0	0	0	0	3 (1%)	0	4 (<1%)	..
Yes	68 (24%)	21 (32%)	13 (11%)	5 (16%)	22 (24%)	71 (24%)	17 (53%)	217 (24%)	..
Missing	0	1 (2%)	1 (1%)	0	1 (1%)	0	0	3 (<1%)	..
Hypertension									<0·0001
No	220 (78%)	43 (66%)	108 (91%)	27 (87%)	71 (78%)	230 (78%)	15 (47%)	714 (78%)	..
Yes	62 (22%)	21 (32%)	10 (8%)	4 (13%)	20 (22%)	63 (22%)	17 (53%)	197 (22%)	..
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)	..
Chronic metabolic or endocrine disease									0·30
No	246 (87%)	57 (88%)	109 (92%)	29 (94%)	80 (88%)	253 (86%)	25 (78%)	799 (88%)	..
Unknown	0	0	0	0	0	1 (<1%)	0	1 (<1%)	..
Yes	36 (13%)	7 (11%)	9 (8%)	2 (6%)	11 (12%)	39 (13%)	7 (22%)	111 (12%)	..
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)	..
Chronic immunosuppression									<0·0001
No	274 (97%)	64 (98%)	117 (98%)	26 (84%)	91 (100%)	282 (96%)	31 (97%)	885 (97%)	..
Unknown	6 (2%)	0	0	0	0	0	0	6 (1%)	..
Yes	2 (1%)	0	1 (1%)	5 (16%)	0	11 (4%)	1 (3%)	20 (2%)	..
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)	..
Obesity									<0·0001
No	258 (91%)	60 (92%)	113 (95%)	26 (84%)	74 (81%)	256 (87%)	29 (91%)	816 (89%)	..
Unknown	1 (<1%)	0	4 (3%)	0	0	3 (1%)	0	8 (1%)	..
Yes	23 (8%)	4 (6%)	1 (1%)	5 (16%)	17 (19%)	34 (12%)	3 (9%)	87 (10%)	..
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)	..
Thrombocytopenia									<0·0001
No	234 (83%)	62 (95%)	116 (97%)	29 (94%)	91 (100%)	279 (95%)	31 (97%)	842 (92%)	..
Unknown	1 (<1%)	1 (2%)	0	0	0	2 (1%)	0	4 (<1%)	..
Yes	47 (17%)	1 (2%)	2 (2%)	2 (6%)	0	12 (4%)	1 (3%)	65 (7%)	..
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)	..

Data are n (%) unless otherwise specified. Demographics were self-reported by participants; comorbidities were either self-reported or recorded from medical records. *Other ethnicities self-reported were Arabic (n=1, in Greece) and Romani (in Albania [n=2], Kosovo [n=1], and Romania [n=1]).

Table 1: **Participant demographics and comorbidities by country**

used Poisson regression with robust SEs to examine the associations between country, age, or sex and confirmed or probable acute infections for the arboviruses with an acute case frequency of more than 2%, presenting risk ratios (RRs) with 95% CIs.

Time to hospital discharge was presented with the Kaplan–Meier method and tested with Cox regression with comparison by age group (age <40 years, 40–60 years, and >60 years) and by sex for acute arbovirus infections with a case frequency of more than 2%. p values of less than 0·05 were considered significant. The full statistical analysis plan is described in the study protocol. Statistical analyses were performed with R (version 4.2).

