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THE IMPERATIVE OF *AEDES* MOSQUITO TESTING FOR ENHANCED DENGUE SURVEILLANCE IN THE UNITED STATES

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ABSTRACT. Dengue virus (DENV) poses an escalating public health threat in both endemic and non-endemic regions, driven by globalization, urbanization, and climate change. Early detection of dengue outbreaks remains a critical component in mitigating the impact of this rapidly spreading arboviral disease. Traditional surveillance approaches rely primarily on clinical case reports, which often lag behind the actual onset of transmission. Because of the lag, intensive vector control response would be delayed and become ineffective to contain the transmission promptly. This calls for the need for mosquito-based DENV surveillance even in low transmission or epidemic-prone settings. This review explores the growing body of evidence supporting mosquito-based DENV detection as a proactive surveillance method, particularly in areas with low transmission or at risk of outbreak due to travel-associated cases. A comprehensive literature search was conducted using several academic and public health databases, including PubMed, Web of Science, Scopus, Google Scholar, and ProQuest. Seventeen studies showed presence of DENV in *Aedes* mosquitoes in the absence of reported human cases. In the United States, four studies reported DENV positive *Ae. aegypti* in places where there were no reported human cases. Among various mosquito surveillance tools, BG-Sentinel traps have been indicated to be effective in capturing host-seeking invasive *Aedes* mosquitoes. Increasing evidence indicates that detecting DENV in field-caught mosquitoes can serve as an early warning signal—preceding reported human dengue cases by several weeks. This entomological approach provides direct insights into the virus's presence in mosquito populations before human infections are formally diagnosed. The review recommends integrating mosquito-based DENV detection into local, state and national arbovirus surveillance systems, especially in non-endemic regions vulnerable to autochthonous transmission. This approach can enhance the timeliness and precision of outbreak responses.

KEY WORDS *Aedes* mosquitoes, BG-Sentinel traps, dengue, low transmission, mosquito testing, surveillance

INTRODUCTION

Dengue fever is a mosquito-borne viral disease that has emerged as one of the most significant public health threats in tropical and subtropical regions (WHO 2024). The global incidence of dengue has reached unprecedented levels in recent years, marking 2024 as the most severe year on record. According to the World Health Organization (WHO), the global dengue burden dramatically increased from 505,430 in 2000 to over 5.2 million in 2019 and 14.3 million in 2024, with Asia bearing the highest burden (75%), followed by Latin America and Africa (WHO 2024). The Americas experienced an unprecedented dramatic surge in 2024, with over 12 million cases reported more than double the number recorded in 2023 (PAHO 2024). This reflects both improved surveillance and actual increases in transmission. Currently, dengue is endemic in more than 100 countries across Asia, the Americas, Africa, and the Western Pacific, where periodic explosive outbreaks place immense strain on health systems.

Factors contributing to this rise include rapid urbanization, increased human mobility, climate change, and inadequate vector control (Ryan et al. 2024, Feng et al. 2025). The true burden is likely much higher, as many cases go unreported or misclassified due to asymptomatic infections and limited diagnostic capacity in low-resource settings (WHO 2023). Although most dengue-endemic regions report seasonal or recurrent outbreaks, certain areas experience low or sporadic transmission, making disease detection and control more challenging (Ng 2011, Lee et al. 2016).

Dengue is primarily transmitted by *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse), both of which have long been established in many US states. *Aedes aegypti* is most prevalent in the southern states, particularly across the Gulf Coast, Arizona, and parts of California, while *Ae. albopictus* has a broader distribution extending from the southeastern states through the mid-Atlantic and as far north as parts of the Midwest (Powell and Tabachnick 2013). Surveillance for these species intensified during the Zika virus response in 2016–2017 (Kraemer et al. 2015, CDC 2017). Although local dengue transmission in the continental United States remains limited, the presence of these vectors, combined with substantial numbers of travel-associated cases, highlights the risk that imported infections can seed local outbreaks when conditions are favorable (Hunsperger et al. 2023, CDC 2025a). Between 2010 and 2021, a total of 7,528 confirmed or probable travel-associated dengue cases were reported in the United States. (Wong et al. 2023). Approximately 90% of these cases were linked to travel outside US states or territories. In 2019, the highest incidence per million air trips was associated with travel to the Caribbean (56.8 per million), Central America (49.7), and Asia (39.6), underscoring how global dengue outbreaks directly shape imported case patterns in the United States (CDC 2025b).

Dengue is endemic in Puerto Rico and the US Virgin Islands, where all four dengue virus serotypes have circulated over the past decades (CDC 2025a). These territories experience periodic outbreaks, often linked to seasonal increases in *Ae. aegypti* abundance and favorable climatic conditions. In contrast to the continental United States—where most infections are travel-associated and only sporadic local transmission occurs—Puerto Rico and the US Virgin Islands report sustained transmission and represent the primary foci of dengue virus circulation under US jurisdiction (CDC 2025b).

