D4.1 Evaluating citizen-based surveillance and pathogen sampling strategies for early warning systems

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R=Document, report; **DEM**=Demonstrator, pilot, prototype; **DEC**=website, patent fillings, videos, etc.; **OTHER**=other
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Executive Summary

This report examines strategies for mosquito vector surveillance and pathogen sampling that involve participation by citizen scientists or other members of the public. It evaluates the extent to which citizen scientists can successfully participate directly in pathogen sampling, as well as how citizen science data on vectors can be used to improve pathogen sampling strategies, proposing ways in which citizen science and pathogen sampling tools can be efficiently used in combination as part of early warning systems. It concludes that public participation in these forms has the potential to improve early warning systems.

Project overview

As our planet heats up due to climate change, outbreaks of zoonotic diseases are increasing and expanding to new parts of the world, in particular Europe. IDAlert aims to tackle the emergence and transmission of zoonotic pathogens by developing novel indicators, innovative early warning systems and efficient tools for decision-makers, and by evaluating adaptation and mitigation strategies to build a Europe that is more resilient to emerging health threats.

IDAlert is a five-year Research and Innovation Action (RIA) project coordinated by Umeå University (Sweden). The consortium comprises 19 organisations from Sweden, Germany, France, Spain, Greece, The Netherlands, Italy, UK, and Bangladesh, with world leading experts in a wide range of disciplines including zoonoses, infectious disease epidemiology, social sciences, artificial intelligence, environmental economics, and environmental and climate sciences.

1. Introduction

The increasing emergence and re-emergence of arboviruses such as Usutu virus (USUV), West Nile virus (WNV), and Sindbis virus (SINV) highlights the urgent need for effective early warning systems to protect public and wildlife health. Surveillance strategies capable of rapidly detecting and monitoring these pathogens are essential to mitigating their impacts (1,2). This report examines strategies for mosquito vector surveillance and pathogen sampling that involve participation by citizen scientists or other members of the public. It evaluates the extent to which citizen scientists can successfully participate directly in pathogen sampling, as well as how citizen science data on vectors can be used to improve pathogen sampling strategies, proposing ways in which citizen science and pathogen sampling tools can be efficiently used in combination as part of early warning systems.

We start by focusing on arbovirus surveillance in the Netherlands, where work is being done using honey dipped FTA cards as a method for enable citizen scientists to participate directly in pathogen sampling. We then examine how citizen science is being used across multiple study sites to monitor vector mosquito populations, and how this surveillance could be leveraged to guide pathogen sampling. We conclude that public participation in both aspects of arbovirus surveillance can be an effective and valuable way to improve early warning systems.

2. Advancing arbovirus surveillance in the Netherlands: Field evaluation of FTA cards and citizen science integration

This section evaluates citizen-based surveillance and pathogen sampling strategies designed to enhance existing early warning systems in the Netherlands. Specifically, we focus on the field deployment of honey-dipped FTA cards. FTA cards are cotton-based cellulose paper used for collecting mosquito saliva and excreta. Along with other cotton-based cellulose paper devices, these cards offer a novel approach for improving arbovirus detection by facilitating collection and transport of nucleic acids material at room temperature with no impact on the quality of the samples (3). In recent years FTA cards dipped in honey have been successfully used inside mosquito traps to support arbovirus surveillance in the field where the cards have proven useful for detecting USUV, WNV, DENV and ZIKV (1,3-5).

Here we show that, by leveraging citizen science networks involved in bird and mosquito monitoring in The Netherlands (6,7) and accessible methods such as postal services, these strategies offer scalable solutions to increase surveillance capacity, reduce logistical constraints, and provide timely responses during arbovirus outbreaks.

The findings presented in this report contribute to understanding the practicality and effectiveness of integrating innovative sampling tools with community-based initiatives. This evaluation also highlights the potential for such methods to complement traditional mosquito surveillance frameworks, ultimately supporting proactive public health measures.

2.1 Objectives of the field testing and citizen scientist integration

- Enhance arbovirus surveillance in the Netherlands by incorporation of novel honey-dipped cards onto mosquito sampling and integration with a citizen science network committed to bird and mosquito national surveillance.
- Assess the suitability of FTA cards for mosquito saliva and excreta sample preservation and downstream analyses (e.g., species identification, pathogen detection, sequencing).