	Albania (n=282)	Bosnia and Herzegovina (n=65)	Croatia (n=119)	Greece (n=31)	Kosovo (n=91)	Romania (n=293)	Serbia (n=32)	Total (N=913)
Insect or tick bites ≤21 days before symptom onset								
Yes	31 (11%)	41 (63%)	22 (18%)	4 (13%)	15 (16%)	104 (35%)	18 (56%)	235 (26%)
No	196 (70%)	5 (8%)	78 (66%)	1 (3%)	76 (84%)	131 (45%)	8 (25%)	495 (54%)
Unknown	54 (19%)	17 (26%)	18 (15%)	26 (84%)	0	58 (20%)	6 (19%)	179 (20%)
Missing	1 (<1%)	2 (3%)	1 (1%)	0	0	0	0	4 (<1%)
International travel ≤21 days before symptom onset								
Yes	10 (4%)	10 (15%)	10 (8%)	3 (10%)	16 (18%)	28 (10%)	6 (19%)	83 (9%)
No	271 (96%)	52 (80%)	108 (91%)	27 (87%)	75 (82%)	261 (89%)	26 (81%)	820 (90%)
Unknown	1 (<1%)	2 (3%)	0	1 (3%)	0	4 (1%)	0	8 (1%)
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)
Tick-borne encephalitis virus vaccination								
Yes	0	0	2 (2%)	0	0	0	0	2 (<1%)
No	277 (98%)	60 (92%)	112 (94%)	29 (94%)	91 (100%)	292 (100%)	32 (100%)	893 (98%)
Unknown	5 (2%)	4 (6%)	4 (3%)	2 (6%)	0	1 (<1%)	0	16 (2%)
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)
Yellow fever virus vaccination								
Yes	1 (<1%)	0	2 (2%)	1 (3%)	0	1 (<1%)	0	5 (1%)
No	276 (98%)	60 (92%)	115 (97%)	27 (87%)	91 (100%)	290 (99%)	32 (100%)	891 (98%)
Unknown	5 (2%)	4 (6%)	1 (1%)	3 (10%)	0	2 (1%)	0	15 (2%)
Missing	0	1 (2%)	1 (1%)	0	0	0	0	2 (<1%)
Japanese encephalitis virus vaccination								
Yes	0	0	0	0	0	0	0	0
No	277 (98%)	60 (92%)	117 (98%)	29 (94%)	91 (100)	292 (>99%)	32 (100%)	898 (98%)
Unknown	5 (2%)	4 (6%)	1 (1%)	2 (7%)	0	1 (<1%)	0	13 (1%)
Missing	0	1 (4%)	1 (1%)	0	0	0	0	2 (<1%)

Data are n (%).

Table 2: **Participant self-reported exposures by country**

Role of the funding source

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

Results

We screened 2896 participants admitted to hospital with an arbovirus-compatible syndrome from May 1 to Oct 31 during 2016–19. 929 participants were recruited and 913 met the full protocol-defined criteria for inclusion in the clinical study. Of these, 850 were alive at discharge and 847 had samples suitable for analysis by the reference laboratories (figure 1). Of the 913 included participants, 282 (31%) were included in Albania, 65 (7%) in Bosnia and Herzegovina, 119 (13%) in Croatia, 31 (3%) in Greece, 91 (10%) in Kosovo, 293 (32%) in Romania, and 32 (4%) in Serbia (table 1). The most frequent comorbidity was chronic cardiovascular disease (217 [24%]) followed by chronic metabolic or endocrine disease (111 [12%]), and obesity (87 [10%]; table 1).

Most participants across all country sites (820 [90%]) had no history of international travel within 21 days before symptom onset (table 2). 65 (7%) participants reported a tick bite within 21 days before symptom onset (data per country by tick bite *vs* insect bite are not shown). One (<1%) participant reported a previous TBEV infection (in Romania) and one (<1%) reported a previous CCHFV infection (in Albania). No previous infections were reported for TOSV or WNV. Two (<1%) of 913 participants reported being vaccinated for TBEV and five (1%) had been vaccinated for yellow fever virus.