In the continental United States, in 2024, locally acquired cases were reported in Florida (91 cases), California (18), and Texas (1) (CDC 2025a). During same year, a record number of dengue cases were identified among US travelers (3,483 cases), which is an 84% increase compared to 2023. In 2025, as of October 7, a total of 2,560 dengue cases has been reported (CDC 2025b). This trend is expected to continue with increased dengue activity in endemic US territories in 2025, according to CDC (2025b). The highest numbers of travel-associated cases in 2024 were reported in Florida (1,016) followed by California (648), and New York (327). In California, the first two locally acquired cases were documented in 2023, followed by 18 confirmed cases in 2024 (CDPH, 2025b), indicating a potential expansion of transmission risk into regions previously unaffected.

In low transmission settings, traditional surveillance systems—often reliant on symptomatic human case detection—may fail to identify silent circulation of the virus, especially due to the high proportion of asymptomatic or mild cases (Asish et al. 2023). In low dengue transmission settings, the high proportion of asymptomatic or clinically mild infections (30–50%) enables substantial silent circulation of the virus that remains undetected by routine case-based surveillance (Rodríguez-Barraquer et al. 2019, Zhang et al. 2023). As a result, dengue transmission may continue unnoticed, creating the potential for explosive outbreaks once conditions become favorable. Testing mosquitoes for the presence of dengue virus (DENV) in these areas offers a complementary strategy to human-based surveillance. It serves as a crucial tool for early outbreak detection, risk assessment, and targeted vector control interventions.

Early detection of dengue outbreaks is therefore a critical component in mitigating the impact of this rapidly spreading arboviral disease. Traditional surveillance approaches rely primarily on clinical case reports, which often lag the actual onset of transmission (Kyle and Harris 2008). Increasing evidence indicates that detecting DENV in field-caught mosquitoes can serve as an early warning signal—preceding reported human dengue cases by several weeks (Lau et al. 2017, Loroño-Pino et al. 1999). This entomological approach provides timely insights into the virus's presence in mosquito populations before human infections are formally diagnosed. This review explores the growing body of evidence supporting mosquito-based DENV detection as a proactive surveillance method, particularly in areas with low transmission or at risk of outbreak due to travel-associated cases. It also evaluates the importance of DENV detection in mosquito vectors as a surveillance tool in low transmission settings, critically examining the operational merits for effective vector control programs, and how to integrate it into comprehensive public health surveillance frameworks.

MATERIALS AND METHODS

This literature review employed a systematic approach to identify and synthesize peer-reviewed and authoritative grey literature concerning *Aedes* mosquito testing as a critical component of dengue surveillance in the United States and other parts of the world with low transmission of the diseases. In addition, we compared different *Aedes* trapping surveillance tools to identify the most effective trap for urban settings where invasive *Aedes* are fast spreading.

A comprehensive literature search was conducted using several academic and public health databases, including PubMed, Web of Science, Scopus, Google Scholar, and ProQuest, in addition to grey literature from authoritative sources such as the Centers for Disease Control and Prevention's (CDC) Morbidity and Mortality Weekly Report (MMWR). Search terms included combinations and variations of "*Aedes aegypti*," "*Aedes albopictus*," "dengue surveillance," "mosquito testing," "vector surveillance," "trapping," "United States," "early detection," and "virus detection". The search was limited to English-language publications and focused on peer-reviewed studies, program evaluations, surveillance reports, and national guidelines primarily relevant to the US context and other places with similar epidemiological settings.

Data screening

Studies were included if they focused on mosquito-based dengue surveillance in the United States or with similar non-endemic low transmission setting, utilized entomological and/or virological methods for DENV detection in *Aedes* species, and provided primary data or evaluations relevant to public health practice. Inclusion criteria were (1) original research articles or surveillance reports focusing on *Aedes* mosquito testing methods or their application in dengue surveillance, (2) studies conducted within the United States or in ecologically relevant contexts, and (3) publications in English. Exclusion criteria included articles without original data, conference abstracts lacking full text, and studies unrelated to *Aedes* vectors or dengue virus. Publications were

