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PUBLIC HEALTH GUIDANCE

Public health guidance for assessing and mitigating the risk of locally-acquired *Aedes*-borne viral diseases in the EU/EEA

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Abbreviations

ADE	Antibody-dependant enhancement
BSL	Biology safety level
CHIKV	Chikungunya virus
DENV	Dengue virus
DHF	Dengue haemorrhagic fever
DSS	Dengue shock syndrome
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunoassay
EMA	European Medicines Agency
EQA	External quality assessment
EU	European Union
EURL-PH-VBV	European Reference Laboratory for public health on vector-borne viral pathogens
EVD-Net	Emerging and Vector-borne Diseases network
IVD	In vitro diagnostic
JEV	Japanese encephalitis virus
NA	Not applicable
NAAT	Nucleic acid amplification test
NFP	National Focal Point
NITAG	National Immunisation Technical Advisory Group
RT-PCR	Reverse-transcription polymerase chain reaction
SoHO	Substances of human origin
VectorNet	European network for medical and veterinary entomology
WHO	World Health Organization
YFV	Yellow fever virus
ZIKV	Zika virus

Executive summary

In recent decades, the epidemiology of mosquito-borne virus infections has been changing in several European countries. Locally acquired (i.e. autochthonous) cases of dengue, chikungunya virus disease and Zika virus disease have been reported in the Mediterranean regions of Europe. Between 2021 and 2024, the number of autochthonous dengue outbreaks and cases increased considerably. Intensive international travel has facilitated the importation of mosquito-borne viruses from endemic, tropical and sub-tropical areas while secondary, local transmission of the viruses from imported cases is predominantly driven by invasive *Aedes* mosquito species. Established populations of the Asian tiger mosquito (*Ae. albopictus*) have been reported in 13 countries in the European Union. The yellow fever mosquito (*Ae. aegypti*) has also been detected in parts of the European Union (e.g. Cyprus, Canary Islands (Spain) and Madeira (Portugal)). The summer weather conditions in recent years have been favourable for invasive *Aedes* mosquito propagation in European countries, and for the multiplication of *Aedes*-borne viruses in the vectors. Due to climatic changes, periods with favourable environmental conditions for the geographical spread and multiplication of invasive *Aedes* mosquitoes are expected to become longer. Warmer weather also facilitates the local transmission of viruses by these mosquitoes. Therefore, the risk of autochthonous outbreaks of *Aedes*-borne viral diseases is foreseen to increase in Europe. Meanwhile, the level of preparedness and experience with prevention and control of *Aedes*-borne viral disease varies in the European countries. This public health guidance provides information to support the assessment and mitigation of the risk of locally acquired *Aedes*-borne viral diseases for public health experts in the European Union/European Economic Area (EU/EEA).

The guidance was produced following consultation with experts in this field. Public health experts were consulted from countries affected by autochthonous transmissions of *Aedes*-borne viral diseases (i.e. France, Italy, Portugal and Spain) and from at-risk countries with varying epidemiological situations (i.e. Belgium, Germany and Greece). In addition, laboratory experts, medical entomologists, and ECDC experts on emerging and vector-borne diseases, substances of human origin, and emergency preparedness and response support were involved in the production of the guidance. In April 2024, a workshop was organised during which the main principles of the guidance were developed. The workshop was followed by a written consultation with the experts involved. An advanced draft of the guidance was then shared with the National Focal Points from the Emerging and Vector-borne Diseases Network and the Substances of Human Origin – Blood Network for comments.

The guidance provides key information on *Aedes*-borne viruses (particularly dengue virus, chikungunya virus and Zika virus), the principles of the laboratory diagnosis of these viruses and the epidemiology of *Aedes*-borne viral disease in Europe. Areas at risk of autochthonous, mosquito-borne transmission in the EU/EEA are divided into four risk levels (i.e. Level 1 to Level 4, and two sub-levels for Level 2 and 3). Areas without established vectors of *Aedes*-borne viruses are categorised as Level 1. Predisposed areas, where vectors of *Aedes*-borne viruses are established (i.e. there is evidence of over-wintering and reproducing populations of *Aedes* vectors), but where vector-borne transmission of the respective pathogen has not been detected in the current transmission season are categorised as Level 2. Within this level, two sub-levels were identified, depending on the receptivity and the vulnerability of an area. Receptivity is determined by the presence and density of *Aedes* vectors and other ecological and climatic factors favouring *Aedes*-borne virus transmission. Vulnerability is defined by the influx of infected travellers and the capacity of the health system to detect infections timely and deploy measures to prevent onward transmission. Predisposed areas with low receptivity and/or vulnerability (e.g. only locally established vectors, low vector density, unfavourable climate, low number of travellers from Level 3 and Level 4 areas) are considered to be Level 2a, whereas areas with medium to high receptivity and vulnerability are considered to be Level 2b. Areas which have experienced sporadic, autochthonous transmission in previous years are also considered to be at this risk level. Risk areas which are affected by autochthonous transmission of *Aedes*-borne viruses in the current transmission season are categorised as Level 3, with two sub-levels, depending on the number of outbreaks/clusters and the traceability of transmission chains. If there has been at least one confirmed autochthonous case of an *Aedes*-borne viral disease as a result of local and probable mosquito-borne transmission in the area during the current year, but the number of cases/clusters is considered to be low and transmission chains are traceable, the area is considered to be Level 3a. If the number of cases/clusters is considered to be high, involving overwhelming tracing capacity, the area is considered to be Level 3b. Endemo-epidemic areas, where autochthonous transmission of *Aedes*-borne viral diseases is not dependent on importation of the viruses, are categorised as Level 4.

For each risk level the public health guidance outlines triggers for re-assessment, as well as relevant surveillance, prevention, preparedness, response and control actions (including laboratory preparedness, awareness raising and capacity building, multi-sectoral coordination and vector management activities). At Levels 1 and 2, public health actions focus on surveillance and prevention, while at Levels 3 and 4 more emphasis is placed on response and control measures. As risk levels are defined by the epidemiological, entomological and environmental conditions, triggers for re-assessment of categorisations are also specified in the document. Concise summaries of disease and entomological surveillance for *Aedes*-borne viral diseases and their vectors are also provided in the guidance, as well as explanations of the suggested prevention, preparedness, response and control actions.

The guidance has been developed on the basis of previous experience and currently applied practices in countries affected by locally acquired transmissions of *Aedes*-borne viral diseases in the EU/EEA. The measures applied are based on international and national guidelines against *Aedes*-borne viral diseases and general public health measures against mosquito-borne diseases. However, scientific evidence is often missing for the specificity, effectiveness and efficiency of the measures in the European context. The main gaps in knowledge and relevant topics for research are also discussed in the guidance. Links to further information on surveillance, preparedness and response (control) actions are provided in Annex 1.

Background and scope

Human arboviruses carried by mosquito vectors in the *Aedes* genus are having a major public health impact in tropical and sub-tropical countries. Citizens of the EU/EEA are predominantly exposed to *Aedes*-borne virus infections during trips to countries or regions with active vectorial transmission of these viruses. Importations of *Aedes*-borne viruses to the EU/EEA by travellers are frequently reported. Until recent years, autochthonous (i.e. locally acquired) cases associated with vector-borne transmission from imported cases were rarely reported. However, the geographical distribution of the Asian tiger mosquito (*Aedes albopictus*) has extended considerably in Europe in recent decades, and the environmental and ecological parameters (average temperature in summer months, mosquito abundance) may provide favourable conditions for local, mosquito-borne transmission of *Aedes*-borne viruses from imported cases.

Increasing numbers of autochthonous dengue cases have been reported in recent years in some western/southern European countries, indicating a growing threat to public health in the EU/EEA. ECDC aims to support the EU/EEA countries in strengthening their surveillance, prevention and control activities against autochthonous transmission of *Aedes*-borne viral diseases (dengue, chikungunya virus disease and Zika virus disease). This public health guidance is intended to support the assessment of the risk of locally acquired *Aedes*-borne viral diseases and suggest suitable public health actions and activities for prevention and control. The scope of the public health guidance is to:

- provide concise descriptions of the most important *Aedes*-borne viruses and the epidemiological situation for *Aedes*-borne viral diseases in the EU/EEA;
- describe risk levels for transmission of *Aedes*-borne virus infection in different geographical areas;
- provide concise descriptions of the suggested surveillance, prevention and control measures;
- describe limitations of the guidance, missing scientific evidence, gaps in knowledge and topics for operational research;
- provide links to relevant documents on public health activities to counteract *Aedes*-borne viral diseases.

Key facts about *Aedes*-borne viral diseases

Aedes-borne viruses

The term '*Aedes*-borne viruses' refers to a broad ecological/epidemiological category of different arboviruses that are transmitted by mosquitoes in the *Aedes* genus. Due to the high diversity of *Aedes* mosquito species globally and the arboviruses detected in them, there are many viruses in this category. The detection of an arbovirus in an *Aedes* vector, or even the demonstration of the ability of a vector species to transmit a defined virus in laboratory experiments (vector competence) cannot be considered as evidence for a vectorial role in natural (field) conditions.

This public health guidance focuses on three diseases (dengue, chikungunya virus disease and Zika virus disease) caused by *Aedes*-borne viruses that are not endemic in EU/EEA countries on the European mainland, even though local outbreaks connected to autochthonous, mosquito-borne transmission have been reported in some countries and further outbreaks are expected in the coming years.

Dengue virus (*Orthoflavivirus denguei*, DENV) is the causative agent of dengue, which is the most widespread mosquito-borne viral disease affecting humans worldwide. Although up to 80% of all dengue infections can remain asymptomatic, commonly reported clinical symptoms include acute, high fever, headache, myalgia, arthralgia, maculopapular rash and haemorrhage. A portion of cases (usually <5%) can be severe and these are commonly referred to as 'dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS)'. Severe dengue is characterised by an increase in vascular permeability that can lead to life-threatening hypovolemic shock. A fraction of DHF/DSS cases may be fatal (particularly in children and adolescents). DENV has four distinct serotypes. Immunity to any of the four serotypes is probably life-long, but this does not confer protective immunity to the other three serotypes. Subsequent infection by a different DENV serotype may contribute to the development of DHF/DSS through antibody-dependant enhancement (ADE), potentially together with host and virus genetic factors and host comorbidities. The median incubation period for dengue is 5.6 days (3–14 days) [1]. Viraemia reaches high titres two days before the onset of symptoms, and it is generally high enough to infect mosquitoes for the next six days [1,2]. In addition, people who are infected with DENV without developing clinical symptoms are also infectious to mosquitoes [2-4].