530 (58%) of 913 participants were admitted with suspected meningitis or encephalitis or both, 318 (35%) with fever plus myalgia or arthralgia or both, and 234 (26%) with fever and severe headache (table 3). Most participants were administered antibiotics (727 [80%]), corticosteroids (379 [42%]), or a combination of antibiotics and corticosteroids (340 [37%]; figure 2). 222 (24%) participants received antivirals (either alone or in combination), including acyclovir in 205 (22%) of 913 participants; ganciclovir in five (2%); ribavirin in four (<1%); inosine pranobex in four (<1%); and aciclovir ointment, antiretrovirals, oseltamivir, and valganciclovir in one (<1%) patient each. 55 (6%) of 913 received blood products, comprising fresh frozen plasma (19 [2%]), platelets (eight [1%]), whole blood (seven [1%]), intravenous immunoglobulin (six [1%]), red blood cell transfusion (one [<1%]), and other blood

	All participants (N=913)	Participants with confirmed or probable acute infection			
		WNV (n=53)	TBEV (n=16)	Toscana virus (n=44)	CCHFV (n=1)
Meningoencephalitis syndrome					
Suspected encephalitis	330 (36%)	33 (62%)	9 (56%)	13 (30%)	1 (100%)
Suspected meningitis	356 (39%)	31 (58%)	14 (88%)	18 (41%)	0
Suspected meningitis, encephalitis, or both	530 (58%)	43 (81%)	16 (100%)	22 (50%)	1 (100%)
Fever with at least one additional sign or symptom	383 (42%)	10 (19%)	0	22 (50%)	0
Acute febrile illness with neurological syndrome					
Fever and focal neurological symptom	66 (7%)	2 (4%)	0	4 (9%)	0
Fever and severe headache	234 (26%)	6 (11%)	0	14 (32%)	0
Acute febrile illness with myalgia or arthralgia syndrome					
Fever and myalgia	304 (33%)	8 (15%)	0	18 (41%)	0
Fever and arthralgia	239 (26%)	4 (8%)	0	18 (41%)	0
Acute febrile illness with haemorrhagic syndrome					
Fever and skin rash	126 (14%)	6 (11%)	0	6 (14%)	0
Fever and haemorrhage	22 (2%)	0	0	0	0
Fever and thrombocytopenia	147 (16%)	4 (8%)	0	8 (18%)	0

114 diagnoses of confirmed or probable acute arbovirus infection were made in 110 patients (four had positive serology for two infections). CCHFV=Crimean–Congo haemorrhagic fever virus. TBEV=tick-borne encephalitis virus. WNV=West Nile virus.

Table 3: **Clinical syndromes and symptoms on presentation**

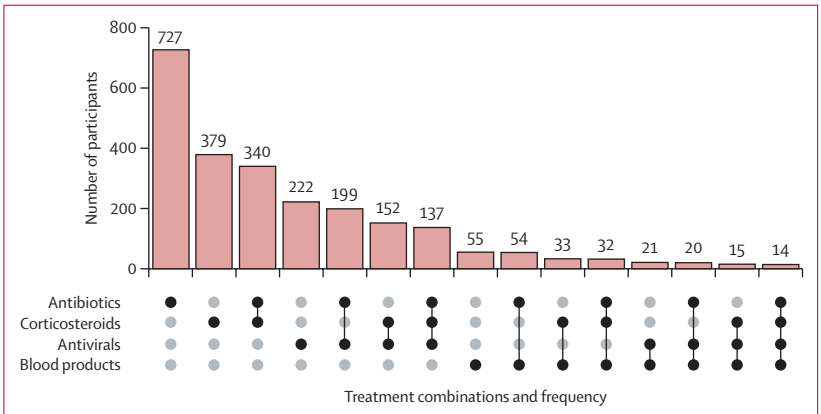

Figure 2: **Number of participants with arbovirus-compatible symptoms given each treatment and combination of treatments**

products (14 [2%]). The six patients who received intravenous immunoglobulin had meningoencephalitis and were given corticosteroids (five [1%]), antibiotics (four [<1%]), or aciclovir (four [<1%]), alone or in combination.

The median length of hospital stay was 9 days (IQR 6–14). 113 (12%) participants were admitted to the intensive care unit (ICU), with a median stay of 8 days (4–14). At discharge, 850 (96%) of 887 with available data were alive (figure 1), giving a fatality rate of 4%.

