LABORATORY PREPAREDNESS AND RESPONSE WITH A FOCUS ON ARBOVIRUSES IN EUROPE

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ABSTRACT

Background: The global health burden of arboviruses is continuously rising, which results in increasing pressure on local and (inter)national laboratory infrastructures. Timely and accurate diagnosis of cases is one of the main pillars for public health and clinical responses to an arbovirus emergence.

Aims and sources: This narrative review aims to summarize recent advances and to identify needs in laboratory preparedness and response activities, with a focus on viruses transmitted by arthropods in Europe. The review is based on evidence extracted from PubMed searches, Public Health and clinical laboratory experiences from the authors and the authors’ opinions substantiated by peer-reviewed scientific literature.

Content: We illustrate the importance of inter-epidemic laboratory preparedness activities to ensure adequate Public Health and clinical responses. We describe the status of arbovirus endemicity and emergence in Europe thereby highlighting the need for preparedness for these viruses. We discuss the components and pitfalls of an adequate laboratory preparedness and response and the broader context of the current landscape of international research, clinical and laboratory preparedness networks. The complexity of arbovirus laboratory preparedness and response is described.

Implications: Outbreak preparedness plans need to look beyond national reference laboratories, to include first-line responding onsite hospital laboratories and plans for strengthening of such local capacity and capability as required depending on the nature of the outbreak. In particular, the diagnosis of arbovirus infections is complicated by the existence of geographic overlap of circulation of numerous arboviruses, the overlap in clinical manifestation between many arboviruses and other aetiologies and the existence of cross-reactivity between related arboviruses in serology testing. Inter-epidemic preparedness activities need strong national and international networks addressing these issues. However, the current mushrooming of European preparedness networks requires governance to bring the European preparedness and response to a next level.

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Background

In the past decade arthropod-borne viral diseases have continued their worldwide geographic expansion and thereby exert an increasing pressure on global health [1]. Arthropod-borne viruses (in short arboviruses) are viruses that replicate in and are transmitted by arthropods, such as mosquitoes, ticks and sandflies, between vertebrate hosts. Arboviruses can cause severe disease in humans and animals and are maintained in complex multi-component life cycles. Through globalization of travel and trade, increasing population density, and possibly under the influence of climate change (novel) arbovirus diseases have expanded considerably [2,3]. Recent examples of large outbreaks in humans resulting from a fast geographic expansion of arboviruses upon
introduction in naive areas with suitable vectors are the emergence of chikungunya virus (CHIKV) and Zika virus (ZIKV) in the New World in 2013 and 2015 respectively [4,5], the latter leading to the declaration of a Public Health Emergency of International Concern by WHO in the period 1 February to 18 November 2016 [6,7].

During the past decade, arboviruses have been expanding to and within Europe, with autochthonous transmission of dengue virus (DENV) in Croatia, France and Madeira (Portugal), CHIKV in France and Italy, West Nile virus (WNV) in central and southern Europe, and the first human cases with Crimean–Congo haemorrhagic fever (CCHF) in Spain [8–17]. In addition in 2016, Usutu virus (USUV), a mosquito-borne bird flavivirus with proven zoonotic potential, has rapidly expanded its geographic coverage in Europe in a multi-country outbreak of multiple virus lineages in birds [18–20]. A recent study in Italy indicated that human USUV infection may not be a sporadic event. USUV infections in patients with or without neurological impairments occurred more frequently than WNV infections in a 4-year period in Italy [21]. Acute USUV infections have been detected in blood donations in Germany and Austria, raising blood safety concerns [22,23].

A risk assessment by WHO-Europe indicated that the risk for an outbreak with ZIKV in Europe should not be underestimated, in particular in countries with established presence of the vectors Aedes aegypti and Aedes albopictus [24,25], although, in contrast to Ae. aegypti, field and laboratory evidence do not point to a significant role of Ae. albopictus in the transmission of ZIKV [26–31]. While both Aedes vectors are established in some parts of south and southeast Europe, other parts of Europe have the established presence of other exotic mosquito vectors [24] in addition to autochthonous vector species, e.g. various Culex species that vector WNV, USUV and Japanese encephalitis virus (JEV) [32–34]. The 2016 USUV outbreak in northwest Europe was similar to the explosive outbreak with the closely related WNV lineage 2, in central Europe in 2008/09 and in Greece in 2010 after a few years of limited local circulation [35]. It has been speculated that the expanding emergence of USUV might be a prelude to the emergence of WNV, both with a similar avian–mosquito lifecycle and both being introduced to naive regions via viraemic migratory birds (humans are dead-end hosts for WNV and USUV) [36].