2.2 Methods

Citizen scientist participants

In the Netherlands, the Vogeltrekstation (Dutch Centre for Avian Migration and Demography) count with a citizen scientist network of hundreds of well-trained volunteers that regularly assisted in ringing wild birds for their monitoring (bird counts and overall ecological status). In addition to their ecologic activities, this network of highly skilled bird ringer volunteers has been essential in the detection and monitoring of arbovirus circulation among birds by carrying out comprehensive active bird sampling for the arbovirus surveillance schema (6,7). Furthermore, since 2020, volunteer ringers at strategic bird sampling locations in different provinces have been working on mosquito monitoring as well, enabling a national wide mosquito sampling during the arbovirus season (June-August).

In this study, a total of thirteen citizen scientists were included in the field evaluation at their designated bird ringing locations across the Netherlands in addition to nine locations in Rotterdam (Figure 1). Participants received a structured document with the protocol for assembling and disassembling the BG-PRO mosquito traps (Biogents, Germany) as well as the materials for the duration of the field test (17 weeks). In addition, multimedia tutorial with instructions were made available to all participants.

Figure 1. Relative location of study sites and citizen science bird ringing sites.

Honey-dipped cards

FTA cards (3cmx3cm) with honey-water 3:1 ratio (0.3g/10mL blue dye) were prepared, frozen and distributed to designated bird ring locations across the Netherlands where surveillance for mosquitoes and arboviruses has been conducted over the past eight years. The Limit of detection of arboviruses (USUV and SINV) in the honey-baited cards were verified in house prior the shipment to the sampling locations. The new sample matrix was incorporated into ongoing weekly sampling and subsequent testing of bird samples, whole genome amplification and sequencing, aiming for consensus genome generation and molecular identification / typing (Figure 2).

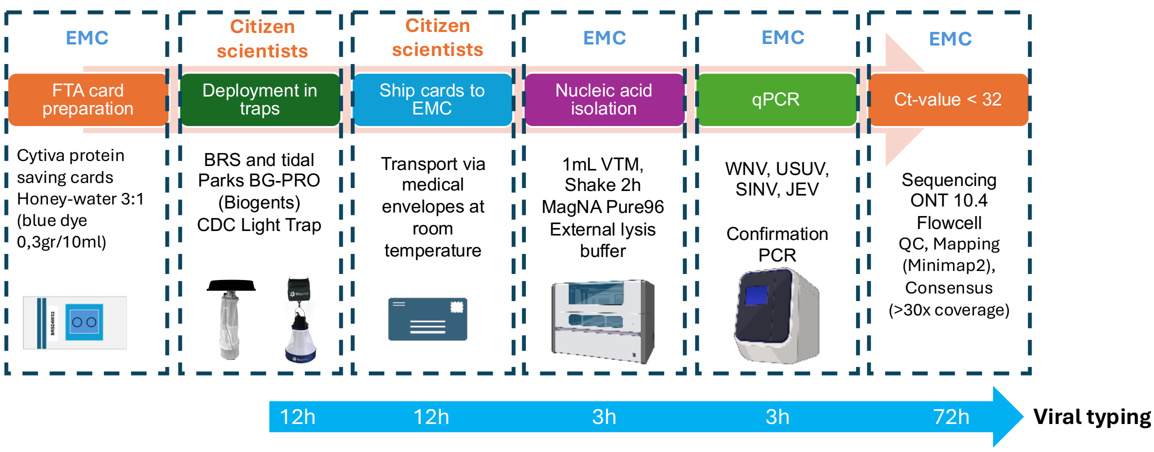

Figure 2. Experimental design of the field evaluation of honey baited cards.

The field test evaluation

The study was conducted in 2024 across 24 locations in the Netherlands from weeks 23-39, including a bird-ringing network with citizen scientists (n=15) and a prospective mosquito surveillance study in the City of Rotterdam (n=9). Mosquito trapping was conducted over a 12/24h period using CO2-baited BG-Pro traps (Bio-gents AG, Regensburg, Germany), FTA cards were placed inside the traps (Figure 3). Next, participating birders returned the cards via postal services. Weekly testing of FTA cards for arbovirus detection was carried out at Erasmus MC Rotterdam. Later, mosquitos were collected, sorted, identified and their feeding status determined and tested for arbovirus.