Of the 847 participants who had samples suitable for analysis by the reference laboratories, 110 (13%) were diagnosed with an acute arbovirus infection (table 3). Case rates of acute arbovirus infection in the participants who had an available sample for testing are presented per studied virus and country in figure 3. Four of the 110 participants had serological test results indicating coinfection or cross-reactivity. The laboratory result in one participant (with WNV and TOSV in Bosnia and Herzegovina) was most likely explained by coinfection, and the results in three participants (with WNV and TBEV in Romania [n=2] and Serbia [n=1]) were most likely explained by antibody cross-reactivity or coinfection. Of the 110 participants, 49 (45%) had an arbovirus discharge diagnosis from a local clinician, 48 (44%) were diagnosed by local clinicians as having a non-arbovirus infection, and seven (6%) were diagnosed by local clinicians as having a non-communicable disease. Of the 37 patients who died in the study, one (3%) was diagnosed with an acute arbovirus infection by the reference laboratories. The fatality rate among the 110 participants with confirmed or probable acute arbovirus infections was therefore 1%.

53 (6%) of 847 participants were diagnosed with an acute WNV infection by the reference laboratories (table 3). The median age of these patients was 59·0 years (IQR 40·7–71·1), 30 (57%) were male, and 23 (43%) were female. 49 (92%) infections were locally acquired and four were in patients who reported international travel within the 21 days before symptom onset. Most WNV infections (37 [70%] of 53) occurred in Romania, with five (9%) in Bosnia and Herzegovina, four (8%) in Serbia, three (6%) in Albania, two (4%) in Croatia, and two (4%) in Kosovo. 24 (45%) of the 53 patients were enrolled in 2018 (appendix 2 p 3). Risk factors for hospitalisation with WNV were being enrolled in Romania (RR 14·1, 95% CI 4·4–45·2), Serbia (13·8, 3·2–59·3), or Bosnia and Herzegovina (7·5, 1·9–30·3) compared with Albania, and increasing age (1·4 per decade, 1·2–1·6; appendix 2

