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A MOSQUITO`S GUIDE TO VIRAL EMERGENCE

GLOBAL RESEARCH COLLABORATION TO STRENGTHEN PREPAREDNESS IN SWEDEN AND ABROAD

EBA

A Mosquito`s Guide to Viral Emergence
Global research collaboration to strengthen preparedness in Sweden and abroad

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to
The Expert Group for Aid Studies (EBA)

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Sammanfattning

Myggburna virus utgör ett växande hot mot global hälsa och utveckling, särskilt i låg- och medelinkomstländer där resurser för övervakning och smittskydd ofta är begränsade. Samtidigt har dessa virus fått ökad betydelse även i Europa, där klimatförändringar, resande och global handel skapat nya möjligheter för vektorburna sjukdomar att etablera sig.

I min avhandling ”A Mosquito's Guide to Viral Emergence” undersökte jag tre viktiga myggburna virus: japanskt encefalitvirus (JEV), Usutuvirus (USUV) och chikungunyavirus (CHIKV). Dessa virus illustrerar olika steg i den globala sjukdomscykeln – från endemisk spridning i Asien till potentiell etablering i Europa.

Fältstudier i Hanoi, Vietnam, utfördes i nära samarbete mellan svenska och vietnamesiska forskare. Genom mer än ett års månatliga mygginsamlingar analyserades artfördelning, säsongsvariation och miljöfaktorer som påverkar förekomsten av Culex-myggor – vektorer som sprider JEV. Resultaten visade att Culex quinquefasciatus dominerade i urbana- och förortsområden och att klimatförhållanden som temperatur och luftfuktighet påverkade populationstätheten. Inget JEV påvisades, men resultaten gav värdefull kunskap för övervakning och riskbedömning.

I Sverige genomfördes laboratoriestudier som visade att svenska Culex pipiens-myggor kan infekteras och effektivt sprida USUV, men inte JEV. Detta indikerar att nordliga arter kan stödja spridning av vissa virus under rätt förhållanden, men också att specifika biologiska barriärer kan begränsa andra. Tillsammans med studier av CHIKV:s evolution i Asien visar resultaten hur klimat, global rörlighet och biologisk anpassning samverkar för att forma framtida sjukdomshot.

För svensk utvecklingspolitik och internationellt samarbete är dessa resultat viktiga. De understryker behovet av:

- Stärkt forskningssamarbete mellan svenska institutioner och universitet i endemiska regioner.
- Långsiktig kapacitetsuppbyggnad inom diagnostik och övervakning i låg- och medelinkomstländer.
- Integrering av vektorburna sjukdomar i klimatanpassnings- och hälsoprogram inom svenskt bistånd.
- Stöd till tvärvetenskapliga “One Health”-initiativ som sammanför människo-, djur- och miljöhälsa.

Avhandlingen visar att global hälsa är ömsesidigt beroende – kunskap från endemiska länder stärker svensk beredskap, och svensk forskning kan samtidigt bidra till lokal motståndskraft i utvecklingsländer.

Abstract

Mosquito-borne viruses represent a growing global health concern, particularly in regions where health infrastructure and surveillance capacity are limited. Yet, the risk is increasingly global: climate change, trade, and travel are enabling these viruses to expand into new ecological and geographical zones.

In my dissertation A Mosquito's Guide to Viral Emergence, I investigated three important mosquito-borne viruses: Japanese encephalitis virus (JEV), Usutu virus (USUV), and chikungunya virus (CHIKV). These viruses illustrate different stages in the global disease cycle – from endemic circulation in Asia to potential establishment in Europe.

Field studies in Hanoi, Vietnam, were conducted in close collaboration between Swedish and Vietnamese researchers. Through more than a year of monthly mosquito collections, species distribution, seasonal variation, and environmental factors affecting the presence of Culex mosquitoes – vectors that transmit JEV – were analyzed. The results showed that Culex quinquefasciatus dominated in urban and suburban areas and that climatic conditions such as temperature and humidity affected population density. No JEV was detected, but the results provided valuable knowledge for surveillance and risk assessment.

In Sweden, laboratory studies showed that Swedish Culex pipiens mosquitoes can be infected with and efficiently transmit USUV, but not JEV. This indicates that northern species can support the spread of certain viruses under the right conditions, but also that specific biological barriers can limit others. Together with studies of CHIKV evolution in Asia, the results show how climate, global mobility, and biological adaptation interact to shape future disease threats.