Humans are the main amplifying host of the virus. Although bites from infected mosquitoes are the predominant mode of transmission, non-vectorial transmissions (e.g. nosocomial, sexual, intrapartum, perinatal and mucocutaneous transmission, as well as transmission through breastfeeding, occupational (medical/laboratory), blood transfusion, bone marrow transplant and organ transplant) have also been reported [5]. Non-human primates may be involved in the sylvatic cycle of dengue in south-eastern Asia, western Africa and South America [6]. The virus circulates between humans in rural and urban areas. The primary vectors are the yellow fever mosquito (*Aedes aegypti*) and the Asian tiger mosquito (*Aedes albopictus*). According to laboratory experiments, the time required for the mosquito to become infective after feeding on a viraemic host (i.e. the extrinsic incubation period), is about ten days at 27°C (but longer at lower temperatures). The optimal daily mean temperature for dengue virus transmission by *Ae. albopictus* in temperate zones of the northern hemisphere is 24–26°C; however, transmission can occur within the temperature range of 12–30°C [7,8]. Dengue is endemic in more than 100 countries in Africa, the Americas, South and South-east Asia, and the Western Pacific region [9,10]. Dengue viruses are often transported by infected travellers. All four serotypes now co-circulate in many places in the world. The number of dengue cases has been increasing considerably over the past few decades, and large outbreaks have been reported [9]. Imported cases of dengue are frequently reported in travellers returning to the EU from endemic areas [11-13]. These may generate local, mosquito-borne transmission events in non-endemic areas where competent vectors are present, and the climatic/weather conditions support virus multiplication in them [14-16].

Chikungunya virus (*Alphavirus chikungunya*, CHIKV) has three major genetic lineages and, until the spread of the East-Central-South African serotype to Asia in 2006 and the Asian type into the Americas in 2013, these three genetic lineages reflected the geographical distribution of the virus (West Africa, East-Central-South Africa and Asia). Up to 28% of CHIKV infections remain asymptomatic [17], but in most cases the disease is characterised by a sudden onset of fever, chills, headache, myalgia, nausea, photophobia, incapacitating joint pain and petechial or maculopapular rash which develops 3–7 days (1–12 days) after infection [18]. Recovery after the acute phase (about 10 days) is common, typically resulting in a life-long immunity against subsequent CHIKV infections [18]. However, the chronic form of CHIKV disease, characterised by recurrent joint pain, may develop in about 5–80% of cases [18]. General complications are rare and include myocarditis, hepatitis and ocular and neurological disorders. Risk factors for more severe disease include intrapartum infection during the perinatal weeks for the neonates, advanced age (>65 years) and co-morbidities. In the elderly, arthralgia can evolve to a chronic rheumatoid arthritis syndrome. Meningoencephalitis affects primarily neonates. The reported average case fatality for CHIKV disease is low, however, excess mortality temporarily associated with different CHIKV disease outbreaks has been observed [19]. Humans are the major vertebrate hosts of CHIKV worldwide, although in Africa and Asia wild primates can also be included in the transmission cycle. The main vectors of CHIKV to humans are the *Ae. aegypti* and *Ae. albopictus* mosquito species.

Mutations in the E1 and E2 glycoproteins of CHIKV can affect viral fitness for dissemination by *Ae. albopictus* [20]. Virus titres in the blood of humans can be very high at the beginning of the illness, lasting for around six days (three to 10 days) [21]. Viraemic humans (both symptomatic and asymptomatic) can infect mosquitoes feeding on them. The extrinsic incubation period varies between two and 15 days [22]. Mother-to-child transmission has also been reported in women who developed the disease within the final week prior to delivery. There is no evidence that the virus is transmitted through breast milk. Transmission of chikungunya virus through transfusion and transplantation has not been reported, but asymptomatic blood donors with high titre viraemia have been detected through CHIKV nucleic acid amplification test (NAAT) screening [23,24].

Zika virus (*Orthoflavivirus zikaense*, ZIKV) has two genetic lineages: the African lineage and the Asian lineage. Over the last two decades, the geographical distribution of ZIKV has expanded to new areas in the Pacific and the Americas. Humans infected with ZIKV often remain asymptomatic. However, infections can manifest as ZIKV disease after 3–10 days of incubation [25]. ZIKV disease usually involves a short (2–7 days) and self-limiting illness with typically mild symptoms of maculopapular rash, with or without mild fever, arthralgia, fatigue, non-purulent conjunctivitis/conjunctival hyperaemia, myalgia and headache [25]. However, among women infected during pregnancy, congenital central nervous system malformations of the foetus (such as microcephaly) and foetal losses were notified during several recent ZIKV disease outbreaks [26]. Unusual increases of Guillain–Barré syndrome incidence, coinciding with the ZIKV disease outbreaks, were reported in several countries in the Americas and in French Polynesia [25]. Although serological surveys in Africa and Asia have detected specific antibodies in various animal species, no animal reservoir for ZIKV is known in Europe. Globally, *Ae. aegypti* is the main vector of ZIKV, but other *Aedes* species can also transmit the virus. The role of *Ae. albopictus* as vector was evidenced by the autochthonous transmission of ZIKV in France in 2019 [27]. Asymptomatic infected humans develop viraemia levels that might infect mosquitoes, however the susceptibility of *Ae. albopictus* and *Ae. aegypti* lines to ZIKV strains can vary. The extrinsic incubation period (i.e. the time interval between a mosquito feeding on virus-infected blood and shedding the virus through its saliva) varies between five and 24 days, depending on temperature and vector species. Beyond vector-borne transmission, maternal-foetal transmission can also occur, most probably by trans-placental transmission and during delivery, when the mother is infected. Sexual transmission has also been documented in several instances [26]. It is also suspected that ZIKV can be transmitted through transfusion of blood or blood components [28].

Epidemiology of dengue, chikungunya virus disease and Zika virus disease in Europe

To date, dengue, chikungunya virus disease and Zika virus disease are not endemic in mainland EU/EEA and the vast majority of the cases are reported among travellers infected in endemic countries in the tropics and sub-tropics. However, when the environmental conditions are favourable, in areas where the competent vectors are established, viraemic travel-related cases may lead to local transmission of the virus. The probability of local transmission after importation events depends on the abundance and activity of the *Ae. albopictus* and *Ae. aegypti* population (which varies seasonally and itself depends on factors such as human population density, the availability of water-holding containers for the development of the aquatic mosquito larvae, rainfall and temperature). It also depends on protective measures (such as protection against mosquito bites and other mosquito control measures), and temperature, which governs the speed of reproduction of the viruses inside the mosquitoes (see section entitled '*Aedes*-borne viruses').

From 2010 to 2024, 579 autochthonous cases of dengue were reported in mainland EU/EEA involving transmission by *Ae. albopictus*, with an increasing number of outbreaks occurring in the most recent years (71 cases in 2022, 130 cases in 2023 and 304 cases in 2024) [14]. These cases were reported by Italy (305), France (240), Spain (24) and Croatia (10).

From 2007 to 2024, 861 autochthonous cases of chikungunya virus disease were reported in mainland EU/EEA. These cases were reported by Italy (829) and France (32) [29,30].

From 2016 to 2024, three autochthonous Zika virus disease cases transmitted through local mosquito bites were reported by France (Hyères city, Var department) [27].

Dengue, chikungunya virus disease and Zika virus disease are notifiable at EU level and therefore surveillance is compulsory in EU/EEA countries. The surveillance system adopted can vary, depending on the probability of virus activity and resources available [31].

Laboratory diagnosis of *Aedes*-borne virus infections

The recommended and preferred laboratory diagnostic tests for the detection of *Aedes*-borne virus infections depend on several factors, including: i) the clinical information and case history, ii) the available specimen (e.g. blood/plasma, urine, saliva, cerebrospinal fluid, amniotic fluid, semen, and breast milk), and iii) the capabilities of the laboratory.

In the acute stage of infection, the direct detection of the virus or its components (i.e. nucleic acid and/or antigens) can be attempted. Molecular assays, particularly nucleic acid amplification by reverse-transcription polymerase chain reaction (RT-PCR) (using various platforms) are the most widely used methods for the detection of viral RNA in diagnostic samples. The advantage of RT-PCR-based techniques are the high sensitivity, high specificity, rapidness and availability/easy set-up in most laboratories. Beyond potential test sensitivity or specificity issues connected to sequence diversity of the target viruses, probably the most important limitation of molecular tests is that the presence of the virus in the patient is limited in time and may rapidly decrease below detection limits during the course of the infection. Therefore, the diagnostic window is usually five to seven days after the disease onset of dengue, chikungunya virus disease or Zika virus disease. However, it also depends on the type of specimen (e.g. DENV RNA is detectable in urine for a longer period than in blood and ZIKV RNA has been detected in semen samples several weeks after infection). In general, the positive result of a molecular test is highly informative and allows case confirmation, but the negative predictive value of such tests is low, especially during later phases of infection.

The subsequent determination of a partial or complete genome sequence of the virus(es) in the specimen is encouraged for the identification of genotype/serotype, molecular epidemiology and test result confirmation/validation purposes. Tests for the detection of DENV non-structural protein 1 (NS1) antigen in blood by immunochromatographic or enzyme immunoassay techniques are also used for direct diagnosis in patients with acute infection. The isolation of *Aedes*-borne viruses (e.g. in cell cultures) is not used in routine diagnostics nowadays (due to the long turn-around time and extensive resources required – e.g. BSL-3 laboratory facilities), however, subsequent isolation of viruses from a positive clinical specimen is encouraged for reference and research purposes.