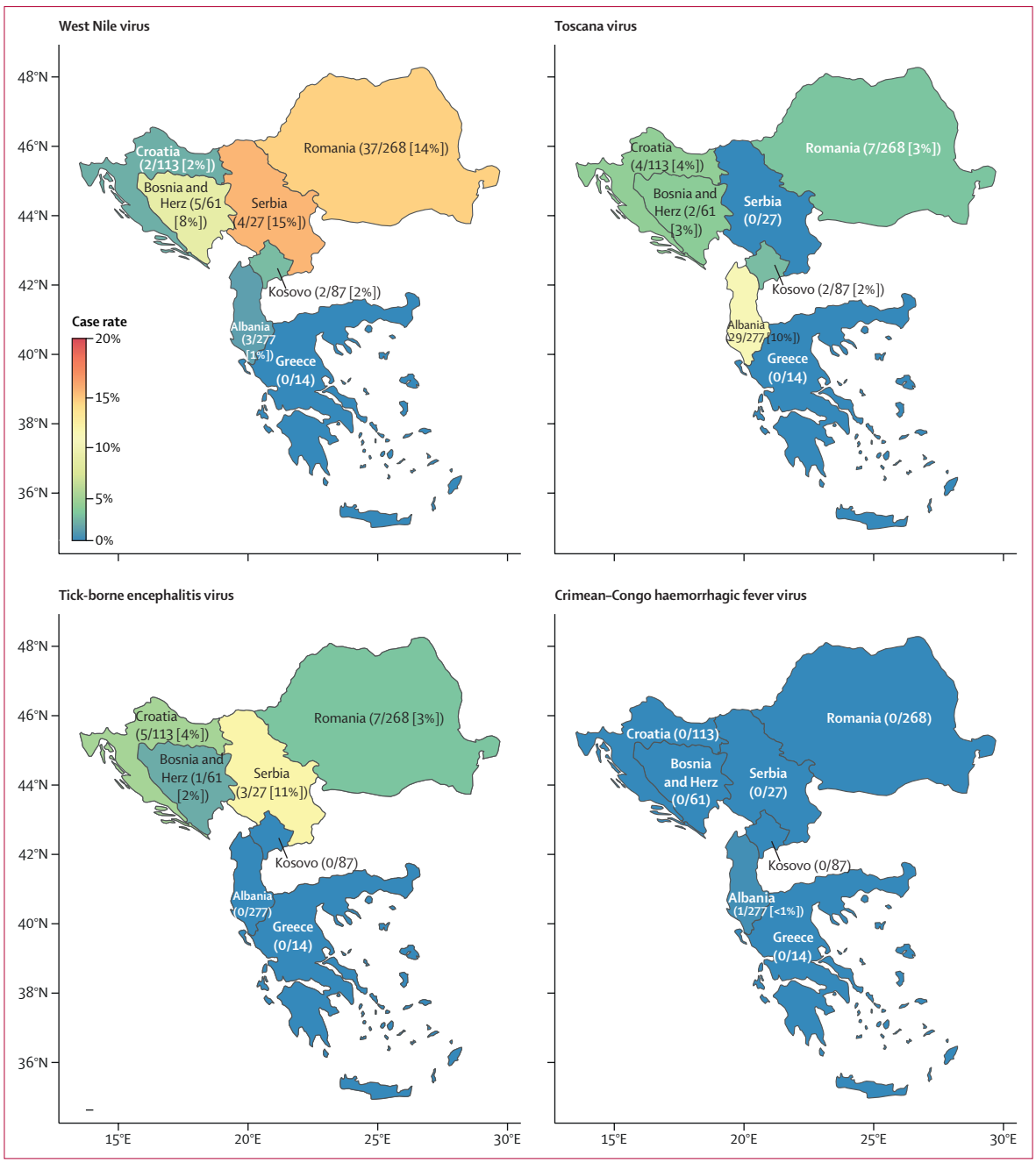

Figure 3: **Case rates of the studied viruses per country**
Data are the proportion of participants diagnosed with an acute arbovirus infection out of all participants admitted to hospital with an arbovirus-compatible syndrome who had a sample available for testing by the reference laboratories, per country. Herz=Herzegovina.

p 3). Most patients with WNV (43 [81%] of 53) presented with suspected meningoencephalitis (table 3). 37 (70%) had a local clinician-attributed arbovirus discharge diagnosis. 41 (77%) received antibiotics, 31 (59%) received corticosteroids, and 24 (45%) received antivirals (appendix 2 p 6). The median length of hospital stay was 12·5 days (IQR 8·0–17·3). 11 (21%) were admitted to the ICU with a median ICU stay of 14 days (3·5–16·0). One (2%) participant with WNV died. Participants older than 60 years had a lower probability of being discharged alive than participants younger than 40 years (hazard ratio 0·39, 95% CI 0·19–0·81; p=0·011; appendix 2 pp 7–9).

16 (2%) of 847 participants were diagnosed with acute TBEV infection (table 3). The median age of these participants was 58·8 years (IQR 38·6–64·8), 11 (69%)

were male, and five (31%) were female. Seven (44%) were enrolled in Romania and in 2019 (appendix 2 p 3). 15 (94%) of the 16 participants with a TBEV infection had locally acquired infections, and all presented with suspected meningoencephalitis (table 3). Seven (44%) had a local clinician-attributed arbovirus discharge diagnosis. 12 (75%) received antibiotics, seven (44%) received corticosteroids, and three (19%) received antivirals (appendix 2 p 10). The median length of hospital stay was 13·0 days (IQR 10·0–14·5) with no ICU admissions or deaths.