For Swedish development policy and international cooperation, these results are important. They underscore the need for:

- Strengthened research collaboration between Swedish institutions and institutions in endemic regions.
- Long-term capacity building in diagnostics and surveillance in low- and middle-income countries.
- Integration of vector-borne diseases into climate adaptation and health programs within Swedish development aid.
- Support for interdisciplinary "One Health" initiatives that bring together human, animal, and environmental health.

The dissertation shows that global health is mutually interdependent – and that knowledge from endemic countries strengthens Swedish preparedness, and Swedish research.

Abbreviations and terms

Explanation	Abbreviation
Aedes (mosquito genus abbreviation)	*Ae.*
Artropod borne viruses	Arboviruses
Centers for Disease Control and Prevention	CDC
Chikungunya fever	CHIKV
Culex (mosquito genus abbreviation)	*Cx.*
Dengue virus	DENV
East/Central/South African (CHIKV genotype)	ECSA
US Food and Drug Administration	FDA
Indian Ocean lineage	IOL
Japanese encephalitis virus	JEV
Ribonucleic acid	RNA
Reverse transcription quantitative polymerase chain reaction	RT-qPCR
Usutu virus	USUV
World Health Organization	WHO

Why is it important to study mosquitoes and viruses?

In the complex world of disease transmission, mosquitoes often take center stage as unwitting actors in the spread of viruses that threaten human and animal health (World Health Organization, 2022, WHO, 2020, Tsai, 1997, Solomon, 1997, Harden et al., 1967). Imagine this: a tiny mosquito, often overlooked in the grand scheme of nature, carrying within it the potential to cause widespread disease, earning it the title of the world's deadliest animal (Li et al., 2025). Mosquito-borne viruses, also called Arboviruses, are a central concern for both health and development. Diseases such as Japanese encephalitis[1], dengue[2], Zika[3], and chikungunya fever[4] cause hundreds of thousands of infections each year and remain entrenched in the world’s poorest regions. The spread of these viruses is increasing, especially in rapidly growing urban areas, through increased contact between wild and domestic life, and due to the observed climate change. Historically mosquitoes and mosquito borne diseases haven been linked to the tropics, but the presence of disease-spreading mosquitoes in Europe is a growing concern due to the establishment and spread of invasive mosquito species, the emergent spread of native European species, and the growing evidence that a number of local mosquitoes are able to transmit emerging viruses (Lawson et al., 2022, Folly et al., 2022, Brugman et al., 2018, Lumley et al., 2018, Hesson et al., 2014, Engler et al., 2013, Medlock et al., 2012). This emphasises the urgent need for an expanded understanding of viral transmission dynamics. And the impact extends beyond illness: outbreaks reduce labor productivity, disrupt education, and impose long-term

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[1] Sjukdomsinformation om japansk encefalit — Folkhälsomyndigheten