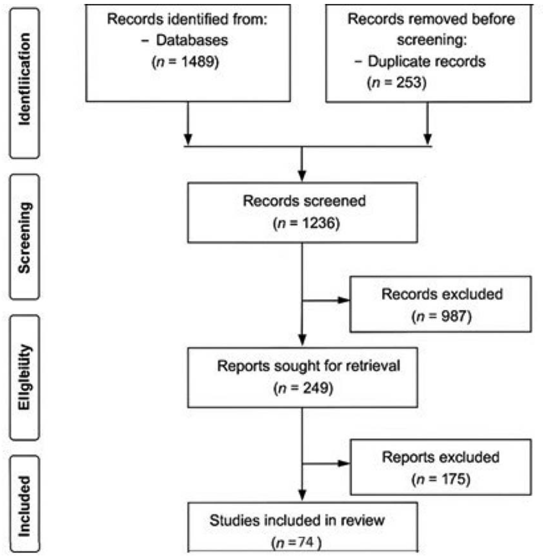

Fig. 1. Literature review workflow (*n* refers to the number of studies).

excluded if they focused solely on other mosquito species (e.g., *Anopheles*), viruses other than DENV, or if they were editorials, opinion pieces, or non-English sources with no translation.

Data extraction and synthesis

Data extracted from eligible studies encompassed mosquito sampling techniques, diagnostic testing methodologies such as real-time reverse transcriptase polymerase chain reaction (PCR), viral isolation, immunoassays, human case reports, geographic focus, and documented impacts on dengue surveillance outcomes. Emphasis was placed on studies integrating molecular diagnostics for dengue virus detection in field-collected *Aedes* mosquitoes and those demonstrating incorporation of entomological surveillance with public health interventions.

The overall selection process is illustrated in Fig. 1, which outlines the flow from article identification to inclusion. A total of 1,489 records were initially identified; after deduplication, 1,239 remained for screening. Of these, 249 were deemed eligible for full-text review, and 74 high- to moderate-quality studies that focused in low dengue transmission settings were included in the final synthesis.

Additionally, gray literature from public health agencies-CDC, the Pan American Health Organization (PAHO), the World Health Organization (WHO) and state-level public health agencies was included to capture practical implementation frameworks and program evaluations. Inclusion criteria prioritized studies that focused on *Ae. aegypti or Ae. albopictus* surveillance and testing, were relevant to the US context or generalizable to US conditions. Studies were included in our analysis if they described methodologies for detecting dengue virus or evaluating vector abundance. Studies were excluded if without mosquito testing results, non-English publications without translation, and articles solely focused on clinical surveillance.

For each study, key data were extracted concerning surveillance approaches (e.g., mosquito trapping techniques, testing results), geographic location, integration with health systems, and outcomes related to early outbreak detection or intervention decision-making. Studies were thematically analyzed to trace how mosquito testing has influenced dengue risk monitoring over time and how it is currently positioned within integrated vector management frameworks.

RESULTS

Adult *Aedes* mosquito surveillance tools

Table 1 summarizes the most commonly used mosquito trapping tools for *Aedes* mosquitoes. Field evaluations of adult *Aedes* mosquito surveillance tools indicate that BG-Sentinel traps are the most effective for capturing host-seeking females, especially when combined with BG-Lure and CO_2. CDC light traps, on the other hand, consistently underperformed in catching *Aedes*, although they remain effective for *Anopheles* and *Culex* species. Gravid-targeted traps such as the Gravid Aedes Trap (GAT), efficiently captured gravid *Ae. aegypti*, but not so much with host-seeking mosquitoes. The Prokopack aspirator proved highly effective for collecting resting adult mosquitoes indoors, making it valuable for assessing indoor density and human exposure risk. However, it is shown to be not so effective in collecting host-seeking mosquitoes. Overall, trap choice depends on the target species, surveillance goal, and field conditions, with BG-Sentinel preferred for adult *Aedes* monitoring in urban settings.

DENV positive *Aedes* mosquitoes without human case reports

At least seventeen studies showed the presence of DENV in *Aedes* mosquitoes in the absence of reported human cases (Fig. 2 and Table 2). In the United States, four studies reported DENV positive *Ae. aegypti* in places where there were no reported human cases. One study from Manatee County, Florida reported the detection of complete DENV-4 genomes in field-collected adult *Ae. aegypti* through metaviromic sequencing in the absence of a local DENV-4 human case in this county over a 2-year period (Boyles et al. 2020).

Several studies from South America and Asia detected DENV in field-collected *Aedes* mosquitoes without concurrent clinical cases, including detections in larvae, males, or adults reared from field eggs—entomological stages most consistent with vertical transmission (Table 2). These reports span multiple serotypes (DENV-1–4), methods (RT-PCR, qRT-PCR, sequencing, and in some cases

Table 1. Comparative evaluation of commonly used mosquito surveillance traps for *Aedes* mosquitoes.