44 (5%) of 847 participants were diagnosed with acute TOSV infection (table 3). The median age of these participants was 52·1 years (IQR 33·3–61·1), 32 (73%) were male, and 12 (27%) were female. Most were enrolled in Albania (29 [66%]) or Romania (seven [16%]) and almost half (21 [48%]) in 2019 (appendix 2 p 3). 42 (95%) were locally acquired. Increasing age (RR 1·2 per decade, 95% CI 1·1–1·4) and male sex (1·9, 1·0–3·6) were risk factors for hospitalisation with TOSV (appendix 2 p 4). 22 (50%) presented with suspected meningoencephalitis, 18 (41%) with fever and myalgia, and 18 (41%) with fever and arthralgia (table 3). Only six (14%) had a local clinician-attributed arbovirus discharge diagnosis. 40 (91%) received antibiotics, 21 (48%) received corticosteroids, and eight (18%) received antivirals (appendix 2 p 11). The median length of hospital stay was 10·5 days (IQR 7·0–14·0). Seven (16%) required ICU admission of a median duration of 4·0 days (3·0–4·5). There were no deaths, and the log-rank test showed no significant differences in probability of hospital discharge by age group or sex (appendix 2 pp 12–14).

There was one (<1%) participant with CCHFV infection of 847 included (table 3): a man enrolled in Albania in 2016 who had not travelled in the 21 days before symptom onset and presented with suspected meningoencephalitis. He received antibiotics and corticosteroids and was discharged after 22 days without ICU admission.

Discussion

We identified that endemic arbovirus infections contribute more to adult hospital admissions in southeast Europe during the summer months than is attributed by local clinicians. This finding suggests a need to improve awareness of ongoing risk and clinical presentation of endemic arboviruses, as well as to improve access to diagnostic assays. It also highlights the need to consider arboviruses in the differential diagnosis of travellers returning from southern Europe with compatible signs and symptoms. Although arbovirus-associated mortality was low, the length of hospital stay shows that arbovirus infections place a burden on individuals and health systems in the region. The high proportion (58%) admitted to hospital with symptoms of central CNS infection (ie, meningoencephalitis) also underscores a need to explore the risk of long-term sequelae for these arboviruses.

TOSV infections have been reported previously in Croatia, France, Greece, Italy, Kosovo,[6] Romania, and Spain.[6,26] In this study, we report the first human infections identified in Albania. Risk factors for TOSV infection were increasing age and male sex, in line with other studies,[27] although the participants with TOSV in our study were older than reported in serosurveillance surveys, most likely reflecting the older age of the symptomatic and hospitalised population included. Most TOSV studies have been done in Italy and France.[6] TOSV is not notifiable at the European level, despite being identified as a leading cause of meningoencephalitis in regions around the Mediterranean in the summer months.[6] Our data show that TOSV might also present as a febrile illness with myalgia and arthralgia. Our data highlight the risk of TOSV infection in southeast Europe, yet only one site tested for TOSV, which could be partly due to limited access to TOSV diagnostic assays in the region. Limited access to diagnostics might also explain the low case numbers described previously.[6]

The high incidence of acute WNV infection, with most cases included in Romania, is consistent with European surveillance system data.[28] Most cases in Europe are WNV lineage 2a.[2] There was a 2% WNV fatality rate in our cohort, which is lower than the 12% reported for the large outbreak that affected several European countries in 2018.[29] This difference might be explained by difficulties in recruiting and consenting the most severe infections in our study, which could have biased inclusion to less severe infections. Alternatively, the high fatality rate of notified infections might reflect under-ascertainment. The WNV risk factors identified in our study agree with existing data showing older age as a risk factor for more severe arbovirus disease.[28]

Of the 16 acute TBEV infections, only one was imported, consistent with the trend of most European TBEV infections being acquired locally.[5] We found low rates of TBEV vaccination, which is consistent with previous reports.[30] TBEV vaccination policies and recommendations vary between countries and the vaccine is generally self-funded.[30] Some countries recommend the vaccine to people in or visiting high-risk areas. In Serbia, access is limited by the requirement of permission from the national Medicines and Medical Devices Agency for import.[30] With a risk of long hospitalisation and long-term sequelae, further studies into cost-effectiveness of vaccination strategies, including reimbursement for low-income households in endemic regions, is warranted.