[2] Sjukdomsinformation om denguefeber — Folkhälsomyndigheten

[3] Sjukdomsinformation om zikavirusinfektion — Folkhälsomyndigheten

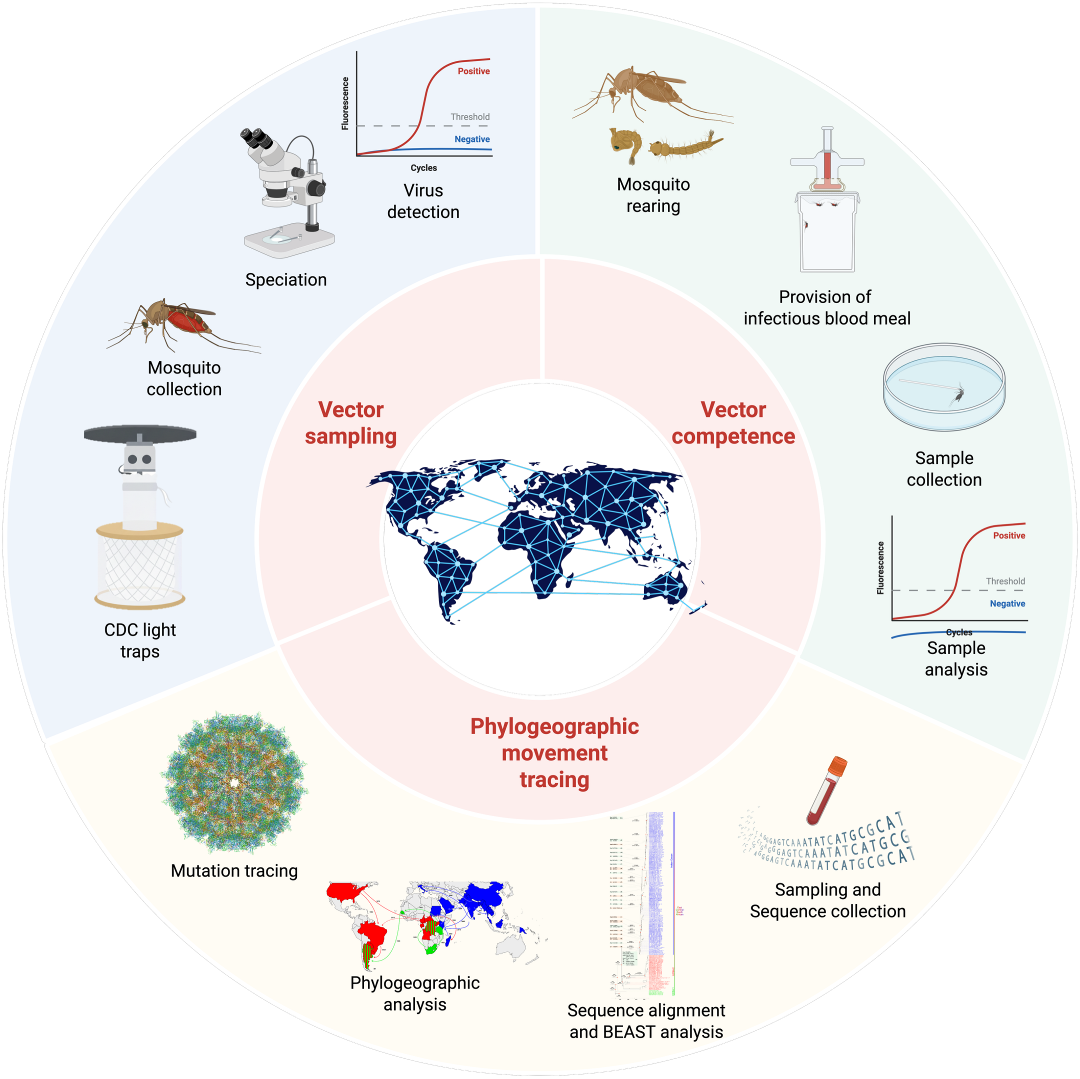
[4] Sjukdomsinformation om chikungunyavirusinfektion — Folkhälsomyndigheten

socioeconomic costs (Franklinos et al., 2019, Chilakam et al., 2023). In agricultural areas, animal infections also affect food security and income stability.

In Asia, Japanese encephalitis virus (JEV) remains a leading causative agent of viral neurological disease, particularly among children (Lindahl, 2012). Despite effective vaccination, transmission persists in many areas due to uneven vaccine coverage and the persistence of vector breeding sites (Mackenzie, 2005, Heffelfinger et al., 2017). Similarly, emerging flaviviruses such as Usutu virus (USUV) are expanding across Europe (Weissenböck et al., 2013, Weissenböck et al., 2003, Mani et al., 1998, Bakonyi et al., 2007, Chvala et al., 2007, Lühken et al., 2017, Kuchinsky et al., 2021, Vilibic-Cavlek et al., 2020, Steinmetz et al., 2011, Becker et al., 2012, Jöst et al., 2011, Rouffaer et al., 2018, Calzolari et al., 2012, Calzolari et al., 2010, Klobucar et al., 2021, Klobucar et al., 2016, Roesch et al., 2019), while the spread of chikungunya virus (CHIKV) illustrates how globalization allows viruses to spread rapidly between continents (CDC, 2022, Powers, 2018, Staples et al., 2009, Vairo et al., 2019).

Climate change amplifies these risks (Bellone and Failloux, 2020, Murrieta et al., 2021, Mellor and Leake, 2000, Wilson, 2010, Van de Vuurst and Escobar, 2023). Warmer temperatures extend the reproductive period of mosquitoes, while altered rainfall patterns create new breeding habitats. Urbanization and inadequate infrastructure compound these effects by creating stagnant water sources in densely populated areas (Lühken et al., 2023).

Figure 1. Overview of projects and methodologies



Source: Developed by the author.