The indirect diagnosis of *Aedes*-borne virus infections is based on the demonstration of the immune response to the virus antigens, most frequently through the detection of anti-viral antibodies in samples. Several platforms and assays are available, including ELISA tests, immunofluorescence assays, and chemiluminescence immunoassays for the detection of virus-specific IgM and IgG antibodies in serum or plasma. The overall advantage of serological tests is the wide diagnostic window, as antibodies may persist for several weeks or months after infection. The timing of the infection can be estimated through the detection of different immunoglobulin types (e.g. IgM and IgG antibodies), the determination of the antibody titres, the determination of antibody kinetics in paired sera (e.g. seroconversion) or antibody avidity tests. However, the accurate determination of the time of infection is often challenging if only serological data are available. One of the main disadvantages of serological tests for the diagnosis of *Aedes*-borne virus infections is the cross-reactivity between related viruses. DENV and ZIKV have antigenic structures of viral proteins similar to other viruses in the *Orthoflavivirus* genus. For the diagnosis of autochthonous infections in Europe, cross-reactions with endemic viruses in the area, particularly West Nile virus, Usutu virus and the tick-borne encephalitis virus, should be taken into consideration.

For imported cases, further orthoflavivirus infections (e.g. yellow fever virus (YFV) and Japanese encephalitis virus (JEV)) should be excluded. Antibodies against CHIKV may also cross-react with other alphaviruses (e.g. Sindbis virus), which may influence test specificity, however, serological cross-reactivity between alpha and flaviviruses is not expected. The different serological methods vary in specificity. The 'gold standard' for the determination of serotype-specific antibodies is usually the virus neutralisation test (or plaque reduction test), simultaneously performed on the serum sample with potentially cross-reacting viruses. However, it can be challenging to perform such tests in some laboratories, due to technical capabilities (e.g. access to BSL-3 laboratory facilities, availability of viruses, cell lines) and capacities. Previous infections or immunisations (e.g. vaccinations against tick-borne encephalitis, yellow fever, dengue or Japanese encephalitis) should also be taken into consideration during the interpretation of serological results.

Diagnostic test kits (with CE label) for the most important *Aedes*-borne viruses (e.g. CHIKV, DENV, YFV, ZIKV) are marketed in the EU/EEA; however, availability may vary. Careful planning of procurements and stockpiling can ensure sufficient supply of in vitro diagnostics (IVDs). Some laboratories may use assays developed in-house. The IVDs should comply with the regulation (EU) 2017/746 on in vitro diagnostic medical devices [32]. Laboratory diagnostic capabilities for the above-mentioned diseases should be developed and maintained irrespective of the risk levels. The European Reference Laboratory for public health on vector-borne viral pathogens (EURL-PH-VBV) provides support for the development and maintenance of laboratory diagnostic capabilities on *Aedes*-borne viruses (e.g. provision of laboratory guidelines, protocols, reference materials, confirmatory services and training) to the EU/EEA countries. Diagnostic capabilities should be regularly monitored through quality assurance and external quality assessments (EQAs).

The demand for laboratory diagnostic tests may rapidly increase during an outbreak. Therefore, the preparedness and response plan should describe procedures for the allocation of resources to ensure, and – in case of an upsurge in requirements – laboratory capacities. Protocols for sample collection, testing and timely reporting should be specified (including the financing of laboratory tests).

Regular communication between general practitioners, specific clinics (e.g. infectious disease clinics, vector-borne disease clinics, travel medicine clinics), hospitals, public health departments and laboratories are essential to understand the rationale behind the choice of the laboratory diagnostic method, the availability of assays and the expectations and limitations of test results. In outbreak situations, communication and coordination between clinicians, virologists, epidemiologists and crisis managers should be enhanced.

Methodology of the guidance production

The number of published scientific reports on public health actions in the context of autochthonous, vector-borne transmissions of *Aedes*-borne viruses in the EU/EEA is low. This public health guidance is based on the knowledge and experience of topic experts from the EU/EEA. Experts have been invited to contribute on the basis of their independent scientific capacities. ECDC's National Focal Points for Emerging and Vector-borne Diseases (NFPs) from four countries with autochthonous transmission of *Aedes*-borne viruses (France, Italy, Portugal and Spain) were consulted to identify two national experts to be involved in the development of the guidance. For the representation of at-risk countries with different epidemiological situations and risk levels, experts were also invited from three other countries (Belgium, Germany and Greece, one expert from each) to help develop the document. To obtain state-of-the-art information on the virological and laboratory diagnosis aspects of *Aedes*-borne virus infections, two virology laboratory experts were invited, as well as two medical entomologists to provide expert opinions on the vector monitoring, management and control issues. All the experts invited provided declarations of interest, which were assessed for conflict of interest according to ECDC's policy on scientific integrity and independence [33]. No conflicts of interests were identified for the participants.

A workshop was organised on 16–18 April 2024 at ECDC (Solna, Sweden) where the aforementioned experts and ECDC's own experts discussed the content of the public health guidance. A draft risk matrix table was developed and shared with the participants prior to the workshop. The input from the experts at the workshop was included in the draft risk matrix, which was subsequently reviewed by the meeting participants. The other parts of the guidance were drafted by ECDC experts in consultation with the external experts, based on published scientific papers, national and international reports, protocols and guidelines.

The draft public health guidance was submitted for a stakeholder consultation between 14 June and 14 September 2024. The document was shared with the NFPs of the EVD-Net and with the NFPs in the Blood group of the Substances of Human Origin (SoHO) network. The input obtained through the stakeholder consultation was included in the final version of the guidance.

The risk matrix and suggested public health actions

Definition of terms used to describe areas of transmission for *Aedes*-borne virus infection

Risk levels

Four risk levels (Level 1 to Level 4, and two sub-levels for Levels 2 and 3) for transmission of *Aedes*-borne viruses are defined in a risk area. For each risk level the guidance outlines the relevant surveillance activities, prevention and control measures.

Definition of terms

- A **risk area** is a geographical area where individuals are exposed to the risk of being infected with locally acquired *Aedes*-borne pathogens.
- **Introduction of a vector** is any detection of a vector species in the environment in any area where the vector has not been established.
- **Established status of vectors** is attributed when a population of mosquitoes has been observed over-wintering and reproducing locally in the environment within or near the same locality in at least one municipality within the risk area. Evidence for an established population of the species is the presence of the insect (at any life stage) in the outdoor environment before the coldest month of the year, and of living immature stages or a growing adult population thereafter.
- The mosquito **transmission season** for *Aedes*-borne viruses mainly depends on environmental factors, in particular temperature. The optimal daily average temperature for dengue and chikungunya virus transmission in temperate zones of the northern hemisphere is 24–26°C for *Ae. albopictus* and 28–29°C for *Ae. aegypti.* However, transmission can occur within the temperature range of 12–30 C for *Ae. Albopictus* and 13–34°C for *Ae. aegypti* [7,8].
- **Receptivity** of a risk area is determined by the presence and density of *Ae. albopictus* and/or *Ae. aegypti* and other ecological and climatic factors favouring *Aedes*-borne virus transmission.
- **Vulnerability** of a risk area is defined by the influx of infected travellers and the capacity of the health system to timely detect infections and deploy measures to prevent onward transmission.
- **Laboratory capacity** for *Aedes*-borne viral infections refers to the ability of EU/EEA laboratories to handle the volume of diagnostic testing, surveillance testing, and research activities required to manage and control outbreaks.
- **Laboratory capabilities** for *Aedes*-borne viral infections refer to the range and complexity of diagnostic tests, surveillance, and research methodologies that EU/EEA laboratories can perform to detect, identify and study these infections.
- **Monitoring** consists of procedures implemented for temporary or continuous observation (e.g. of species dynamics) and is not followed by any additional activities.
- **Surveillance** consists of procedures developed in response to a risk and carried out to support subsequent actions.
- **Haemovigilance** refers to a set of organised surveillance procedures relating to serious adverse or unexpected events or reactions in donors or recipients, and the epidemiological follow-up of donors (according to Directive 2002/98/EC) [34]. It covers the entire transfusion chain, from the donation and processing of blood and its components to their provision and transfusion to patients and their follow-up. It includes the monitoring, reporting, investigation and analysis of adverse events related to the donation, processing and transfusion of blood, and actions taken to prevent their occurrence or recurrence.
- **Biovigilance** extends the term haemovigilance beyond blood to incorporate monitoring of adverse events and reactions associated with other substances of human origin (SoHO), such as tissue, organs, and cells used for transplantation [35]. It should be noted that for this guidance, having systems in place for haemovigilance and biovigilance does not imply routine testing for *Aedes*-borne viruses in SoHO.
- **Risk Level 1**: An **area without established vectors** is a risk area where *Ae. albopictus* and/or *Ae. aegypti* are not (yet) established and autochthonous vector-borne transmission of *Aedes*-borne viral diseases has never been detected.
- **Risk Level 2**: A **predisposed area** is a risk area where *Ae. albopictus* and/or *Ae. aegypti* are established but where vector-borne transmission of the respective pathogen has not been detected in the current transmission season. The risk for vector-borne transmission in predisposed areas is influenced by the receptivity and vulnerability of the area.
  - **Risk Level 2a**: Predisposed areas with low receptivity and/or vulnerability (e.g. only locally established vectors, low vector density, unfavourable climate, low number of travellers from Level 3 and Level 4 areas).

  - **Risk Level 2b**: Predisposed areas with medium to high receptivity and vulnerability (e.g. widely established vectors, high vector density, favourable climate, high number of travellers from Level 3 and Level 4 areas). Areas with sporadic, autochthonous transmissions in the previous years are also included in this risk level.

Classifying areas into Level 2a or 2b can be challenging, because both receptivity and vulnerability may vary markedly on the local scale (depending on factors such as population density and land-use), and it is difficult to define a clear threshold between low and medium (or medium and high) receptivity, and between low and medium (or medium and high) vulnerability. Furthermore, these indicators vary strongly on a seasonal basis.