Viraemic travellers returning from endemic regions to naive regions with competent local vectors are thought to have initiated the outbreaks with CHIKV and ZIKV in the Americas and the local transmission events with DENV and CHIKV in Europe [9,16,17,37,38]. Globally the number of yearly travellers has risen from 450 million in 1990 to nearly 950 million in 2010. European Union (EU) Tourism Statistics indicate that in 2014 EU residents >15 years of age made an estimated 1.2 billion trips (accounting for 2.6 billion nights), of which 6.2% were to destinations outside the EU. Destinations outside Europe made up 14.6% of all EU outbound trips: 1.8% to Latin America, 3.6% to North America, 4.7% to Asia, 0.5% to Oceania and 4.0% to Africa, although the distributions of travel destinations may differ significantly for travellers from different countries [39]. Outbreaks and/or geographic expansion of arboviruses globally are reflected in (periodic) increases in arbovirus diagnosis in returning travellers. An illustrative example is the increase in reported yellow fever cases (n = 4) in European Union travellers in the period August 2016 to March 2017, which reflected the increased activity of yellow fever virus (YFV) in South America [40]. Some virus infections in returning travellers (e.g. CHIKV, ZIKV, DENV) constitute a risk for further spread if competent vectors are present [2,16,17,41]. The majority of ZIKV cases imported into the EU/European Economic Area (n = 2130) since 2015 were found in France (54%) and Spain (14%) where Ae. albopictus has an endemic presence [41,42], indicated by WHO-Europe as a risk factor for autochthonous transmission [25]. One of the other identified factors in a European country’s risks for a ZIKV outbreak was the ability of a country to robustly detect ZIKV introduction and local transmission [25].

In addition to the above examples of emergence of arboviruses, several other human pathogenic arboviruses are endemic to Europe, such as the tick-transmitted viruses tick-borne encephalitis virus (TBEV) and CHF virus and mosquito-borne viruses like Sindbis virus in northern Europe and WNV in the Balkans and northern Italy. These show occasional peaks in incidence due to variable local biotic and abiotic drivers of emergence [43–50]. Awareness among clinicians and targeted multi-component surveillance is needed to monitor the epidemiology of these viral infections [51].

The emergence of arbovirus disease in the human population is the result of complex processes usually involving animal reservoirs, arthropods and humans, whereas in a few cases the pathogen has completely adapted to an urban human—mosquito—human cycle (i.e. CHIKV, DENV, urban YFV and ZIKV) [2]. Although the timing is, the nature and geography of emerging disease events are often not completely unexpected [3,52,53], e.g. the emergence of CHIKV and ZIKV in the Americas and the geographic expansion of WNV, USUV and TBEV in Europe. In this light the world might be facing the emergence of YFV in Asia and of JEV in Africa. Indeed, in April 2017 the first case of autochthonous JEV infection was reported from Angola [54]. These continuously changing dynamics of arbovirus emergence and the rise in its global health burden will increasingly exert pressure on local and (inter)national laboratory infrastructures. As diagnostics are the pillars of surveillance, individual patient care and (clinical) outbreak response, this asks for inter-epidemic laboratory preparedness.

**Laboratory response: disease detection**

As human arbovirus disease is an end point of a complex infection cycle involving vectors and reservoir hosts, timely detection of arbovirus infections requires multidisciplinary collaboration, including ecologists, entomologists, veterinarians and wildlife disease experts. Laboratory preparedness and response therefore can be seen as a continuum of activities, one of which is the routine diagnostic capacity for evaluation of illness in humans (Fig. 1). For common diseases known to be endemic in a region, diagnostic capacity needs to be available in—or rapidly accessible for—routine clinical laboratories. For rare, exotic diseases, diagnostics is generally referred to specialized (inter)national reference laboratories. These reference centres have the expertise to support preparedness and response in their broadest sense, including access to diagnostics for rare viruses and laboratories for Risk Group 3 and 4 pathogens, and research-based monitoring of the evolution of viruses to ensure diagnostic accuracy and development of improved diagnostic platforms. For emerging disease threats with epidemic potential, diagnostic capacity available at reference centres ideally would need to be deployable to clinical laboratories to scale up local laboratory capacity.