Feature	BG-Sentinel (BGS)	CDC light trap	Gravid *Aedes* Trap (GAT)	Prokopack aspirator	References
Target Species	*Aedes. aegypti*, *Ae. albopictus*	*Anopheles*, *Culex* (low for *Aedes*)	*Aedes* spp. (gravid females)	Resting *Ae. aegypti*, *Ae. albopictus*	Kröckel et al. 2006; Williams et al. 2006; Barrera et al. 2014; Vazquez-Prokopec et al. 2009; Maciel-de-Freitas et al. 2014; Barrera et al. 2020; Eiras et al. 2021; Vazquez-Prokopec et al. 2022; Reegan et al. 2024
Attractants	BG-Lure, CO_2, convection air currents	Light, CO_2	Water + organic infusion (hay/leaf)	Battery-operated	Maciel-de-Freitas et al. 2006; Vazquez-Prokopec et al. 2009; Eiras et al. 2021; Vazquez-Prokopec et al. 2022; Reegan et al. 2024
Effectiveness for *Aedes* spp.	High; best performing adult trap	Low–moderate; underestimates density	High for *Ae. aegypti*/*albopictus*, esp. low-density	High efficiency for indoor resting females	Williams et al. 2006; Barrera et al. 2014; Vazquez-Prokopec et al. 2009; Barrera et al. 2020; Eiras et al. 2021; Vazquez-Prokopec et al. 2022. Degener et al. 2014; Petrie and Beier 2022; Reegan et al. 2024
Correlation with Human Risk	Strong correlation with human landing collections	Poor correlation	Moderate correlation with adult abundance	Strong; reflects indoor resting density	Maciel-de-Freitas et al. 2006; Barrera et al. 2014; Vazquez-Prokopec et al. 2009; Barrera et al. 2020; Eiras et al. 2021; Vazquez-Prokopec et al. 2022
Cost	High (∼5–10× CDC light trap)	Low	Low	Low (manual device)	Reiter, 2001; Vazquez-Prokopec et al. 2009; Barrera et al. 2020; Eiras et al. 2021; Vazquez-Prokopec et al. 2022
Power Requirement	Continuous power source	Battery	None	None (manual aspiration)	Barrera et al. 2014; Vazquez-Prokopec et al. 2009; Barrera et al. 2020; Eiras et al. 2021; Vazquez-Prokopec et al. 2022
Field Suitability	Best for arbovirus surveillance	Lightweight, robust	Simple, low-maintenance	Excellent for indoor surveys in endemic settings	Barrera et al. 2014; Vazquez-Prokopec et al. 2009; Barrera et al. 2020; Eiras et al. 2021; Vazquez-Prokopec et al. 2022
Other Vectors	Inefficient for *Anopheles*	Good for *Anopheles*/*Culex*	Minimal (mostly *Aedes*)	May catch *Culex* and other resting species	Barrera et al. 2014; Vazquez-Prokopec et al. 2009; Barrera et al. 2020; Eiras et al. 2021; Vazquez-Prokopec et al. 2022

Fig. 2. Map showing the location of studies that documented dengue virus presence in *Aedes* mosquitoes while without reported human cases.

virus isolation), and *Aedes* species (*Ae. aegypti* and *Ae. albopictus*).

In São Paulo, Brazil, DENV-3 RNA was detected in pools of male *Ae. albopictus* collected from urban sites with no prior history of human dengue cases (Guedes et al. 2010). In Veracruz, Mexico, larval *Ae. aegypti* from ovitrap collections tested positive for DENV-2 and DENV-3 by RT-PCR (Martínez et al. 2014). No DENV-2 human cases were reported locally during the surveillance period. In Sri Lanka, larval *Ae. aegypti* collected from multiple districts tested positive for DENV-1 and DENV-4 (Gunathilaka et al. 2017). No human cases of DENV-4 were reported during the same month. Similarly, detection of DENV-2 in immature *Ae. aegypti* from urban breeding sites with no human dengue cases was reported in Makkah, Saudi Arabia (Ali et al. 2022). In Iran, DENV RNA was detected in field-collected adult *Ae. albopictus* in Sistan and Baluchestan Province, with no reports of local human cases confirmed by the national surveillance system (Mubbashir et al. 2018). *Aedes. aegypti* was the primary vector in 70% of these reports, followed by *Ae. albopictus* in 20%, and mixed-species collections in 10%.

DISCUSSION

Our review on studies that documented DENV–positive *Aedes* in the apparent absence of contemporaneous human case reports indicates that low-level, cryptic virus maintenance in vector populations is plausible across diverse eco-epidemiological settings. Evidence from both *Ae. aegypti* and *Ae. albopictus*, with viral RNA and, in some instances, virus isolation and genomes recovered, signals that collectively support vertical (transovarial) transmission and/or transient introductions that fail to trigger recognized human outbreaks. Collectively, both laboratory and field studies reinforce the importance of proactive entomological surveillance in receptive, non-endemic settings such as the United States, where early detection of dengue virus in mosquitoes could serve as a sentinel warning system and inform timely vector control interventions.