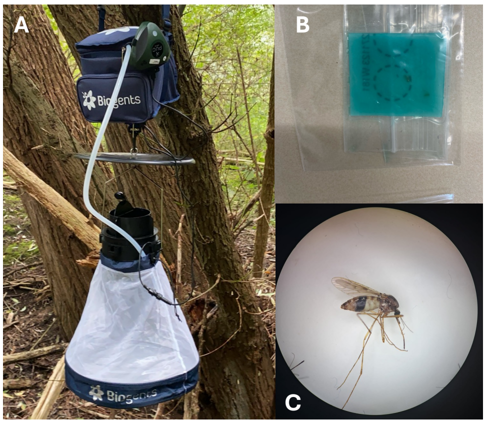

Figure 3. Field deployment of honey baited cards. Mosquito trap, B. Honey baited card, C. Female mosquito honey and blood fed during sorting and identification.

2.3 Results of field evaluation

Rapid integration of developed strategies onto surveillance initiatives

The rapid integration of developed strategies into surveillance initiatives proved highly effective. Citizen scientists quickly adopted the newly prepared collection cards, achieving a return rate of 60–100% within the expected timeframe. Incorporating this new method had minimal impact on the participants' work schedules during active surveillance.

Feedback from citizen scientists indicated only minor challenges, such as occasionally forgetting to remove cards from the freezer before heading to the field and minor handling issues with the cards. Importantly, the deployment and collection of traps, as well as mosquito handling, were unaffected. After collection, mosquitoes were stored at -20°C, while FTA cards were quickly collected and shipped for analysis, ensuring the integrity of both specimen types until processing.

Fast detection of arboviruses in FTA cards

A total of 287 FTA cards were collected during the study. Among the mosquitoes sampled, 38% fed on the honey bait, 62% did not feed, and around 4% were blood-fed. Our laboratory testing revealed 19 positive FTA cards, detecting either USUV or SINV, with one card showing evidence of both viruses (Figure 4).

Figure 4. Relative location of detected arboviruses among study sites. 24 different locations at eight different provinces in the Netherlands.

Mosquito pools associated with USUV-positive FTA cards matched findings for *Culex pipiens / torrentium* species pools. However, SINV was detected only on FTA cards, which were particularly effective for sequencing, with Ct values ranging from 23 to 36. Mosquito pools proved more sensitive than FTA cards for detecting USUV.
Sequencing showed that SINV clustered within clade A genotype I (Figure 5) but differed from the strain identified in 2022 in the Netherlands in a wild bird (8).