Unveiling mosquito-borne virus transmission dynamics, vector competence, and genetic evolution

The overarching aim of my thesis was to explore how environmental conditions, mosquito ecology, and viral characteristics shape the risk of disease emergence — and how this knowledge can inform preparedness and development strategies. Viral transmission is how viruses spread from one host to another, involving a virus entering a new body, replicating, and exiting to infect others. This happens via direct contact (skin, droplets from coughs/sneezes), indirect contact (surfaces), airborne spread (aerosols), vectors (insects), or vertical transmission (mother to child). Vectors are living organisms like mosquitos or ticks, that can carry pathogens between humans and animals to varying degrees, being competent — can transmit — or incompetent — cannot transmit — vectors. In this work mosquitoes as vectors were the main focus.

The thesis addressed three key questions:

1. *What drives mosquito abundance and diversity in urban and peri-urban Hanoi, Vietnam?* How do temperature, rainfall, and habitat type influence mosquito populations, and what implications does this have for JEV transmission risk?
2. *Can Swedish* Culex pipiens *mosquitoes serve as vectors for JEV or USUV?* What biological or environmental barriers determine whether viruses can replicate and transmit in northern climates?
3. *How do evolutionary processes shape the emergence and global spread of CHIKV?* What can genomic analyses reveal about the patterns and drivers of virus dispersal?

Together, these studies link local ecological understanding with global surveillance needs, illustrating how capacity building and collaboration between Sweden and partner countries can advance shared preparedness goals.

Field surveillance in Vietnam

Despite the significant disease burden, understanding of JEV transmission dynamics remains limited. This study therefore aimed to elucidate the spatio-temporal patterns of *Culex* mosquitoes[5] in and around Hanoi, Vietnam, over one year by trapping and identifying mosquitoes and subsequently analysing them for JEV (Krambrich et al., 2024d). The goal was to improve understanding of fluctuations in the number of JEV vector mosquitoes, thereby supporting effective disease control and prevention.

Between 2020 and 2021, monthly mosquito collections were carried out for 13 months at five sites across Hanoi, representing urban, peri-urban, and rural gradients. CDC light traps were set overnight outdoors. The 4,829 captured mosquitoes were morphologically identified and sorted by genus and species, and pools of *Culex* mosquitoes were screened for JEV RNA using RT-qPCR[6]. Local meteorological data—temperature, rainfall, and relative humidity—were obtained from the collection sites for each sampling event.

Cx. quinquefasciatus was the most abundant species. Overall mosquito abundance peaked from February to May and declined throughout the remainder of the year. The study found no significant difference in *Culex* mosquito numbers between urban and suburban areas but observed significantly higher counts in peri-urban areas. Urban sites showed higher average monthly temperatures but lower humidity compared with suburban and peri-urban areas. Screening of viral RNA in vector mosquito pools did not detect JEV RNA in any of the 4,673 analysed Culex mosquitoes.

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[5] *Culex* mosquitos usually domiate the local mosquito population in Hanoi, Vietnam. They are a large group (genus) of mosquitos that includes many common “house” and night-biting species, some of which transmit diseases. Several *Culex* species are important vectors of viral diseases, including West Nile virus, Usutu virus, Japanese encephalitis virus, and other encephalitides.

[6] A laboratory technique of molecular biology.

Although JEV RNA was not detected, continued surveillance remains essential due to potential variations in infection rates in the field.

Vector competence of Swedish mosquitoes

Swedish *Cx. pipiens* mosquitoes were assessed for their ability to transmit two emerging arboviruses—Usutu virus (USUV) and Japanese encephalitis virus (JEV)—to evaluate the risk of local transmission should these viruses be introduced into Sweden (Krambrich et al., 2024b, Krambrich et al., 2024a). This intrinsic ability of an arthropod to acquire, replicate, and transmit a specific pathogen to a new host is called vector competence. Vector competence experiments using colonized mosquitos originating from Gothenburg showed that Swedish *Cx. pipiens* are susceptible to USUV infection and capable of transmitting the virus. USUV was detected in saliva as early as 7 days post-feeding, indicating a short extrinsic incubation period. Infection, dissemination, and transmission rates varied over time but a proportion of mosquitoes could transmit the virus at all examined time points, underscoring a potential risk of local USUV spread under suitable conditions, particularly in the context of climate change.

In contrast, when the same mosquito population was exposed to JEV (Nakayama strain), viral RNA was consistently detected in bodies, but no evidence of virus transmission capacity was observed. Infection rates remained stable but dissemination was minimal, indicating that under the tested conditions Swedish *Cx. pipiens* mosquitoes are not competent vectors for JEV genotype III. Together, these findings show that while USUV transmission by native Swedish mosquitoes is plausible, JEV transmission appears unlikely without specific environmental conditions, highlighting the need for continued surveillance as climate and ecological shifts may facilitate the emergence or expansion of both viruses.