In low transmission regions, where reported human cases are infrequent or seasonal, the risk of underestimating true dengue circulation is high. These areas may harbor small but persistent foci of infected mosquitoes or asymptomatic individuals who silently maintain viral presence, a phenomenon documented in both endemic and non-endemic areas (Duong et al. 2015, Bosch et al. 2017, CDC 2025a). Recent advances in trapping technologies such as BG-Sentinel traps have improved the efficiency, sensitivity, and spatial coverage of adult *Aedes* surveillance, facilitating systematic mosquito collection and virologic testing. Moreover, the presence of competent vectors, particularly *Ae. aegypti*, coupled with reports of travel-associated cases indicate that the potential for transmission exists even in the absence of confirmed human cases, highlighting the critical role of combined entomologic and virologic surveillance in low-transmission settings.

Effective adult *Aedes* mosquito collection technique

Among the most widely used mosquito surveillance tools, BG-Sentinel traps are especially effective for capturing *Ae. aegypti* and *Ae. albopictus* – the two prominent DENV vectors, because they mimic human scent and utilize visual and olfactory cues to attract mosquitoes (Maciel-de-Freitas et al. 2006,

Table 2. Summary of literature that reported the presence of dengue infected mosquitoes in the absence of reported human cases.

Study	Location	Mosquito species	DENV serotype	Detection method	Human cases reported	Key findings
Buckner et al. 2013	Florida, USA (Key West)	*Aedes aegypti*, *Ae. albopictus*	DENV-1	RT-PCR*	No human cases	8.33% (3/36) positivity rate for *Ae. aegypti* and 11.11% (2/ 18) for *Ae. albopictus*.
Boyles et al. 2020	Florida, USA (Manatee Co.)	*Ae. aegypti*	DENV-4	RT-PCR, full genome assembly	No human cases	The first documented case of such a phenomenon in the absence of a local DENV4 human case in this county over a 2-year period
Smartt et al. 2013	Florida, USA (Manatee Co.)	*Ae. aegypti* adults from field	DENV-4	RT-PCR, sequencing	No human cases	1–4% DENV positivity rate. First US detection; strain matched Haitian lineage; suggests vertical maintenance
Bosio et al. 1992	Indianapolis, Houston, New Orleans (lab derived from US populations)	*Ae. albopictus* (lab progeny)	DENV-1	Experimental, lab-based transovarial infection	No human cases	11 to 41% vertical transmission rate; Demonstrated intra-familial variation
Espinosa et al. 2014	Argentina	*Ae. aegypti*	DENV-2	Real-time RT-PCR	No human cases	1/15 mosquito poos were positive for DENV-2
Guedes et al. 2010	Brazil	Adult *Ae. aegypti* reared from field eggs	DENV-1-3	Virus isolation & RT-PCR	No human cases	9/83 pools were positive for DENV. Out of 9 positive pools, 5 for DENV-1, 2 for DENV-2 and 2 for DENV-3 isolated from adult
Gunathilaka et al. 2017	Sri Lanka	*Ae. aegypti* and *Ae. albopictus* larvae	DENV-2	RT-PCR	No human cases	Vertical transmission detected – 9.8% DENV positivity rate in *Ae. albopictus* larvae and ~8.1% in *Ae. aegypti* larvae
Martínez et *al*. 2014	Mexico	*Ae. aegypti* larvae and adults	DENV-1	RT-PCR	No human cases	2/226 pools were positive for DENV-1 with no clinical dengue cases at the time of sampling
Mubbashir et al. 2018	Pakistan	*Ae. albopictus* larvae	DENV-1	RT-PCR	No human cases	vertical transmission was detected with 4/1340 positivity rate for DENV
Eisen et al. 2014.	Mérida, Yucatán, Mexico	*Ae. aegypti*	Unspecified DENV	RT-PCR	No human cases	0.7–5.4% DENV positivity rate despite no concurrent clinical cases

Table 2. Continued.

Study	Location	Mosquito species	DENV serotype	Detection method	Human cases reported	Key findings
Lee et al. 2010	Singapore	Adult *Ae. aegypti*, *Ae. albopictus*	DENV-1	RT-PCR	No human cases	Field detection during inter-epidemic period
Ferreira-de-Lima et al. 2020	São Paulo, Brazil	*Ae. aegypti* adults	DENV-3	qRT-PCR	No human cases	2/573 (0.3%) pools tested positive for DENV; cryptic virus circulation documented
Ali et al. 2022	Makkah, Saudi Arabia	*Ae. aegypti* adults	Unspecified DENV	RT-PCR	No human cases	6/259 (2.3%) pools tested positive for DENV; indicated both vertical and cryptic circulation
de Figueiredo et al. 2010	Brazil	*Ae. aegypti* adults	DENV-1-3	RT-PCR	No human cases	6/154 (3.8%) mosquito pools tested for DENV. Over two years without linked human cases
Mweya 2025	Tanzania	*Ae. aegypti* adults	DENV unspecified	RT-PCR	No human cases	0.6% (4/631) DENV positivity rate reported

* Real-time polymerase chain reaction.