In this guidance, it is advised to spatially differentiate between Levels 2a and 2b at the (lowest) spatial resolution for which it practically makes sense to differentially apply the recommended actions, and to consider the highest seasonal risk (e.g. that of the (late) summer months when mosquito biting densities are at their highest, extrinsic incubation periods are shortest and many travellers are returning from summer holidays in affected destinations) in order to differentially classify an area into Level 2a or 2b.

- **Risk level 3**: An **affected area** is a risk area with autochthonous transmission of *Aedes*-borne viruses in the current transmission season. Two types of affected areas are differentiated according to the number of outbreaks/clusters and the traceability of transmission chains:
  - **Risk Level 3a**: There has been at least one confirmed autochthonous case of an *Aedes*-borne viral disease as a result of local mosquito-borne transmission in the area during the current year. However, the number of cases/clusters is considered to be low and transmission chains are traceable.
  - **Risk Level 3b**: The number of cases/clusters is considered to be high and this is overwhelming tracing capacity.
- **Risk Level 4**: An **endemo-epidemic area** is a risk area where autochthonous transmission of *Aedes*-borne viral diseases is not dependent on importation of the viruses (i.e. over-wintering of viruses). In addition, introduction of additional, exotic viruses or strains may also initiate local outbreaks.

Summary of risk area characteristics

Risk Level	Risk area type	Vector established	Autochthonous transmission during the current season	Presence of *Aedes*-borne viruses all over the year
1	Area without established vectors	No	No	No
2	Predisposed area	Yes	No	No
3	Affected area	Yes	Yes	No
4	Endemo-epidemic area	Yes	Yes	Yes

Public health activities at different risk levels

Risk level 1 - Area without established vectors

Description and triggers for re-assessment

- *Ae. albopictus* or/and *Ae. aegypti* are not established in the area (but may be/have already been introduced).
- Public health actions focus on surveillance of imported cases and mosquito vectors.
- Re-assessment frequency: annual.
- Re-assessment triggers: establishment of *Ae. albopictus* or/and *Ae. aegypti* (according to vector surveillance data). → Move to Risk Level 2a.

Public health actions and interventions

Disease surveillance

- Surveillance for notifiable *Aedes*-borne viral diseases and reporting of imported and autochthonous cases at national and EU level (necessary);
- SoHO: generic haemovigilance and biovigilance systems (necessary).

Laboratory diagnosis

- Provide professional guidelines for laboratory testing and clinical management of *Aedes*-borne viral diseases.

The production of guidelines for laboratory testing and clinical management of *Aedes*-borne viral infections requires the formation of an expert committee, possibly interdisciplinary (virologists, laboratory scientists, clinical specialists of infectious diseases, and public health experts) who, based on literature reviews and diagnostic and clinical data, identify effective diagnostic methods and management/treatment protocols. The laboratory testing guidelines should address protocols for sample collection, handling, and storage and recommend appropriate diagnostic assays based on performance metrics, such as sensitivity and specificity. The implementation of guidelines should include offering support and consultation to laboratories and clinicians and regular updates of the guidelines based on new evidence and technological advancements.

- Ensure there are laboratory capabilities within the country for diagnosis.

Key components of laboratory capabilities are: (i) range of diagnostic methods, including molecular methods, next-generation sequencing, serological testing including neutralisation tests, and viral isolation and culture; (ii) number of target pathogens included in diagnostic tests; (iii) availability of advanced techniques (e.g. viral genome sequencing and analysis to track viral mutations and phylogenetic relationships, use of metagenomic approaches to identify novel or co-circulating pathogens in samples); (iv) expertise in bioinformatics for data analysis and interpretation; (v) research and development sector conducting studies to understand pathogen biology and to develop/update diagnostics tests; (vi) quality assurance and control – i.e. adherence to/development of international standards and guidelines (WHO, ECDC) and participation in/organisation of proficiency testing; (vii) collaboration with public health authorities, academic institutions and international organisations to share data, resources, and expertise; (viii) training and capacity building – i.e. providing training and support to other laboratories and public health entities to enhance overall diagnostic capabilities.

- Ensure that laboratory capacity is sufficient to diagnose imported and potential locally acquired cases.
- Monitor the efficacy of laboratory diagnostic methods.

The efficacy of laboratory diagnostic methods can be monitored through the following activities: (i) participation in EQA programmes and inter-laboratory comparisons; (ii) adherence to quality control procedures (use of internal controls, routine check and validation of reagents and calibration and maintenance of equipment; (iii) validation and verification of new methods before implementation; (iv) monitoring and analysis of test results; (v) correlation of test results with clinical data and retesting of samples that produce unexpected or critical results; (vi) obtaining and maintaining certifications and accreditations from relevant authorities to ensure adherence to best practices.

- Ensure that access to laboratory testing is available for patients (including financial aspects of testing costs).

Awareness raising and capacity building

- Promote awareness among healthcare professionals of *Aedes*-borne viral diseases so that this will be considered in the differential diagnosis of travellers returning from affected areas.
- Educate travellers to affected areas on how to reduce the risk (e.g. protect against infection during travel, consider pre-travel-vaccination in accordance with national guidelines).

Multi-sectoral collaboration and coordination

- Competent SoHO authorities should consider measures to prevent transmission to SoHO recipients through transfusion or transplantation from donors returning from affected areas.

Measures for blood, tissues, cells and organ safety could include the implementation of policies for the deferral or testing for *Aedes*-borne viruses of donors who have travelled to or resided in *Aedes*-borne virus affected areas and also the possible use of SoHO pathogen inactivation methods, where applicable.

- Consult with the National Immunisation Technical Advisory Groups (NITAGs) and/or other competent body to define vaccination recommendations for residents and/or travellers to affected areas.

In addition to personal protective measures, vaccination is a supplementary means of protecting against disease. As of 2025, two vaccines were available against dengue (Dengvaxia® and Qdenga®) and two against chikungunya virus (Ixchiq® and Vimkunya®), having received marketing authorisation from the European Medicines Agency (EMA) for use in the EU. There is no vaccine against Zika virus disease available in the EU/EEA. Vaccines should be used in accordance with manufactures' instructions, based on the recommendations from NITAGs or other competent bodies (e.g. travel medicine vaccines). Vaccine recommendations will address priority target groups for vaccination, vaccination schedule, vaccine co-administrations, programmatic consideration for the deployment of the vaccine and risk communication. A strategy for post marketing studies should be clearly defined.

Vector management (entomological surveillance and control activities)

- Develop a surveillance/monitoring plan for invasive *Aedes* mosquitoes in the area.
- Monitor the introduction of new invasive *Aedes* mosquitoes at points of entry.

Early detection of new invasive mosquitoes increases the opportunity for appropriate and timely response measures and *Aedes*-borne disease prevention. For this purpose, a surveillance network must be set up to allow the early detection of an introduction/initial spread of invasive *Aedes* species before it establishes permanent populations and spreads over a wide area. The network should optimise resources by intelligently targeting the surveillance operations at as many previously defined high-risk sites as possible and at points of entry. Such points of entry could be storage sites for imported used tyres, shelters/greenhouses for imported plants, such as *Dracaena* spp. (Lucky Bamboo), large parking lots at country borders, highways and road axes that originate in colonised areas, ports, airports and railway stations.

- Stimulate active and passive monitoring of vectors (e.g. reporting by citizens)

While active monitoring mostly involves the trapping and reporting of mosquitoes by governmental, academic or commercially-run organisations, passive monitoring of vectors could consist of informing the general public (and/or children at schools) and requesting feedback from them (e.g. through mobile-phone-based reporting systems and/or by asking them to send in collected mosquito specimens for identification).

- Model ecological niche suitability of the area for *Ae. albopictus* and *Ae. aegypti*

While observations on the presence and absence of mosquitoes are essential to gain insight into the distribution and spread of invasive *Aedes* mosquitoes, it is not economically feasible to monitor each location. Ecological niche modelling provides estimates of the distribution status for those areas with no information currently available, by establishing statistical relationships between vector distribution data and predictor covariates. This technique could also help to make estimates of future ecological suitability in a changing climate.

- Develop and apply vector control at points of entry, possibly including disinfection protocols on vessels and aircrafts arriving from areas where invasive *Aedes* mosquitoes are present, to mitigate the risk of introducing new vectors.

Control measures include the use of larvicides in stagnant water, community education programmes, and the implementation of disinfection protocols at ports and airports. At points of entry, vector surveillance and control measures should aim to prevent the introduction of invasive *Aedes* mosquitoes by intercepting them before or on arrival. This may include implementing inspection and disinfection protocols on vessels and aircraft arriving from countries where these vectors are known to have already been established. In addition, the implementation of specific measures for high-risk goods coming from these countries could be considered: e.g. the prevention of water collection by covering used tyres or storing them indoors, and use of residual adulticidal applications if water is detected. For plants transported with water, such as lucky bamboo, consider applying larvicidal treatments to the water surrounding the plants and conduct mosquito surveillance both inside and outside greenhouses.

- Identify and deploy appropriate vector control interventions after detecting the introduction of new vector species in order to prevent their establishment.

Rapid and targeted action is essential to prevent the spread of invasive mosquitoes and reduce the risk of their establishment. By implementing effective control measures immediately, the potential for these vectors to establish and proliferate in new areas can be minimised, safeguarding public health.

Links to further information on surveillance, preparedness and response (control) actions are provided in Annex 1.

Risk level 2 - Predisposed area

Risk level 2a - Predisposed area with low receptivity

Description and triggers for re-assessment

- *Ae. albopictus* or/and *Ae. aegypti* are established in the area.
- The vector density is generally low (as described in ECDC's technical report 'Guidelines for the surveillance of invasive mosquitoes in Europe' [36].
- The climatic conditions are generally unfavourable for *Aedes*-borne virus transmission.
- Public health actions focus on surveillance of imported cases and mosquito vectors, prevention and preparedness.
- Re-assessment triggers:
  - weather conditions becoming favourable for *Aedes*-borne virus transmission (based on meteorological data and potentially modelling). → Move to Risk Level 2b;
  - vector densities becoming higher (based on mosquito surveillance data). → Move to Risk Level 2b;
  - detection of the first autochthonous case(s) of an *Aedes*-borne viral disease. → Move to Risk Level 3a.