The laboratory response to an emerging event needs to be timely, i.e. as early as possible, and accurate, i.e. with high sensitivity and specificity [55–58]. Timeliness can be assured by thorough preparedness. Laboratory preparedness should comprise a range of inter-epidemic activities in which barriers and challenges for reference laboratories to rapidly implement diagnostics to emerging pathogens could be addressed. For an accurate response, the essential basic questions for diagnostic triage (Table 1) need to be known and if (partially) unknown, these knowledge gaps would need to be systematically identified. Awareness of the existing diagnostic knowledge gaps is important to define a proper sampling strategy, for an adequate choice of type of test to use and for a correct interpretation of laboratory results and so correct confirmation or ruling out of an infection [55–58]. Furthermore, it can
provide guidance to the clinical and public health response where the identified critical knowledge gaps can be addressed [51]. This requires intensive integration and collaboration between these, traditionally often autonomously operating, disciplines. For example, during the first phase of the emergence of ZIKV in the Americas, the lack of knowledge on the infection kinetics of ZIKV in various population groups (i.e. pregnant women) was identified by reference laboratories as a crucial gap to be addressed [57] and this issue was a topic of research in numerous clinical studies during the course of the outbreak [59–63].

**Laboratory preparedness**

While in theory there is good coverage of clinical diagnostic laboratories and reference centres across Europe [64,65], a challenge is how to focus the preparedness activities, in view of the expanding list of arboviruses of relevance for Europe and the threat of local outbreaks. Optimal laboratory preparedness constitutes a multi-component approach.

**Foresight and the establishment of generic approaches to diagnostic preparedness**

A challenging question is how to prioritize the choice of pathogens for which to develop toolboxes. Prioritization exercises like the WHO R&D blueprint that prioritizes diseases likely to cause epidemics in the future could provide guidance to these inter-epidemic activities. The January 2017 blueprint included four arboviruses: ZIKV, CCHFV, Rift Valley fever virus and severe fever with thrombocytopenia syndrome virus [66]. Another tool that has been developed to inform preparedness activities is the ECDC online tool for the prioritization of infectious disease threats [67]. Furthermore, numerous short-lists identifying and classifying emerging virus threats have been published in the past two decades [68–73]. The availability of toolboxes for high-risk virus groups would facilitate the laboratory response to novel emerging viruses as well, e.g. the genus orthobunyavirus, family Peribunyaviridae is known to be prone to yielding novel (re-assorted) arboviruses of importance to veterinary and public health [74–76] while a wide range of studies in bats and rodents has taught us that there is still a lot ‘out there’ to surprise the world [71,77,78].

**Mapping and overcoming logistic and sharing barriers**

Although there is widespread capacity to develop primer/probe combinations for (RT-)PCR detection, an obstacle for rapid deployment and implementation of laboratory response to an emerging event are the dissemination logistics for international sharing of materials critical for diagnostic set-up and validation, due to accumulating restrictive regulations fuelled by biosecurity
concerns ('dual-use') [79] and the Nagoya Protocol on Access and Benefit-sharing [80]. Inter-epidemic preparation of and negotiation on so-called umbrella permits and Memorandums of Understanding together with internationally generally accepted Standard Operating Procedures for shipment should facilitate these issues in outbreak situations.