Reegan et al. 2024). These traps have been demonstrated to outperform others in terms of the number of *Aedes* captured, making them highly suitable for DENV surveillance in endemic and non-endemic areas (Amos et al. 2020). CDC light traps, while useful for general mosquito surveillance, are less effective for day-biting species such as *Ae. aegypti*, as these traps typically attract nocturnal mosquitoes. Therefore, reliance solely on CDC light traps in dengue-focused studies can lead to underrepresentation of key vectors and a misinterpretation of transmission risk (Li et al. 2016).

The introduction of the BG-Sentinel trap markedly improved dengue vector surveillance in the last decade by enhancing adult *Aedes* collection for risk monitoring. Importantly, BG-trapped mosquitoes have been successfully tested for natural DENV infection in field settings across the United States, Brazil, Australia, Colombia, and Asia – directly linking entomological surveillance with real-time outbreak detection (Figueiredo et al. 2013, Ritchie et al. 2013, Kim et al. 2017, Carrasquilla et al. 2021). While some of these studies did not consistently report the exact number of DENV-positive mosquitoes, they collectively demonstrate the utility of BG-Sentinel traps for xenomonitoring and early warning, often detecting viral activity before clinical cases were fully recognized.

Overall, the integration of BG-Sentinel traps into mosquito surveillance programs enhances the ability to detect DENV infections in real-time, facilitating prompt interventions and better management of outbreaks. Hence, testing mosquitoes for DENV can serve as a direct indication of active viral circulation in the environment before human cases become apparent. For example, studies in Singapore (Lee et al. 2012) and Taiwan (Huang et al. 2010) have shown that entomological DENV detection precedes clinical outbreaks by several weeks, allowing for proactive vector control and public health responses. Drawing on lessons from both endemic and low-transmission settings (Duong et al. 2015, Bosch et al. 2017, CDC, 2025b), optimal dengue surveillance in low transmission areas therefore involves integrating entomological, virological, and epidemiological data. This holistic approach helps overcome limitations inherent in any single method and provides a more comprehensive understanding of local transmission risk.

Rationale for mosquito testing in low transmission areas

In non-endemic areas prone to dengue introduction, dengue surveillance traditionally relies on the detection of clinically apparent human cases. However, this approach often underrepresents the actual transmission dynamics due to several key challenges: subclinical infections, delays in case reporting, limited diagnostic infrastructure, and the focal nature of dengue outbreaks. These limitations make it difficult to detect early signs of viral circulation and to implement timely interventions (Vong et al. 2010, Simmons et al. 2012, Koa et al. 2016).

Several studies both from endemic and non-endemic settings indicate that mosquito-based DENV detection, while logistically demanding, can provide critical early warning of focal transmission. For instance, in Manatee County, Florida, *Ae. aegypti* pools tested positive for dengue virus during routine molecular surveillance despite no locally acquired human cases at the time (Boyles et al. 2020). These findings highlight that infections in mosquitoes may precede recognition of human cases, underscoring mosquito testing as a proactive surveillance tool. However, it is important to recognize resource constraints and competing public health priorities that limit molecular surveillance in many local vector control agencies. Our argument is not for unchecked universal testing, but for strengthening testing capacity when logistical capacity and epidemiological risk justify it — for example, in jurisdictions with risk factors such as established *Aedes* populations, recent imported dengue cases, or proximity to active dengue transmission.

Indeed, mosquito-based molecular surveillance – specifically, testing *Ae. aegypti* for dengue virus – offers a complementary tool that can enhance rapid vector control response, even when the likelihood of detecting a positive mosquito is low. Several studies have shown that mosquitoes can test positive for DENV before any human cases are reported (Table 2), suggesting that entomological testing could act as a leading indicator for transmission (Lourenço-de-Oliveira et al. 2018). In non-endemic areas, detecting even a single infected mosquito can be critical for initiating vigorous vector control responses and preventing wider outbreaks from happening (Souza-Neto et al. 2019).

Epidemiological modeling has shown that the force of infection in dengue is not always directly correlated with reported clinical cases, especially in areas where dengue is historically unknown (Rodriguez-Barraquer et al. 2019, Stephenson et al. 2021). This discrepancy highlights the need for integrated surveillance strategies that include dengue-based entomological surveillance to detect viral circulation early. In fact, studies have identified dengue-positive mosquitoes weeks before the first clinical cases appear, emphasizing their role as a sentinel tool for outbreak prediction (Lima-Camara et al. 2014).