Public health actions and interventions

(Activities in italics have been specified at lower level(s) and are also relevant at this risk level.)

Disease surveillance

- *Surveillance for notifiable Aedes-borne viral diseases and reporting of imported and autochthonous cases at national and EU level (necessary).*
- *SoHO: generic haemovigilance and biovigilance systems (necessary).*

Laboratory diagnosis

- Ensure that laboratory capacity is sufficient (including stock of tests in the event of a surge in demand).

Key aspects to be considered are (i) use of high-throughput automated systems to increase the volume of testing; (ii) availability of resources (i.e. diagnostic equipment such as PCR machines, ELISA readers, sequencers and adequate stock of necessary reagents, kits, and other consumables); (iii) staff availability – i.e. sufficient number of trained laboratory personnel to perform tests and manage data and ongoing training programmes to keep staff updated on new testing protocols and emerging viruses; (iv) adequate infrastructure – i.e. sufficient laboratory space to handle increased sample volume during outbreaks and appropriate biosafety levels (BSL-2 to BSL-4); (v) operational capacity – i.e. ability to extend operating hours or work multiple shifts during outbreak situations and systems in place for rapid scale-up of testing capacity in response to outbreaks; (vi) logistical support – i.e. efficient management of supply chains to ensure continuous availability of necessary materials and robust data management systems to handle large volumes of test results and epidemiological data.

- *Provide professional guidelines for laboratory testing and clinical management of Aedes-borne viral diseases.*
- *Ensure there are laboratory capabilities within the country for diagnosis.*
- *Monitor the efficacy of laboratory diagnostic methods.*
- *Ensure that access to laboratory testing is available for patients (including financial aspects of testing).*

Awareness raising and capacity building

- Implement training courses (e.g. on testing, diagnosis, prevention and case management), information and management recommendations.
- Educate travellers coming from affected areas on how to reduce the risk of further transmission (e.g. protect against mosquito bites in accordance with national guidelines).
- Conduct public information campaigns on *Aedes*-borne diseases (e.g. on the need to consult a healthcare professional in the event of symptoms) and on the use of personal protective measures against mosquito bites and source reduction.
- *Promote awareness amongst healthcare professionals of Aedes-borne viral diseases so that this will be considered in the differential diagnosis of travellers returning from affected areas.*
- *Educate travellers to affected areas on how to reduce the risk (e.g. protect against infection during travel, consider pre-travel-vaccination in accordance with national guidelines).*
- *Educate travellers coming from affected areas on how to reduce the risk of further transmission (e.g. protect against mosquito bites in accordance with national guidelines).*

Multi-sectoral collaboration and coordination

- Define response and control measures for imported cases of *Aedes*-borne viral diseases (e.g. vector control at the residence of an imported case during the season).
- Consider drafting a preparedness and response plan for autochthonous transmission of *Aedes*-borne viral diseases.

The preparedness and response plan could be based on the risk levels presented in this document and should delineate roles and responsibilities for activities such as outbreak investigation, surveillance, vector control, risk communication and community engagement, and SoHO safety. Preparedness and response plans are ideally reviewed and revised based upon the lessons learned from past outbreaks.

- Establish a multi-sectoral coordination committee (national or regional) for exchange of information between affected sectors (e.g. public health, SoHO safety, integrated landscape management, medical entomology and vector control), as part of the preparedness plan.
- *Consult with competent authorities for SoHO to consider measures to prevent transmission to SoHO recipients through transfusion or transplantation from donors returning from affected areas.*
- *Consult with the NITAGs and/or other competent bodies to define vaccination recommendations for residents and/or travellers to affected areas.*

Vector management (entomological surveillance and control activities)

- Develop a surveillance/monitoring plan for *Aedes* mosquito presence and abundance in the area (necessary).
- Monitor the densities of invasive *Aedes* mosquitoes in the risk area.

In areas where invasive *Aedes* mosquito species have become established, surveillance of their abundance and further spread is needed for timely risk assessment of pathogen transmission to humans. Where the vector density is generally low, and where the climatic conditions are generally unfavourable for *Aedes*-borne virus transmission, it would be most economical to focus on mosquito abundance/density measurements during the month(s) where the seasonal density may be expected to be at or near its maximum (density typically peaks during the summer months, but it can be bi-modal in areas with hot and dry summers).

- Model disease transmission potential (basic reproduction number) by *Ae. albopictus* and/or *Ae. aegypti*.
- Identify and apply appropriate vector control interventions to mitigate geographical spread and density increase of vectors.

Implementing targeted strategies, such as treatment with registered biocides, environmental management and community education to remove stagnant water bodies, can effectively reduce the density of vectors. Active community involvement is crucial. Encourage citizens to report mosquitoes and participate in local vector control activities. Monitoring and adapting these interventions based on local conditions and vector behaviour are critical for controlling the spread and maintaining low vector densities, thereby protecting public health.

- Promote awareness among the general population and local authorities of how to control mosquito breeding sites.
- *Monitor the introduction of new invasive Aedes mosquitoes at points of entry.*
- *Stimulate active and passive monitoring of vectors (e.g. reporting by citizens).*
- *Model ecological niche suitability of the area for Ae. albopictus and Ae. aegypti.*
- *Develop and apply vector control at points of entry, possibly including disinfection protocols on vessels and aircrafts arriving from areas where invasive Aedes mosquitoes are present, to mitigate the risk of introducing new vectors.*
- *Identify and deploy appropriate vector control interventions after new vector species introductions have been detected to prevent their establishment.*

Links to further information on surveillance, preparedness and response (control) actions are provided in Annex 1.

Risk Level 2b - Predisposed area with high receptivity and vulnerability

Description and triggers for re-assessment

- Vectors of *Aedes*-borne viruses are established and widespread.
- Vector densities are generally high, according to criteria described in ECDC's 'Guidelines for the surveillance of invasive mosquitoes in Europe' [36].
- The environmental and climatic conditions are favourable for autochthonous transmission of *Aedes*-borne viral diseases.
- High number of travellers from Level 3 and Level 4 areas.
- Public health actions focus on surveillance of imported cases, possible autochthonous cases and mosquito vectors, and prevention and preparedness.
- Re-assessment triggers:
  - Environmental conditions becoming unfavourable for *Aedes*-borne virus transmission (based on meteorological data and potentially modelling). → Move to Risk Level 2a.
  - *Aedes* vector densities becoming low (based on mosquito monitoring data). → Move to Risk Level 2a.
  - Number of travellers from Level 3 and Level 4 areas becoming low. → Move to Risk Level 2a.
  - Detection of the first autochthonous case of *Aedes*-borne viral disease. → Move to Risk Level 3a.

Public health actions and interventions

(Activities in italics have been specified at lower level(s) and are also relevant at this risk level).

Disease surveillance

- *Surveillance for notifiable Aedes-borne viral diseases and reporting of imported and autochthonous cases at national and EU level (necessary).*
- *SoHO: generic haemovigilance and biovigilance systems (necessary).*

Laboratory diagnosis

- *Provide professional guidelines for laboratory testing and clinical management of Aedes-borne viral diseases.*
- *Ensure there are laboratory capabilities within the country for diagnosis.*
- *Ensure that laboratory capacity is sufficient (including stock of tests in the event of a surge in demand).*
- *Monitor the efficacy of laboratory diagnostic methods.*
- *Ensure that access to laboratory testing is available for patients (including financial aspects of testing).*

Awareness raising and capacity building

- Advise infected patients (imported cases) and asymptomatic travellers coming from affected countries/Level 3-4 areas to take measures to prevent onward transmission.
- Intensify public information campaigns on *Aedes*-borne diseases (e.g. the need to consult a healthcare professional in the event of symptoms) and to reinforce the use of personal protective measures against mosquito bites and source reduction/larval control of mosquitoes.
- Intensify awareness among healthcare professionals of *Aedes*-borne viral diseases for differential diagnosis of travellers coming from affected areas, and for locally acquired cases (especially in areas where there have been autochthonous transmissions in the past).
- *Implement training courses (e.g. testing, diagnosis, prevention and case management), information and management recommendations.*
- *Conduct public information campaigns on Aedes-borne diseases (e.g. on the need to consult a healthcare professional in the event of symptoms) and on the use of personal protective measures against mosquito bites and source reduction of mosquitoes.*
- *Promote awareness among healthcare professionals of Aedes-borne viral diseases so that this will be considered in the differential diagnosis of travellers coming or returning from affected areas.*
- *Educate travellers to affected areas on how to reduce risk (e.g. protect against infection during travel, consider pre-travel-vaccination in accordance with national guidelines).*
- Educate travellers coming from affected areas on how to reduce the risk of further transmission (e.g. protect against mosquito bites in accordance with national guidelines).

Multi-sectoral collaboration and coordination

- Develop a preparedness and response plan for autochthonous *Aedes*-borne virus transmissions, including surveillance activities (e.g. active case finding, population screening, enhanced mosquito monitoring) and an integrated vector control plan.
- Allocate resources necessary to enable emergency response (i.e. vector control, communication plan).
- Ensure timely and regular exchange of information between affected sectors through the multi-sectoral coordination committee**,** as part of the preparedness plan.
- Community mobilisation, involvement of a broad range of stakeholders.
- *Consult with the NITAGs and/or other competent body to define vaccination recommendations for residents and/or travellers to affected areas.*

- *Define response and control measures for imported cases of Aedes-borne viral diseases (e.g. active case finding and vector control at the residence of an imported case during the season).*
- *Consult with competent authorities for SoHO to consider measures to prevent transmission to SoHO recipients through transfusion or transplantation from donors returning from affected areas.*

Vector management (entomological surveillance and control activities)

- Monitor the densities and seasonal dynamics of invasive *Aedes* mosquitoes in the risk area (necessary).