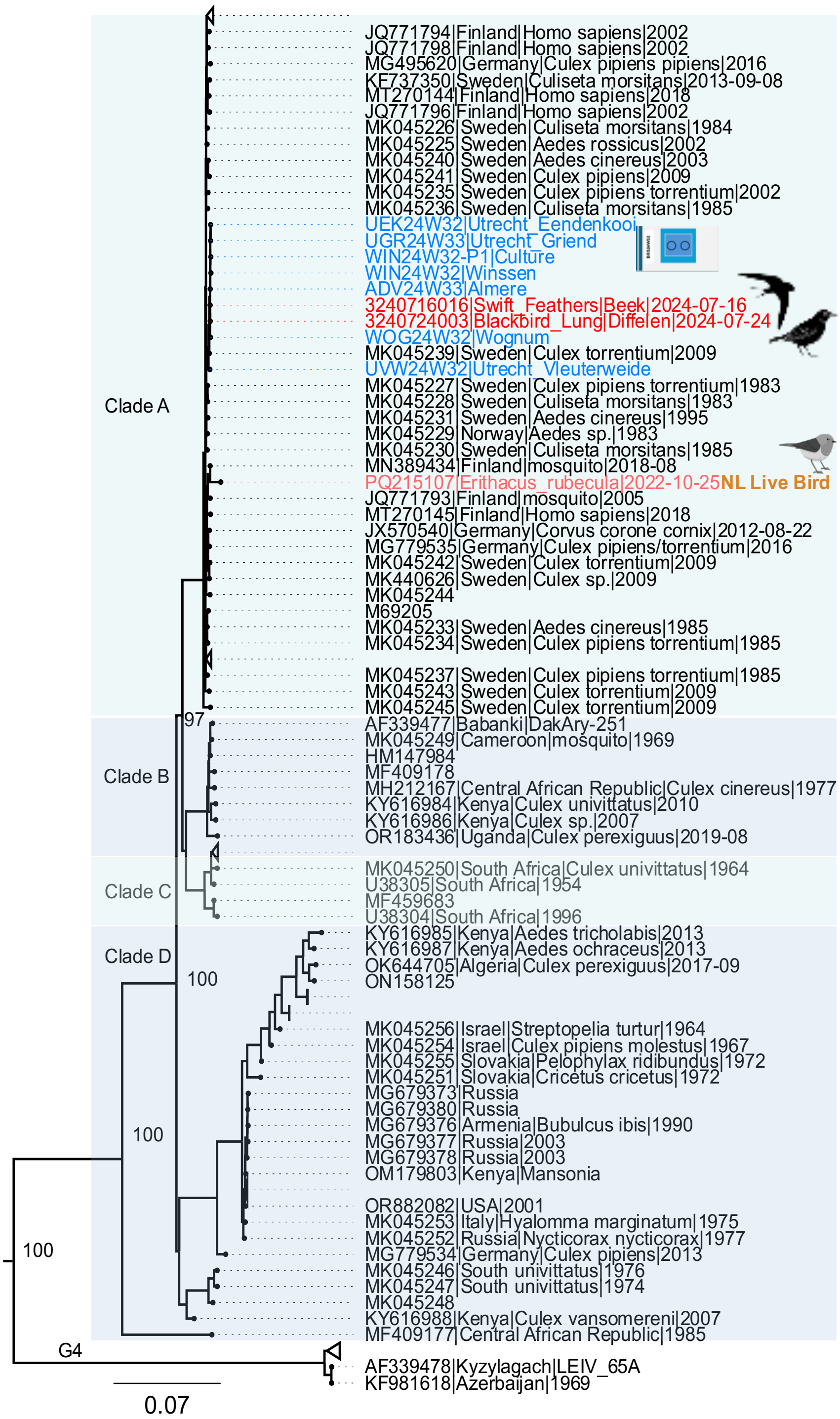

Figure 5. Phylogenetic reconstruction of SINV recovered genomes. In blue, two SINV near complete genomes (ADV24W33 [92%], WIN24W32-P1 [95%]) and five partial genomes (range 40-61% genome coverage) recovered from FTA cards. In red, two SINV near complete genomes recovered from dead birds in 2024 (Week 29-30) in the Netherlands (submitted by Dutch Wildlife Health Centre) and one partial CDS sequence (PQ215107) recovered from a European Robin from the live bird surveillance in 2022 (Streng *et al*, 2024)

The spatial distribution of SINV detections indicated widespread circulation during the sampling period, pointing to a continuous outbreak (Figure 4). The findings highlight FTA cards as a cost-effective and straightforward tool that complements existing monitoring systems. Their use could enhance the speed and efficiency of arbovirus detection, providing near-real-time insights critical for public health responses.

Molecular surveillance from bird and mosquitoes

In addition to molecular analysis on novel FTA-cards, mosquito and bird samples were processed according to our surveillance scheme. Samples USUV positive confirmed (Ct-value <32) were selected for whole genome amplification and subsequent sequencing. Phylogenetic analysis of recovered genomes from 2024 revealed circulation of mostly USUV lineages of Africa 3 in the Netherlands and a single Europe 3 genome (Figure 6). Mosquito recovered genomes describe local transmission of Africa 3 which have been consistently circulating and with increased frequency since 2022, in contrast to previous co-circulation of both Africa 3 and Europe 3 (9).