Diversification of species and evolutionary analyses

Chikungunya virus (CHIKV) is a mosquito-borne RNA virus transmitted mainly by *Aedes aegypti* and *Ae. albopictus*, responsible for outbreaks in over 100 countries since its discovery in Tanzania in 1952 (CDC, 2022, Vairo et al., 2019, Staples et al., 2009, Gould et al., 2017). While rarely fatal, CHIKV can cause long-lasting joint pain, and despite ongoing vaccine development—including an FDA[7]-approved live attenuated vaccine—prevention still relies on mosquito control (Schneider et al., 2023, Valneva SE, 2023, Campos et al., 2020, de Lima Cavalcanti et al., 2022). CHIKV circulates in both forest-dwelling and urban transmission cycles and is divided into the West African, Asian, and East/Central/South African (ECSA) genotypes. One of these genotypes , the Indian Ocean lineage (IOL), is associated with major epidemics in Asia, including Thailand's outbreaks in 1958, 2008–2009, and 2018–2019 (Weaver and Lecuit, 2015, Volk et al., 2010, Hammon et al., 1960, Khongwichit et al., 2021, Wanlapakorn et al., 2014, Division of Epidemiology, 2008 to 2022).

My study looked at the 2018 chikungunya outbreak in Thailand by sequencing virus samples from patients returning to Sweden and comparing them with virus sequences from around the world to understand how the virus spreads and how it changes over time (Krambrich et al., 2024c). Evolutionary analyses revealed CHIKV's global spread over ~70 years, highlighting sub-Saharan Africa as a historical source and Southeast Asia—especially India—as a major hub for interregional transmission, with repeated bidirectional movements involving East Africa, India, Southeast Asia, the Arabian Peninsula, and Europe. Mutation analyses were also made. The evidence suggests that the 2018 Thai strains likely originated in South Asia, possibly Bangladesh, via Myanmar. Overall, the findings underscore CHIKV's complex evolutionary history, global mobility, ecological adaptability, and the roles of climate change,

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[7] US Food and Drug Administration www.fda.gov/about-fda

travel, and urbanisation in facilitating its continued emergence and public-health impact.

Discussion and implications

The study of mosquitoes as viral vectors began in 1881 when Carlos Finlay proposed that yellow fever virus is transmitted by infected mosquitoes (Taylor, 2014). Today, mosquito-borne viruses (arboviruses) such as JEV, USUV, CHIKV, and DENV continue to pose major public health challenges. My thesis examined key ecological, evolutionary, and epidemiological aspects of mosquito-borne viruses, focusing on JEV, USUV, and CHIKV, as well as *Culex* mosquito dynamics. Climate, landscape, human mobility, vector abundance, and viral genetic evolution all shape transmission patterns and geographic distribution.

Future work should expand vector competence studies to additional mosquito species in Sweden and assess how environmental factors such as temperature, humidity, and weather conditions influence vector capacity. Differences between field-caught and colonised mosquitoes, the antiviral RNA interference response, and the role of mosquito microbiota also deserve attention. Such factors may help explain geographic differences in transmission, such as why Swedish mosquitoes cannot transmit JEV while other European mosquitoes can. Surveillance of mosquito abundance, virus prevalence, and genetic diversity remains essential.

Integrating molecular epidemiology and phylogeography can further clarify transmission dynamics and guide targeted interventions. Continued development of vector control tools—including improved insecticides, genetically modified mosquitoes, and environmental management—alongside viral mutation tracking will aid outbreak prediction and strain risk assessment. Investment in vaccines and antivirals is also needed.

As climate change reshapes mosquito habitats, adopting a One Health approach that integrates human, animal, and environmental perspectives, supported by interdisciplinary collaboration, will be crucial for long-term prevention and control of mosquito-borne diseases.

Global collaboration and preparedness in non-endemic regions

These experiments were the first to show that Scandinavian mosquitoes can sustain full USUV transmission cycles, placing Northern Europe within the expanding range of flavivirus ecology. The Swedish component of the research underscores how non-endemic countries (like Sweden) can act as "preparedness laboratories". Preparedness begins before the first outbreak. Having local experimental evidence on vector competence allows public-health agencies to model realistic transmission risks rather than relying on assumptions from other regions.

Swedish funding for biosafety facilities, molecular diagnostics, and entomological training contributes knowledge that can later be transferred to partner nations. International development cooperation can strategically link domestic preparedness and global health solidarity. Investments in northern laboratories can strengthen both Swedish resilience and international capacity-building.