If invasive *Aedes* mosquito species are established over wide areas (risk Level 2b and above) they should be surveyed to assess the abundance/density of the population and its seasonal dynamics (throughout the year). These data, together with other mosquito population parameters (e.g. host preferences), will help to evaluate the vectorial capacity of the mosquito population in the local context.

- Based on the preparedness and control plan: consider preparing *Aedes* vector control activities if entomological indicators suggest there is a need.

Key indicators to monitor include *Aedes* adult female mosquito density, egg abundance (measured in ovitraps), larval and pupal indices and the risk of arbovirus transmission in the area. Evaluation of these indicators is crucial when implementing targeted control measures to prevent the spread of *Aedes*-borne viral diseases.

- Based on the preparedness and control plan: implement larval vector control activities when and where appropriate (e.g. drainage catch basins).

Implementing larval vector control activities, such as targeting drainage catch basins, at appropriate periods and locations is crucial for reducing vector populations. This proactive approach helps to disrupt the mosquito life cycle effectively, reducing the emergence of adult mosquitoes and minimising the risk of *Aedes*-borne disease transmission to the targeted area. It is noted that larval control – i.e. environmental management, source reduction, larviciding, or biological control (community-based and/or top- down approaches) can be conducted when cases are detected, but is more effective when it is consistent and routine rather than as a periodic emergency response.

- Based on the preparedness and control plan: consider implementing ground adult vector control activities around imported confirmed or probable cases, and probable local cases where epidemiological evidence suggests there is a strong likelihood of *Aedes*-borne disease, during the active vector season.

Rapid action in the proximity of reported cases helps to prevent further transmission and protects the community from outbreaks of *Aedes*-borne diseases. Adulticidal products mainly fall within the category of pyrethroids, which are not recommended for use on a large scale. In the case of virus transmission, 'peri- domestic' or 'perifocal' space spray treatments with insecticides can be carried out in and around households where human infection is suspected or has been reported. Different treatment methods (house-to-house application using portable equipment, vehicle-mounted fogging, and cold or thermal fogging) are available, but they must be tailored to the risk scenario, the area to be covered, accessibility and the *Aedes* species.

- Monitor the efficacy of control activities against the target mosquito populations, including insecticide resistance, if appropriate.

Regular monitoring of the effectiveness of control activities against target mosquito vectors, including the assessment of insecticide resistance where applicable, is critical. This ongoing evaluation ensures that interventions remain efficient. In the case of long-term control programmes, rotation of available insecticides should be adopted, as resistance has already been detected in some European populations following insecticide-based mosquito management programmes. Adulticide products mainly fall within the category of pyrethroids which, in the case of *Ae. albopictus* controls, are only recommended in specific cases (e.g. imported cases) and not as a method for use on a large scale.

- *Monitor the introduction of new invasive Aedes mosquitoes at points of entry.*
- *Model disease transmission potential (basic reproduction number) by Ae. albopictus and Ae. aegypti.*
- *Identify and apply appropriate vector control interventions to mitigate geographic spread and density increase of vectors.*
- *Promote awareness among the general population and local authorities of the need to control mosquito breeding sites.*
- *Develop and apply vector control at points of entry, possibly including disinfection protocols on vessels and aircrafts arriving from areas where invasive Aedes mosquitoes are present, to mitigate the risk of introducing new vectors.*
- *Identify and deploy appropriate vector control interventions after detecting the introduction of new vector species to prevent their establishment*.

Links to further information on surveillance, preparedness and response (control) actions are provided in Annex 1.

Risk level 3 - Affected area

Risk level 3a – Affected area with low number of cases

Description and triggers for re-assessment

- At least one autochthonous case detected (i.e. confirmed case according to EU case definition [37], or, if not applicable, according to national case definition).
- The number of autochthonous cases/clusters is low and transmission chains are traceable.
- Public health actions and interventions focus on surveillance, prevention, response and control.
- Re-assessment triggers:
  - 45 days passed since the date of onset of the last autochthonous case. (the 45-day period is calculated as the sum of the days after symptom onset until viraemia levels are sufficient to infect a mosquito (four days), the longevity of an *Aedes* mosquito in natural circumstances (23 days) and the maximum intrinsic incubation period (14 days), with a four-day safety margin for clinical diagnosis of new cases.) → Move to Risk Level 2a or 2b.
  - The number of autochthonous cases and clusters becoming high, which is overwhelming tracing capacity. → Move to Risk Level 3b.

Public health actions and interventions

(Activities in italics have been specified at lower level(s) and are also relevant at this risk level.)

Disease surveillance activities

- Active case finding in the area with confirmed autochthonous transmission (necessary). Ensure timely detection and reporting of imported or autochthonous cases at local, regional and national level (necessary).
- Ensure timely reporting of autochthonous cases at EU level (necessary).
- Apply molecular surveillance/genotyping/serotyping of viruses (necessary).
- Reporting of surveillance data of asymptomatic infections through active case finding and blood donation screening (if applicable and if testing of donations for the relevant virus is implemented in the area as a SoHO safety measure) (necessary).
- Track transmission chains to define the affected areas and transmission dynamics.
- *Surveillance for notifiable Aedes-borne viral diseases and reporting of imported and autochthonous cases at national and EU level (necessary).*
- *SoHO: generic haemovigilance and biovigilance systems (necessary).*

Laboratory diagnosis

- *Provide professional guidelines for laboratory testing and clinical management of Aedes-borne viral diseases.*
- *Ensure there are laboratory capabilities within the country for diagnosis.*
- *Ensure that laboratory capacity is sufficient (including stock of tests in the event of a surge in demand).*
- *Monitor the efficacy of laboratory diagnostic methods.*
- *Ensure that access to laboratory testing is available for patients (including financial aspects of testing).*

Awareness raising and capacity building

- Increase awareness among health professionals for the diagnosis of locally-acquired cases too (diagnostic strategy; case management; reporting, prevention recommendations).
- *Advise infected patients and asymptomatic travellers coming from affected countries/Level 3-4 areas to take measures to prevent onward transmission.*
- *Intensify public information campaigns on Aedes-borne diseases (e.g. the need to consult a healthcare professional in case of symptoms) and to strengthen the use of personal protective measures against mosquito bites and source reduction/larval control of mosquitoes.*
- *Implement training courses (e.g. on testing, diagnosis, prevention and case management), information and management recommendations.*
- *Conduct public information campaigns on Aedes-borne diseases (e.g. the need to consult a healthcare professional in case of symptoms) and on the use of personal protective measures against mosquito bites and source reduction.*
- *Educate travellers to affected areas on how to reduce the risk (e.g. protect against infection during travel, consider pre-travel-vaccination in accordance with national guidelines).*
- *Educate travellers coming from affected areas on how to reduce the risk of further transmission (e.g. protect against mosquito bites in accordance with national guidelines).*

Multi-sectoral collaboration and coordination

- Apply response and control measures in the event of confirmed autochthonous and imported cases of *Aedes*-borne viral diseases.
- Based on consultation with the multi-sectoral coordination committee, and according to the preparedness and response plan, establish a multi-sectoral outbreak emergency response team and organise regular meetings.
- Determine the geographical boundaries for implementation of various response measures in and around the affected area.
- Safety of SoHO: consider implementing measures for blood, tissues, cells and organ safety in affected area, based on risk assessment.

The measures could be based on a risk assessment for donor-derived transmission through SoHO. The measures implemented could consider the supply of SoHO in the affected area. Measures may include temporary suspension of SoHO collection or screening of donors with nucleic acid amplification testing (NAAT) in the affected area, implementing deferral policies or NAAT screening of donors who travelled or stayed in the area, pathogen inactivation methods for certain SoHO components/grafts, and evaluating the impact of these measures on SoHO supplies. The specific measures may vary depending on the size of the affected area and the available supply; for example, suspension of collection in a large area may significantly affect supply. In such cases, testing of donors may be a more appropriate alternative.

- Review and - if necessary - modify/refine the preparedness and response plan.
- Perform in-action and after-action reviews, identify the reasons for ineffective response actions, if applicable.

In-action reviews can be conducted quickly during ongoing outbreaks to ensure that the response is running optimally. Following the conclusion of outbreaks, after-action reviews offer the opportunity for structured learning from the event, so that lessons may be learned and incorporated into future response activities.

- *Consult with competent authorities for SoHO to consider measures to prevent transmission to SoHO recipients through transfusion or transplantation from donors returning from affected areas.*
- *Consult with the NITAGs and/or other competent body to define vaccination recommendations for residents and/or travellers to affected areas.*
- *Develop a preparedness and response plan for autochthonous Aedes-borne virus transmissions, including surveillance activities and an integrated vector control plan.*
- *Define response and control measures for imported cases of Aedes-borne viral diseases (e.g. active case finding and/or vector control at the residence of an imported case during the season).*
- *Allocate the resources necessary to enable emergency response (i.e. vector control, communication plan).*
- *Ensure timely and regular exchange of information between affected sectors through the multi-sectoral coordination committee, as part of the preparedness plan.*
- *Community mobilisation, involvement of a broad range of stakeholders.*

Vector management (entomological surveillance and control activities)

- Implement ground adult vector control activities around cases of *Aedes*-borne disease.
- *Monitor the densities and seasonal dynamics of invasive Aedes mosquitoes in the risk area (necessary).*
- *Monitor the introduction of new invasive Aedes mosquitoes at points of entry.*
- *Model disease transmission potential (basic reproduction number) by Ae. albopictus and Ae. aegypti.*
- *Based on the preparedness and control plan: consider preparing Aedes vector control activities if entomological indicators suggest there is a need to do so.*
- *Based on the preparedness and control plan: implement larval vector control activities when and where appropriate (e.g. drainage catch basins).*
- *Based on the preparedness and control plan: consider implementing ground adult vector control activities around imported confirmed or probable cases, and probable local cases where epidemiological evidence suggests high likelihood of Aedes-borne disease, during the active vector season.*
- *Monitor the efficacy of control activities against the target mosquito populations, including insecticide resistance, if appropriate.*
- *Promote awareness among the general population and local authorities about controlling mosquitoes breeding sites.*
- *Develop and apply vector control at points of entry, possibly including disinfection protocols on vessels and aircrafts arriving from areas where invasive Aedes mosquitoes are present, to mitigate the risk of introducing new vectors.*
- Identify and deploy appropriate vector control interventions after detecting new vector species introductions to prevent their establishment.