The establishment of sequence data-sharing platforms

With the rapid development of next-generation sequencing approaches, next-generation sequencing as a generic tool for agnostic detection of pathogens has great potential for the emerging infectious diseases field. In this field, sharing of data seems suboptimal, for a range of reasons, including practical, legal, ethical and political barriers [81]. The development and use of data-sharing platforms where sequences, preferably linked to essential background information (e.g. date, location, host species, sample type, travel information, clinical manifestation) and bioinformatics workflows, are deposited and shared will contribute to an effective laboratory response and overall response to emerging disease events. The sharing of data regarding emerging infectious diseases is not without problems, as it involves multiple stakeholders with different incentives [82,83]. The mapping of barriers to data sharing to identify possible solutions is widely debated, with the overall agreement that better systems need to be developed [81–84]. Examples of such data-sharing platforms/networks are the WHO-endorsed DengueNet, the Germany hosted Global Initiative for Sharing All Infections (GISAID) [82,83] and the Nagoya Protocol on Access and Benefit-sharing [80]. The development and use of data-sharing platforms where sequences, preferably linked to essential background information (e.g. date, location, host species, sample type, travel information, clinical manifestation) and bioinformatics workflows, are deposited and shared will contribute to an effective laboratory response and overall response to emerging disease events. The sharing of data regarding emerging infectious diseases is not without problems, as it involves multiple stakeholders with different incentives [82,83]. The mapping of barriers to data sharing to identify possible solutions is widely debated, with the overall agreement that better systems need to be developed [81–84]. Examples of such data-sharing platforms/networks are the WHO-endorsed DengueNet, the Germany hosted Global Initiative for Sharing All Infections (GISAID) [82,83] and the Nagoya Protocol on Access and Benefit-sharing [80].

Quality assurance

Diagnostic laboratories need to comply with accreditation schemes (e.g. ISO15189), which requires extensive validation of the assays used, although accreditation requirements differ per country. A specific hurdle to implementation of diagnostics for emerging or newly established infections is that accreditation schemes often do not accept validations done by other laboratories. Clinical samples needed for validation may be difficult to come by when dealing with an emerging disease. In an assessment of the Zika virus (ZIKV) laboratory response in European reference laboratories it became clear that although a majority [84] of laboratories were willing to share their validation data with other laboratories, external validation was only acceptable for 34% of the laboratories [58]. The availability of validation panels and positive controls to assure diagnostic accuracy is generally a major obstacle for a rapid response. Forty-seven per cent of the EU/EEA reference laboratories for ZIKV diagnostics indicated that the availability of well-defined serology validation panels was their biggest challenge for implementation of diagnostics, closely followed by the lack of positive reference materials (43%) [58]. Of 39 European laboratories responding to an Ebola virus laboratory response questionnaire, 12% indicated the availability of positive reference material as a major obstacle for an adequate response to the Ebola virus outbreak in West Africa in 2014/15 [89] (Reusken et al., in press). These issues could be addressed during inter-epidemic activities involving general bio-banking of a wide range of well-defined validation cohorts and the establishment of validation data-sharing platforms. Bio-banking is addressed for instance by the EU H2020 programme EVAg [90]. However, established platforms for timely sharing of validation data are currently lacking. Sharing of such data is mostly done bilaterally between collaborating laboratories or only too late in the response process through peer-reviewed publication, while specialized networks like the ECDC Emerging viral disease expert laboratory network EVD-LabNet [91] and the EU Joint Action EMERGE [92] might act as facilitators. ZIKV emerged in the Americas in May 2015 and the first publications putting serumology test validation data in the public domain appeared >1 year later, with substantial test comparisons even >2 years later [93–96].

Capability building

A laboratory’s capability for accurate diagnosis of endemic and emerging infectious diseases will benefit from training and External Quality Assessments (EQA, proficiency testing). Both EVD-LabNet and EMERGE provide training courses and twinning partnerships, and run EQAs based on needs indicated by their members [97–108]. The role of the diagnostic laboratory in research, Public Health and clinical response to emerging infectious disease events can be trained, optimized (identification of knowledge/response gaps) and secured in multi-disciplinary outbreak simulation exercises [109–111].

Establishment of preparedness networks

All of the above-mentioned inter-epidemic preparedness activities need strong national and international networks addressing these issues. In recent years the European scientific, public health and medical communities have made substantial progress by establishing a number of international networks like the EU H2020 research networks PANDEM [112], COMPARE [113], ERINHA [114] and EVAg [89], and the Public Health oriented ECDC respectively EC DG Santé-endorsed laboratory response networks EVD-LabNet [91] and EMERGE JA [92]. Clinical research response is addressed in the EU research network PREPARE [115] while the public–private partnership in the Zoonoses Anticipation and Preparedness Initiative [116] focuses on the design of new, high-throughput manufacturing processes for delivering effective infectious disease control tools. A putative pitfall of this increasing number of preparedness and response networks is the lack of interoperability between these entities. Establishment of collaboration across the disciplines covered by each of these networks would bring the European preparedness and response to a next level.