Moreover, the role of asymptomatic or mildly symptomatic individuals in maintaining silent dengue transmission underscores the importance of molecular entomologic surveillance (Bosch et al. 2017). These individuals often go undetected by the routine health system reporting but may continue to infect mosquitoes, sustaining local transmission chains (Duong et al. 2015). In such scenarios, mosquito testing becomes a crucial means to reveal hidden viral activity and potential human risk. At an individual level, a modeling study showed that people with asymptomatic infections are approximately 80% as infectious to mosquitoes as their symptomatic counterparts (Ten-Bosch et al. 2018). At a population level, the study estimated that approximately 88% of infections result from people who display no apparent symptoms at the time of transmission. These results suggest that individuals undetected by public health surveillance systems may be the primary reservoir of dengue virus transmission and that policy for dengue control and prevention must incorporate entomological surveillance.

In addition, vector competence and behavior can vary significantly by location and season, impacting transmission risk. Vector competence is also influenced by viral genotype–vector interactions; for example, Asian and American strains of DENV-2 often produce higher mosquito midgut infection and salivary gland dissemination rates compared to other serotypes (Christofferson and Mores 2011). Environmental factors, including temperature and humidity, modulate extrinsic incubation periods, often accelerating transmission potential under warmer conditions (Mordecai et al. 2017). In the United States, *Ae. aegypti* populations in Florida and Texas have been shown to be competent for multiple DENV serotypes under laboratory conditions, supporting their role in sporadic local outbreaks (Richards et al. 2012, Alto et al. 2014). While further entomological studies are required to directly measure vector competence, routine entomological surveillance provides important indirect insights. For example, data on vector presence, abundance, age structure, and detection of naturally infected mosquitoes help infer the potential for local transmission and the functional competence of mosquito populations under real-world conditions. Collectively, these findings underscore that the biological traits and vector competence of *Ae. aegypti*, together with field evidence from routine surveillance, make it the most efficient and epidemiologically important dengue vector to actively monitor, highlighting the need for active monitoring and targeted vector control.

In addition, routine testing of mosquito populations for DENV has been shown to help identify temporal and spatial hotspots, guiding targeted control strategies. For example, entomological surveillance in Brazil and Puerto Rico has identified specific neighborhoods or periods when infected mosquitoes were more common, enabling more effective allocation of resources (PAHO 2019). In low-transmission or epidemic-prone settings, combining routine DENV testing with robust entomological surveillance can help detect emerging mosquito hotspots early, inform proactive vector control, and provide critical data for risk assessment and outbreak preparedness, even before human cases are reported.

The detection of DENV through vertical transmission in areas without known human cases also challenges current surveillance strategies that rely primarily on symptomatic case reporting. It underscores the need for proactive mosquito-based surveillance, particularly in low-transmission or non-endemic settings. Identifying infected mosquitoes in the absence of reported human infections can provide an early warning signal of potential outbreaks, help target vector control efforts, and refine risk assessments (Guedes et al. 2010, Buckner et al. 2013). In this context, vertical transmission is not merely a biological curiosity but a mechanism with practical implications for public health.

In United States, across Florida, Texas and California, where *Ae, aegypti* is fast spreading and local dengue transmission is reported, local vector control agencies have begun to implement entomological DENV surveillance. Testing mosquitoes for DENV serves as a precautionary measure given the increasing incidence of imported cases and the widespread establishment of competent vectors (Kraemer et al. 2015, Sehi et al. 2025). Such proactive monitoring is essential for early detection of local transmission.

However, critics argue that the low infection rate in mosquito pools from historically non-endemic areas reduces the cost-effectiveness of routine testing. For instance, the California Department of Public Health (CDPH) recently advised against promoting *Aedes* mosquito testing for dengue by local vector control agencies (CDPH 2024) due to no documented DENV positivity rates in its non-endemic areas. This guidance underestimates the critical value of such surveillance for early detection and outbreak prevention. Even in regions where local transmission is sporadic, the growing number of imported dengue cases (CDPH 2025b) —coupled with the established presence of competent *Aedes* vectors (Alto et al. 2014, McGregor and Connelly 2021, Sehi et al. 2025)—creates conditions conducive to autochthonous transmission (Kraemer et al. 2019, Kache et al. 2021). Mosquito-based DENV surveillance functions as a sentinel tool, capable of identifying dengue virus circulation in vector populations before human cases are reported, thus enabling timely and targeted public health interventions (Wong et al. 2022). In fact, dengue virus has been detected in *Aedes* mosquitoes in Florida, Texas, and Arizona either before or in the absence of confirmed human infections (Hahn et al. 2017, Lebo et al. 2023). Discounting mosquito testing based solely on low detection rates risks weakening our early warning capacity—particularly at a time when global travel and climate change are expanding the reach of arboviral threats (Messina et al. 2019). Far from being a resource demanding exercise, targeted *Aedes* testing is a forward-looking investment in public health preparedness.