Links to further information on surveillance, preparedness and response (control) actions are provided in Annex 1.

Risk level 3b – Affected area with high number of cases

Description and triggers for re-assessment

- The number of autochthonous cases/clusters is high, which is overwhelming tracing capacity and targeted vector control interventions.
- Public health actions and interventions are focusing on response and control.
- Re-assessment triggers:
  - The number of autochthonous cases and clusters are decreasing. → Move to Risk Level 3a.
  - Sufficient capacities for the tracing of transmission chains. → Move to Risk Level 3a.
  - Autochthonous transmissions have occurred throughout the year, without importation of new viruses. → Move to Risk Level 4.

Public health actions and interventions

(Activities in italics have been specified at lower level(s) and are also relevant at this risk level).

Disease surveillance

- Active case finding in the area with autochthonous transmission if applicable.
- *Ensure timely detection and reporting of imported or autochthonous cases at local, regional and national level.*
- *Ensure timely reporting of autochthonous cases at EU level (necessary).*
- *Apply molecular surveillance/genotyping/serotyping of viruses (necessary).*
- *Reporting of surveillance data for asymptomatic infections through active case finding and blood donation screening (if applicable and if donations are tested for the relevant virus in the area as a SoHO safety measure) (necessary).*
- *Surveillance for notifiable Aedes-borne viral diseases and reporting of imported and autochthonous cases at national and EU level (necessary).*
- *SoHO: generic haemovigilance and biovigilance systems (necessary).*

Laboratory diagnosis

- *Provide professional guidelines for laboratory testing and clinical management of Aedes-borne viral diseases.*
- *Ensure there are laboratory capabilities within the country for diagnosis.*
- *Ensure that laboratory capacity is sufficient (including stock of tests in the event of a surge in demand).*
- *Monitor the efficacy of laboratory diagnostic methods.*
- *Ensure that access to laboratory testing is available for patients (including financial aspects of testing).*

Awareness raising and capacity building

- *Increase awareness among health professionals for the diagnosis of locally-acquired cases too (diagnostic strategy; case management; reporting, prevention recommendations).*
- *Advise infected patients and asymptomatic travellers coming from affected countries/Level 3-4 areas to take measures to prevent onward transmission.*
- *Intensify public information campaigns on Aedes-borne diseases (e.g. the need to consult a healthcare professional in case of symptoms) and to strengthen the use of personal protective measures against mosquito bites and source reduction/larval control of mosquitoes.*
- *Conduct public information campaigns on Aedes-borne diseases (e.g. the need to consult a healthcare professional in case of symptoms) and on the use of personal protective measures against mosquito bites and source reduction.*

Multi-sectoral collaboration and coordination

- Review the response and control strategies and, depending on the resources available and cost-effectiveness, consider modifying the preparedness and response plan (e.g. prioritise places to treat; treat whole neighbourhoods).
- Implement the modified preparedness and response plan.
- Apply modifications in the response and control measures for autochthonous and imported cases of *Aedes*-borne viral diseases.
- *Safety of SoHO: consider implementing measures for blood, tissues, cells and organ safety in affected area, based on risk assessment.*
- *Consult with competent authorities for SoHO to consider measures to prevent transmission to SoHO recipients through transfusion or transplantation from donors returning from affected areas.*
- *Consult with the NITAGs and/or other competent body to define vaccination recommendations for residents and/or travellers to affected areas.*
- *Based on consultation with the multi-sectoral coordination committee, and according to the preparedness and response plan, establish a multi-sectoral outbreak emergency response team and organise regular meetings.*

- *Determine the geographical boundaries for implementation of various response measures in and around the affected area.*
- *Perform in-action and after-action reviews, identify the reasons for ineffective response actions, if applicable.*
- *Allocate the resources necessary to enable emergency response (i.e. vector control, communication plan).*
- *Ensure timely and regular exchange of information between affected sectors through the multi-sectoral coordination committee, as part of the preparedness plan.*

Vector management (entomological surveillance and control activities)

- Intensify ground adult mosquito control (space spraying or residual spraying) with multiple applications in areas of active transmission.

Intensifying ground adult mosquito control through methods such as space or residual spraying with multiple applications is essential in high-risk areas with an increased number of locally acquired cases. This approach not only quickly reduces the adult mosquito populations but also serves to prevent further transmission of *Aedes*-borne diseases in the targeted area.

- *Monitor the introduction of new invasive Aedes mosquitoes at points of entry.*
- *Model disease transmission potential (basic reproduction number) by Ae. albopictus and Ae. aegypti.*
- *Based on the preparedness and control plan: implement larval vector control activities when and where appropriate (e.g. drainage catch basins).*
- *Monitor the efficacy of control activities against target mosquito populations, including insecticide resistance, if appropriate.*
- *Promote awareness among the general population and local authorities on controlling mosquito breeding sites.*
- *Develop and apply vector control at points of entry, possibly including disinfection protocols on vessels and aircrafts arriving from areas where invasive Aedes mosquitoes are present, to mitigate the risk of introducing new vectors.*
- Identify and deploy appropriate vector control interventions after detecting new vector species introductions to prevent their establishment.

Links to further information on surveillance, preparedness and response (control) actions are provided in Annex 1.

Risk level 4 - Endemo-epidemic area

Description and triggers for re-assessment

- Autochthonous transmission of *Aedes*-borne viral diseases in subsequent years, independent from the importation of new viruses.
- *Aedes*-borne viruses are frequently introduced and result in further, autochthonous transmissions and outbreaks.
- Public health actions and interventions are focusing on surveillance, response and control.

Public health actions and interventions

(Activities in italics have been specified at lower level(s) and are also relevant at this risk level.)

Disease surveillance activities

- Apply targeted surveillance (e.g. testing patients with fever) at the beginning of the seasonal enhanced circulation for the timely detection of outbreaks.
- *Ensure timely detection and reporting of imported or autochthonous cases at local, regional and national level.*
- *Ensure timely reporting of autochthonous cases at EU level (necessary).*
- *Apply molecular surveillance/genotyping/serotyping of viruses (necessary).*
- *Reporting of surveillance data on asymptomatic infections through active case finding and blood donation screening (if applicable and if donations are tested for the relevant virus in the area as a SoHO safety measure) (necessary).*
- *Surveillance for notifiable Aedes-borne viral diseases and reporting of imported and autochthonous cases at national and EU level (necessary).*
- *SoHO: generic haemovigilance and biovigilance systems (necessary).*

Laboratory diagnosis

- *Provide professional guidelines for laboratory testing and clinical management of Aedes-borne viral diseases.*
- *Ensure there are laboratory capabilities within the country for diagnosis.*
- *Ensure that laboratory capacity is sufficient (including stock of tests in the event of a surge in demand).*
- *Monitor the efficacy of laboratory diagnostic methods.*
- *Ensure that access to laboratory testing is available for patients (including financial aspects of testing).*

Awareness raising and capacity building

- Organise regular public information campaigns and improve education on *Aedes*-borne diseases to strengthen use of personal protective measures against mosquito bites and source reduction/continued larval control of mosquitoes.
- Increase awareness among health professionals at the beginning of the high intensity transmission season every year.
- Conduct/intensify public information campaigns on *Aedes*-borne diseases (e.g. the need to consult a healthcare professional in case of symptoms) and on the use of personal protective measures against mosquito bites and source reduction at the beginning of the high intensity transmission season every year.
- *Increase awareness among health professionals for the diagnosis of locally-acquired cases too (diagnostic strategy; case management; reporting, prevention recommendations).*
- *Advise infected patients and asymptomatic travellers coming from affected countries/Level 3-4 areas to take measures to prevent onward transmission.*
- *Intensify public information campaigns on Aedes-borne diseases (e.g. the need to consult a healthcare professional in case of symptoms) and to strengthen the use of personal protective measures against mosquito bites and source reduction/larval control of mosquitoes.*

Multi-sectoral collaboration and coordination

- *Consult with the NITAGs and/or other competent body to define vaccination recommendations for residents and/or travellers to affected areas.*
- *Safety of SoHO: consider implementing measures for blood, tissues, cells and organ safety in affected area, based on risk assessment.*
- *Review the response and control strategies and, depending on the available resources and cost-effectiveness, consider modifying the preparedness and response plan (e.g. prioritise places to treat; treat whole neighbourhoods).*
- *Implement the modified preparedness and response plan.*
- *Based on consultation with the multi-sectoral coordination committee, and according to the preparedness and response plan, establish a multi-sectoral outbreak emergency response team and organise regular meetings.*
- *Determine the geographical boundaries for implementation of response measures in and around the affected area.*
- *Perform in-action and after-action reviews, identify the reasons for ineffective response actions, if applicable.*
- *Allocate resources necessary to enable emergency response (i.e. vector control, communication plan).*

- *Ensure timely and regular exchange of information between affected sectors through the multi-sectoral coordination committee, as part of the preparedness plan.*
- *Apply response and control measures in the event of autochthonous and imported cases of Aedes-borne viral diseases*.

Vector management (entomological surveillance and control activities)

- Consider testing the presence of viruses in mosquitoes to determine minimum infection rates.

The infection rates of mosquitoes are often very low, and therefore it makes sense to test pools of mosquitoes, rather than individual mosquitoes. When a pool tests positive, it is not possible to quantify if one or more mosquitoes in that pool was positive. Therefore, the minimum infection rate is calculated by the number of positive pools divided by the total number of mosquitoes tested (x 1000). Cost-effectiveness of the entomo-virological surveillance may be considered before implementation.