We identified one infection of acute CCHFV in Albania, where CCHFV is known to be endemic.[18] Of the studied arboviruses, CCHFV has gained the most attention due to its high fatality rate, an absence of medical countermeasures, and outbreaks affecting several regions in Europe since the 1950s.[18] CCHFV is recognised as a priority disease for research and development by WHO.

We note the inconsistency between local clinical and reference laboratory-confirmed arbovirus diagnoses, particularly for diseases for which local diagnostics are less likely to be available (eg, TOSV). Even when reference diagnostics are available, results might not be accessible to clinicians in time to inform initial treatment decisions. This factor, together with the time required to gain results even from standard hospital assays, and that most participants presented with meningoencephalitis symptoms in our study, might partly explain the high proportion of patients administered antibiotics empirically on admission in our study. Improving access to, and the turnaround time of, arbovirus diagnostics would support clinicians to not start or to stop empirical antimicrobials earlier upon admission to minimise unnecessary antibiotic use.

Strengths of our study are the standardised observational protocol, the simplicity of the protocol's implementation, and the collaborative approach. These strengths, together with the focus on an issue of importance to the region, were key factors for sites in deciding to participate in the study. The frequency of arboviruses detected illustrates a well designed protocol powered to meet its objectives. In addition, we showed that by establishing an observational study with a syndromic design, we could identify emerging travel-imported arboviruses, such as one case of Zika virus in Romania (described previously[31]). Nevertheless, our study also has limitations. The numbers of patients recruited in Greece and, to a lesser extent, Serbia were lower than planned in the protocol, which could limit interpretation of the analyses for these countries. Some of the larger RR estimates and confidence limits suggest sparse data bias. In addition, convenience sampling for site and patient inclusion, the different number and geographical locations of hospitals participating per country, and the variation in their research resource capacity limit the generalisability of the data. Some investigators reported that recruiting critically ill participants was challenging, and others reported challenges in competing demands and sample storage. Therefore, our data might not represent the full disease spectrum and could be an underestimate of the proportion of hospital admissions with an arbovirus illness. Recall bias for self-reported data, such as previous exposure, is another potential limitation. Further, variation in time to presentation and capacity to collect samples at specified timepoints posed challenges to the interpretation of the serological results.[19]

In conclusion, our study identified a higher proportion of hospital admissions with arbovirus-compatible symptoms in the summer months in southeast Europe attributable to endemic arbovirus infections than were diagnosed locally, highlighting the importance of improving awareness of the viruses and access to diagnostics in the region. We showed the feasibility of a coordinated regional approach utilising sentinel site-based observational studies to generate evidence while building capability to respond to emerging epidemics.