It is also important to note that negative mosquito test results do not necessarily indicate absence of dengue viruses. In low transmission areas, DENV prevalence in mosquito populations can be extremely low, and focal or cryptic transmission may occur in the absence of reported human cases. Many studies report detection only after testing hundreds or thousands of mosquito pools (Gu and Novak 2004). For example, in 2024, local vector control agencies in California tested over 13,000 *Ae. aegypti* mosquitoes for dengue virus, yet no positive mosquitoes were detected (CDPH 2025b). Thus, absence of detection should be interpreted cautiously, and ongoing or periodic mosquito surveillance remains critical for early warning, risk assessment, and guiding timely vector control interventions.

Evidence from dengue-endemic countries shows that a large proportion of infections are asymptomatic or mild, enabling silent virus circulation that is not captured by routine case surveillance, and that even asymptomatic individuals can infect mosquitoes (Rodríguez-Barraquer et al., 2019, Duong et al. 2015, Zhang et al. 2023). While non-endemic areas differ in factors such as lower mosquito abundance, reduced human–vector contact, and environmental constraints, the core lesson from endemic settings remains that symptomatic surveillance alone may underestimate true risk. Testing *Aedes* mosquitoes for dengue virus offers a complementary approach to identify silent circulation early, before local transmission establishment or outbreaks occur.

In the United States, the CDC (2020) explicitly noted that local vector control agencies should focus their limited resources on entomological surveillance and vector population reduction, as these efforts have a more direct impact on reducing disease risk. Virus testing in mosquitoes is only justified when there is a credible public health signal, such as confirmed or suspected local human dengue cases, clusters of travel-associated infections in nearby areas, or during an outbreak investigation. While the CDC guidance (CDC 2020) does not strongly advise routine mosquito testing for dengue, there are compelling reasons to reconsider this position in regions with high numbers of travel-associated dengue cases and favorable environmental conditions for *Aedes* vector mosquitoes. Travel-related importations have repeatedly served as the seed for local outbreaks once competent vectors and suitable weather patterns align.

In practice, there is no fixed numerical threshold of travel-associated dengue cases that warrants mosquito testing; however, several US states, including Florida, Texas, and California, have initiated *Aedes* mosquito testing following detection of even a few imported cases (CDC 2025a), particularly when local vector abundance and climatic conditions favor transmission—supporting a risk-based, context-specific approach to entomological surveillance. Routine mosquito testing in such settings could provide an early warning system (Kao et al. 2016)—detecting silent viral circulation before human cases are identified. This is particularly crucial given that a large proportion of dengue infections are asymptomatic or mild, allowing the virus to spread undetected. Integrating viral testing into mosquito surveillance during peak mosquito seasons could enhance data-driven awareness, guide vector control programs, and help prevent outbreaks before they occur. Therefore, even in the absence of confirmed human cases, proactive mosquito testing in areas with high travel associated prevalence and receptive settings should be viewed as a preventive surveillance investment, not a diversion of resources.

It is indeed important to recognize that resource limitations and competing surveillance priorities are key considerations for local vector control programs. Nonetheless, strengthening mosquito-based dengue testing whenever logistical capacity permits —particularly in areas with vector abundance — is essential, as it provides a critical early warning mechanism for identifying focal transmission and informing timely response efforts. When resource constraints limit implementation approach in some vector control agencies, targeted and collaborative approaches can maximize efficiency and reduce long-term public health and economic costs. Strengthening molecular surveillance, optimizing operational programs, and analyzing public health datasets are necessary to refine mosquito testing protocols and ensure their sustainability. Given the expanding geographic range of *Aedes* mosquitoes and the growing incidence of travel-associated dengue cases, states with suitable climates and well-established vector populations need to prioritize and enhance mosquito and molecular surveillance programs.

In conclusion, testing mosquitoes for dengue virus in low-transmission or epidemic-prone areas is an essential component of proactive disease surveillance and control. While ecological differences—such as lower mosquito abundance, reduced human–vector contact, and environmental factors like temperature and humidity—may limit the likelihood of sustained transmission in non-endemic regions, lessons from endemic countries demonstrate that asymptomatic infections and silent circulation can still facilitate virus introduction and spread. Integrated with human case data, environmental monitoring, and regional coordination, mosquito surveillance helps optimize resource deployment and may aid prevent larger outbreaks with far higher public health and economic costs.

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