- *Monitor the densities and seasonal dynamics of invasive Aedes mosquitoes in the risk area.*
- *Monitor the introduction of new invasive Aedes mosquitoes at points of entry.*
- *Model disease transmission potential (basic reproduction number) by Ae. albopictus and Ae. aegypti.*
- *Implement ground adult vector control activities around cases of Aedes-borne disease.*
- *Based on the preparedness and control plan: implement larval vector control activities when and where appropriate (e.g. drainage catch basins).*
- *Monitor the efficacy of control activities against the target mosquito populations, including insecticide resistance, if appropriate.*
- *Promote awareness among the general population and local authorities of the need to control mosquito breeding sites.*
- *Develop and apply vector control at points of entry, possibly including disinfection protocols on vessels and aircrafts arriving from areas where invasive Aedes mosquitoes are present, to mitigate the risk of introducing new vectors.*
- *Identify and deploy appropriate vector control interventions after detecting new vector species introductions to prevent their establishment.*

Links to further information on surveillance, preparedness and response (control) actions are provided in Annex 1.

Knowledge gaps and limitations

Pathogen-host interactions

In general, there is a lack of knowledge about the susceptibility of EU/EEA population to severe *Aedes*-borne virus infections: The immunological landscape of the EU/EEA population in relation to *Aedes*-borne viruses remains poorly characterised. Factors such as pre-existing flavivirus immunity, which may contribute to ADE or cross-protection, need thorough investigation. In particular, the impact of simultaneous or sequential infections (or vaccination) with multiple orthoflaviviruses, or with other pathogens endemic to Europe, on disease severity and immune responses is not well understood. In addition, the impact of demographic shifts, particularly the ageing population and increasing prevalence of chronic diseases, on infection outcomes is not well understood. Genetic factors, such as polymorphisms in Fc receptors or cytokine genes, which may influence disease severity, require comprehensive study in the European context. There is also limited knowledge of the long-term sequelae of *Aedes*-borne viral infections in European populations. The potential for chronic health effects, particularly neurological and autoimmune complications, following acute infections also needs further investigation in the context of European healthcare systems and population demographics.

Diagnosis

There is a need for improved diagnostic capabilities, including the development of multiplex assays for simultaneous detection of multiple *Aedes*-borne viruses and the validation of point-of-care diagnostics suitable for field use in Europe.

Surveillance and response

The disease surveillance systems in the EU/EEA are predominantly based on the detection of symptomatic cases. However, asymptomatic infections with certain *Aedes*-borne viruses (e.g. DENV, ZIKV) may reach 80% and asymptomatic viraemic individuals can also transmit the virus to mosquitoes. Active case finding during an outbreak or serosurveillance studies after an outbreak may give a more precise indication of the number of people infected. The entomological surveillance in many countries is limited or fragmented both spatially and temporally, which reduces the usefulness of the mosquito surveillance data for trend analysis, timely risk assessment and alerting.

Detailed analysis of the locally acquired dengue and chikungunya virus disease outbreaks in the EU could identify the effectiveness and efficiency of the applied public health measures for outbreak mitigation, containment and elimination.

Prevention and treatment

The development of national vaccine recommendations is a competency of the NITAGs, albeit with some particularities with regard to the vaccine recommendations for travellers. Few countries have issued recommendations concerning dengue and chikungunya vaccines for the protection of travellers and/or outbreak management purposes. The effectiveness of Qdengua® vaccine against DENV-3 and DENV-4 serotypes in seronegative individuals still needs to be documented. Vaccines against chikungunya virus have received marketing authorisations based on immunogenicity study results and there is currently a lack of data on the efficacy/effectiveness of the vaccines. Deployment of the above vaccines as part of outbreak management should be closely monitored in terms of safety, effectiveness and vaccination coverage.

The development of broad-spectrum antivirals targeting conserved viral proteins (e.g. NS3 or NS5) of *Aedes*-borne viruses is a research priority. The potential for repurposing existing drugs, such as nucleoside analogues or host-targeting antivirals, requires exploration. In addition, combination therapies to mitigate the risk of antiviral resistance should be investigated.

Vector ecology and vector management

While *Ae. albopictus* is established in several parts of the EU and laboratory experiments indicate vectorial capacity for several arboviruses beyond DENV, CHIKV and ZIKV (e.g. YFV, Rift Valley fever virus), its ability to transmit these viruses under natural conditions in the EU remains unknown.

The potential establishment of *Ae. aegypti* in mainland EU/EEA could significantly alter arbovirus transmission dynamics. Predictive modelling incorporating climate change scenarios, urbanisation patterns and human mobility is needed to assess the risk and potential impact. The competitive interactions between *Ae. aegypti* and established *Ae. albopictus* populations and their implications for virus transmission need investigation.

In addition, little is known about the vectorial capacity of local *Aedes* species native to Europe for various arboviruses. The potential for virus adaptation to new vector species and the impact on transmission dynamics require further investigation. Detailed entomological studies, including vector competence assays and field-based transmission studies, are needed.

There is a sub-optimal understanding of the socio-economic drivers of *Aedes*-borne virus transmission in Europe. The role of factors such as urbanisation patterns, international trade and travel in facilitating vector spread and virus introduction needs more comprehensive analysis to inform policy decisions. The specific adaptations of *Aedes* mosquitoes to European urban environments, including their breeding preferences and dispersal patterns, are also not fully characterised. This knowledge is crucial for developing targeted control strategies and predicting transmission hotspots. The impact of climate change on vector competence and extrinsic incubation periods of *Aedes*-borne viruses in European *Aedes* populations should also be investigated, as well as the potential role of vertical transmission in over-wintering and maintenance of *Aedes*-borne viruses in temperate European climates.

Effectiveness of control interventions for *Aedes*-borne viral infections in the European context also need further assessment. The most effective methods for promoting sustained behaviour change and community participation in vector control efforts in diverse European cultural contexts are not well defined and neither is the efficacy of vector control methods, such as the use of insecticide, the more innovative sterile insect technique, or Wolbachia-based approaches. The cost-effectiveness and sustainability of these interventions in different ecological settings within the EU/EEA need rigorous evaluation. The influence of the mosquito microbiome on vector competence for *Aedes*-borne viruses in European *Aedes* populations, and the potential for microbiome manipulation as a control strategy, could also be investigated. There are also gaps in knowledge on the impact of insecticide resistance on vector control efficacy, and in particular the prevalence and mechanisms of insecticide resistance in European *Aedes* populations, and their implications for current and future vector control strategies.

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Annex 1. Links to further information on surveillance, preparedness and response (control) actions against *Aedes*-borne viral diseases and their vectors

Disease surveillance

ECDC Annual Epidemiological Reports: https://www.ecdc.europa.eu/en/publications-data/monitoring/all-annual-epidemiological-reports

ECDC Surveillance Atlas of Infectious Diseases: https://www.ecdc.europa.eu/en/surveillance-atlas-infectious-diseases

Surveillance systems overviews: https://www.ecdc.europa.eu/en/surveillance-and-disease-data/annual-epidemiological-reports/introduction-annual

Information on *Aedes*-borne viral diseases

Chikungunya virus disease – ECDC webpage: https://www.ecdc.europa.eu/en/chikungunya-virus-disease

Dengue – ECDC webpage: https://www.ecdc.europa.eu/en/dengue

Factsheet on Zika virus disease. – ECDC Factsheet: https://www.ecdc.europa.eu/en/zika-virus-infection/facts/factsheet

Factsheet for health professionals on chikungunya – ECDC Factsheet: https://www.ecdc.europa.eu/en/chikungunya/facts/factsheet

Factsheet for health professionals on dengue – ECDC Factsheet: https://www.ecdc.europa.eu/en/dengue-fever/facts

Zika virus disease – ECDC webpage: https://www.ecdc.europa.eu/en/zika-virus-disease

Laboratory diagnosis

Laboratory testing for dengue virus – WHO interim guidance: https://iris.who.int/bitstream/handle/10665/381187/B09394-eng.pdf?sequence=1

Preparedness and response

Global strategic preparedness, readiness and response plan for dengue and other *Aedes*-borne arboviruses, 2024–2025 – WHO plan: https://www.who.int/publications/m/item/global-strategic-preparedness--readiness-and-response-plan-for-dengue-and-other-aedes-borne-arboviruses

Vector management (entomological surveillance and control activities)

AIM-COST guideline list – A collection of guideline documents used for monitoring/surveillance and control of *Aedes* invasive mosquitoes in Europe and neighbouring countries. https://www.aedescost.eu/GuidelineList

A spatial modelling method for vector surveillance – ECDC technical report: https://www.ecdc.europa.eu/en/publications-data/spatial-modelling-method-vector-surveillance

Aedes aegypti – ECDC Factsheet for experts: https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-aegypti

Aedes albopictus – ECDC Factsheet for experts: https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-albopictus

Aedes japonicus – ECDC Factsheet for experts: https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-japonicus

Aedes koreicus – ECDC Factsheet for experts: https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-koreicus

*Aedes*Risk – A tool to support the decision-making process for surveillance and vector control of dengue, chikungunya and Zika virus disease in Europe**:** https://shinyapps.ecdc.europa.eu/shiny/AedesRisk/

ARBOCARTO – A software package for predictive mapping of population densities of *Aedes* mosquitoes: https://www.arbocarto.fr/en

Determining discriminating concentrations of insecticides for monitoring resistance in mosquitoes – Report of a multi-centre laboratory study and WHO expert consultations: https://www.who.int/publications/i/item/9789240045200

ECDC Guidelines for the surveillance of invasive mosquitoes in Europe: https://www.ecdc.europa.eu/en/publications-data/guidelines-surveillance-invasive-mosquitoes-europe

Guidelines for laboratory and field testing of mosquito larvicides – WHO guidelines: https://apps.who.int/iris/handle/10665/69101

Integrated *Aedes* management for the control of *Aedes*-borne diseases – Journal article: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0006845

Invasive mosquito surveillance effort over 2015–2019 – ECDC map: https://www.ecdc.europa.eu/en/publications-data/invasive-mosquito-surveillance-effort-2015-2019

Manual on prevention of establishment and control of mosquitoes of public health importance in the WHO European Region (with special reference to invasive mosquitoes) – WHO manual: https://iris.who.int/handle/10665/343056

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