PREPARE MERMAIDS ARBO investigator group
Ioannis Andrianopoulos, Catalin Apostolescu, Jurica Arapović, Nazli Ayhan, Rusmir Baljic, Aleksandra Barac, Ljiljana Betica Radić, Silvia Bino, Victoria Birlutiu, Violeta Briciu, Ivica Čabraja, Đurđica Cekinović Grbeša, Xin Hui S Chan, Felicity Chandler, Remi Charrel, Siyu Chen, Maria Chondrogiorgi, Natalie Cleton, Daniel Codreanu, Victor M Corman, Erwin de Bruin, Tomasović Domagoj, Edita Dražić Maras, Emmanuel Denis, Christian Drosten, Irina Magdalena Dumitru, Mario Duvnjak, Simin Aysel Florescu, Ushma Galal, Lynsey Goodwin, Herman Goossens, Corine GeurtsvanKessel, Stela Halichidis, Arjan Harxhi, Lauren Hookham, Peter Horby, Adriana Hristea, Raluca M Hrisca, Kevin Jacobs, Margareta Ieven, Ilias Iosifidis, Puškarić Ivana, Louella M R Kasbergen, Marion Koopmans, Alma Gabriela Kosa, Iris Koshovari, Vasilios Koulouras, Mandy Kuijstermans, Ivan-Christian Kurolt, Arsim Kurti, Dragan Ledina, James L Lee, Katherine Loens, Mihaela Lupse, Santini Marija, Iosif Marincu, Alemka Markotić, Julia Melchert, Pieter Moons, Ruxandra Moroti, Visnja Mrdjen, Cristian M Niculae, Maja Ostojić, Nenad Pandak, Metaxia N Papanikolaou, Pellumb Pipero, Albina Ponosheci Biçaku, Corneliu P Popescu, Edmond Puca, Athina Pyrpasopoulou, Lul Raka, Hamdi Ramadani, Chantal Reusken, Emmanuel Roilides, Iordanis Romiopoulos, Sandra Scherbeijn, Louise Sigfrid, Reina S Sikkema, Goran Stevanovic, Željana Sulaver, Gratiela Tardei, Nijaz Tihic, Skuhala Tomislava, Lance Turtle, Maja Travar, Anca Meda Văsieșiu, Antonija Verhaz, Ivana Vrkic, Ankica Vujovic, Jia Wei, Mihaela Zaharia, Mačak Šafranko Željka.

Contributors
MPGK and PWH were co-chief investigators of this study. LS, CR, CD, PWH, and MPGK led the development of the research questions and study design, informed by the wider study team (LS, JLL, UG, NC [a member of the PREPARE MERMAIDS ARBO investigator group], CR, HG, MI, CD, PWH, and MPGK). LS and JLL led site recruitment, training, study management, and coordination. ED developed the site study training. MI and KL developed and coordinated the sample kits for the sites, and coordinated the shipment of samples to the reference laboratories. EdB, LMRK, FC and RSS of the PREPARE MERMAIDS ARBO investigator group, and MPGK conducted the serological diagnostics. VMC and JM (a member of the PREPARE MERMAIDS ARBO investigator group) conducted the RT-qPCR analysis. The site investigators in the PREPARE MERMAIDS ARBO investigator group recruited patients, documented data, and collected patient samples. XHSC, JW, SC, LH, and UG analysed the data and prepared tables and figures. UG, XHSC, JW, and SC accessed and verified the data. XHSC and LS led on writing the manuscript, with contributions from the wider study team. All authors had access to all the data in the study and had final responsibility for the decision to submit for publication. All authors reviewed and approved publication of the manuscript.

Declaration of interests
LT has received consulting fees from the Medicines and Healthcare products Regulatory Agency, as well as AstraZeneca and Synairgen (paid to their institution); speakers fees from Eisai; and support for conference attendance from AstraZeneca. VBr has received speakers fees and support for conference attendance from, and participated on a data and safety monitoring board for, SC Pfizer Romania. VK declares having received support for conference attendance from MSD, Pfizer, and Menarini, and having participated on a data and safety monitoring board for MSD and Menarini. AMV declares having received consulting fees from Medichub Media; having received lecture fees from Ewopharma Romania, Gilead Sciences, Zentiva, and Alfasigma Romania; having received conference attendance support from Pfizer Romania, MSD Romania, and Ewopharma Romania; having participated in a data and safety monitoring board for MSD Romania; and being President of Asociatia Medicilor Infectionisti Mures. All other authors declare no competing interests.

Data sharing
Formal requests for study data should be made to the corresponding author (LS) outlining the research aims, methods, and the variables

needed. Such requests will be considered by the core PREPARE MERMAIDS ARBO investigator group (LS, PWH, and MPGK). If research questions and methods are considered reasonable, relevant, and valid, the requested, fully anonymised data will be made available under data transfer agreements. The PREPARE MERMAIDS ARBO investigator group will decide about coauthorships after discussion with the interested party.

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Editorial note: The Lancet Group takes a neutral position with respect to territorial claims in published maps and institutional affiliations.

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