Laboratory preparedness for arboviruses

Preparedness and response for arbovirus emergence is quite challenging mainly for three reasons. First, the clinical manifestations of arbovirus infections overlap and are non-specific in the first...
phase of disease. In general, the broad pallet of arbovirus syndromes is classified into four main syndrome groups: febrile disease, arthralgia and/or rash, haemorrhagic syndrome and neurological syndrome [2,51,117]. Second, arbovirus circulation overlaps geographically, which complicates narrowing down the necessary diagnostic panel. Diagnosis of arbovirus infections is often mainly based on serological testing as viraeemia is typically short-lived [2,118–121]. Diagnosis based on serology however has severe drawbacks due to frequent cross-reactivity between antibodies triggered by closely related viruses or their vaccines while secondary infections might boost levels of cross-reactive antibodies due to previous infections/vaccinations, which complicates a proper interpretation of test results. An illustration is provided by the current co-circulation of DENV and ZIKV in the Americas. Overlap in disease spectrum, geographic presence, and widespread YFV vaccination make interpretation of diagnostic serology very challenging. In Europe co-circulation of multiple neurotropic flaviviruses, like TBEV, WNV and USUV, and sometimes locally high vaccination grades for TBEV, represent similar issues [57,118,122,123].

Multiple studies have shown that arbovirus illness is under-diagnosed in returning travellers and in endemic areas [124–128]. A recent study among >2000 Dutch travellers with known clinical and travel history demonstrated that clinicians, irrespective of the likelihood of such an infection, rarely requested arbovirus diagnostics for travellers within Europe and overemphasized arbovirus requests for patients with very severe or very specific presentations whereas the majority of arbovirus infections present in non-specific syndromes [117]. Although commercially available tools exist to provide clinical laboratories and clinicians with decision support regarding the necessary differential diagnostics [129], the complexities of the arbovirus response cannot be reflected in these ranking tools. Therefore an overall underdiagnosis of arbovirus infections is expected and it is simply not feasible (i.e. cost effective) to determine the cause of a disease beyond the most common and treatable aetiology.

Expert laboratories for BSL3 and BSL4 arboviruses in the two European laboratory preparedness networks EVD-LabNet and EMERGE aim to provide expertise and reference [58,102,130,131], but first-line arbovirus diagnostics will also be performed in routine, primary, secondary and tertiary healthcare-associated laboratories, especially in case of an epidemic when scale-up of testing is needed. Although there is a broad European coverage at the country level for priority arboviruses in reference laboratories and the capability for their diagnostics in European reference laboratories has been assessed in the past [97,106,130,132,133], the coverage of and capability for such assays in routine, healthcare-associated laboratories and the existence of pre-arrangements for scale-up need to be assessed as well to address the level of preparedness for larger outbreaks/epidemics. This will affect outbreak response and individual patient care as in large outbreaks (national) reference laboratories will lack the capacity to handle diagnostic requests, while timeliness is often only assured with onsite testing in the absence of a pre-arranged efficient sample transport infrastructure. At the beginning of 2016, the Brazilian government distributed 500 000 PCR kits for molecular testing for ZIKV to 27 laboratories in the country, and in October 2016 3.5 million rapid serology tests were distributed [134]. However, proficiency testing in parallel with the up-scaling of diagnostic capacity is crucial, as major differences in assay performance in EQA assessments of emerging infections have been observed [98–100,133,135–137]. For instance, although ZIKV diagnostics were widely covered in Europe in the first phase of the outbreak, an EQA showed that the capability for molecular diagnosis of a ZIKV infection lacked sensitivity [58,108].

Conclusion

The overall global and European health burden of arboviruses results in increasing pressure on laboratory preparedness and response infrastructures. As timely and accurate diagnosis of cases is one of the main pillars for public health and clinical responses to an infectious disease emergence, inter-epidemic activities could ensure such adequate responses. (Re)emerging infectious disease outbreak preparedness plans should consider the laboratory pillar and be developed in a collaboration between reference laboratories and hospital laboratories, and include planning of the strengthening of such local capacity and capability when needed, e.g. in case of an outbreak overflowing the national reference system. The current mushrooming of European preparedness networks requires governance and the establishment of collaboration and alignment across the disciplines covered by each of these networks in order to bring the European preparedness and response to a next level.

Transparency declaration

The authors declare that they have no conflicts of interest.

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