Placing the Vietnam and Sweden studies side by side illustrates how contrasting environments illuminate complementary aspects of the same global challenge. In Vietnam, the central question was how ecological, and climatic conditions influence mosquito abundance; in Sweden, whether those conditions could allow transmission if the virus were introduced.

By connecting these settings through joint research design and data exchange, the project operationalized Sweden's "mutual learning" principle in development cooperation: knowledge moves both ways. Vietnamese experience with field surveillance and vector entomology informed Swedish risk assessment, while Swedish laboratory infrastructure enabled advanced molecular diagnostics. Such reciprocal collaborations are increasingly recognized as sustainable models of scientific diplomacy—low-cost, high-impact mechanisms for long-term capacity development (Tvedten et al., 2021, Carneiro et al., 2016).

Globalisation, climate change and public health threats

Viral evolution follows human movement and environmental opportunity. The same connectivity that drives economic development—trade routes, tourism, labour migration—also facilitates pathogen spread as seen with CHIKV. For Swedish and European policymakers, this case demonstrates the global interconnectedness of arboviral threats: an outbreak thousands of kilometers away can reshape regional risk landscapes within months. Supporting genomic surveillance capacity in partner countries contributes directly to global health security. Investments in sequencing, bioinformatics, and open data sharing are development-cooperation tools as crucial as vaccine supply or health-system funding.

Climate and Environment Shape Disease Ecology

The combined findings confirm that vector-borne-disease emergence is tightly linked to environmental change. Rising temperatures and shifting rainfall patterns influence mosquito life cycles, while certain urbanization features (e.g. standing water) provide ideal breeding habitats. Climate adaptation policies therefore require explicit integration of vector surveillance indicators. Preparedness requires continuous knowledge exchange.

Capacity Building Is Central to Sustainability

Training, infrastructure, and long-term institutional relationships proved as valuable as the immediate scientific outputs. The project created sustained networks that now facilitate rapid data exchange.

One Health as an Operational Framework

All three studies show that human, animal, and environmental health are inseparable. Pigs act as amplification hosts for JEV; birds maintain USUV cycles; human mobility propagates CHIKV. Cross-sectoral coordination is therefore essential—an area where Swedish development cooperation already holds comparative advantage.

Opportunities to translate science into policy action

My thesis findings suggest several opportunities to translate scientific insight into policy action.

Integrate vector-borne disease risk into climate adaptation programmes

Current Swedish climate initiatives in Africa and Asia already address water management, agriculture, and biodiversity. Including entomological surveillance components would create valuable early-warning systems. Joint projects between the Swedish Meteorological and Hydrological Institute (SMHI), universities, and partner institutions could align climate modelling with mosquito abundance data.

Strengthen research partnerships and data sharing

Programmes such as Sida's bilateral research cooperation (e.g., with Vietnam, Tanzania, and Mozambique) could incorporate vector-borne disease themes. Long-term grants should emphasize training in genomic surveillance, field entomology, and digital data exchange. Building open-access databases for mosquito and virus monitoring would amplify regional and global preparedness.

Invest in bioinformatics and laboratory infrastructure

The ability to detect and characterize viruses early depends on molecular capacity. Support for low-cost sequencing platforms and training in data analysis would empower local laboratories to identify new threats without external dependency. The research presented in this thesis demonstrated how joint analysis between Swedish and Vietnamese teams accelerated both discovery and learning.

Maintain Nordic preparedness

Domestic preparedness is part of Sweden's local security and global contribution. Maintaining vector competence laboratories, reference collections, and biosafety facilities ensures that Sweden can respond rapidly to introductions and share protocols internationally. Such facilities double as training centers for visiting scientists from partner countries.

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Mosquitoes transmit viruses such as Japanese encephalitis and chikungunya. Through field studies in Vietnam and lab tests in Sweden, this DDB examines global virus spread and the potential for mosquito-borne transmission in Sweden. The findings highlight how climate, ecology, and globalization shape infection risks and underscore the need for global collaboration, capacity building, and integrated preparedness for emerging health threats.

Myggor kan sprida virus, t.ex. japansk encefalit och chikungunya. Genom fältstudier i Vietnam och laboratorietester i Sverige undersöker denna DDB spridningen globalt och om och hur olika virus kan spridas via myggor i Sverige. Resultaten visar hur klimat, ekologi och globalisering formar smittrisker och betonar behovet av globalt forskningssamarbete, kapacitetsuppbyggnad och bättre beredskap inför hälsohot.

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