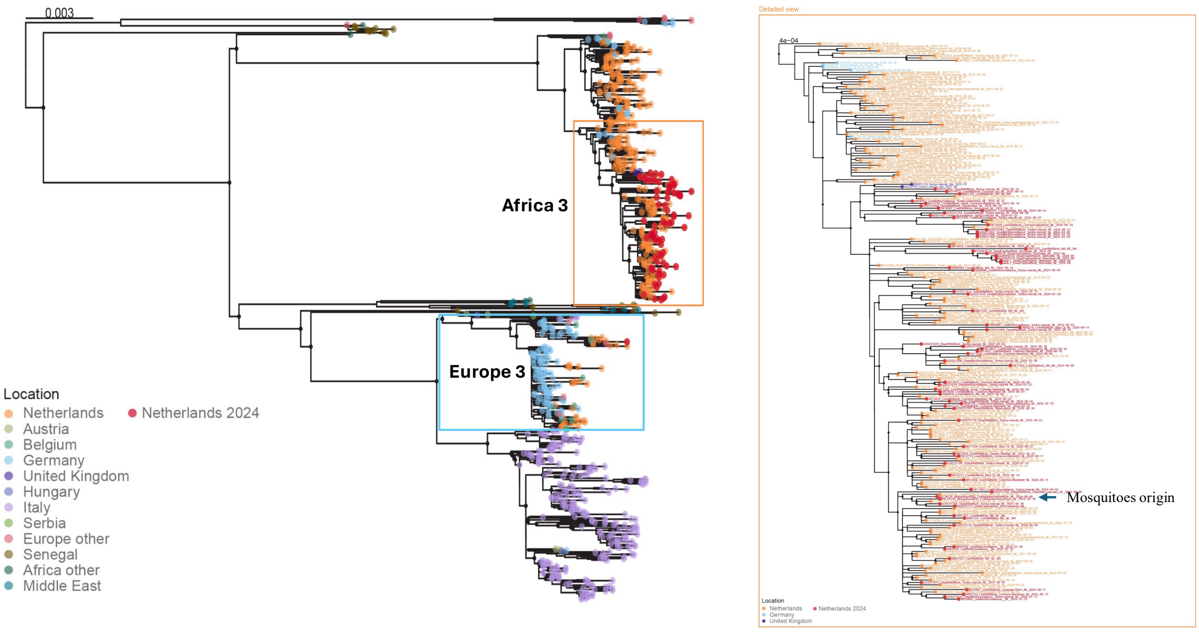

Figure 6. Phylogenetic reconstruction of USUV genomes. Analysis of 57 USUV genomes up to October 2024 including dead wild birds (n=18), captive birds (n=7), live birds (n=30), mosquitoes (n=2).

Improving the time to result and preparedness

Active bird and mosquito monitoring carried out simultaneously across the Netherlands by the volunteer network, provide an extensive framework that aid to map the circulation of arboviruses. By monitoring birds on a bigger network across the country, we can sense host populations with higher mobility (geographically) and seasonal behavior. Thus, sampling and testing birds inform about introduction and circulation of viruses among resident or migratory species. However, determining the origin of the circulating viruses relies on molecular epidemiology and cannot always inform on where the infection of the host (birds) may have occurred. Conversely, by sampling mosquitoes simultaneously, we can determine active local transmission of arboviruses by detection of virus particles among mosquito population.

Since 2020, the active mosquito sampling has been included in the surveillance network and carried by the previously mentioned volunteer ringers. However, confirmation of local transmission by mosquito testing requires a stepwise approach that have included sample collection by the citizen scientist, storage at -20ºC, followed by transport, sorting, identification and mosquito pooling at dedicated entomology laboratory followed by subsequent transport for testing at the reference laboratory. Therefore, this approach although appropriate and continue as gold standard for molecular testing of mosquitoes, is labor intensive and time consuming.

The introduction of honey-baited cards as a new sampling matrix offers significant improvements. This method offers results and typing within two weeks, significantly enhancing the capacity for rapid detection and response during outbreaks. Additionally, this field evaluation has strengthened the skills of citizen scientists, equipping them to conduct the sampling process effectively while providing valuable insights into the logistics of mosquito collection.

In summary, we tested a comprehensive system that integrates rapid arbovirus detection, response, and management with capacity-building among citizen scientists. This dual approach not only accelerates outbreak responses but also empowers local communities to contribute to public health initiatives.

Increasing public engagement and citizen science participation in Rotterdam

Volunteer birders participation has increased over the last year in Rotterdam. In addition to participants from the knowledge center for the distribution and trend of the wild bird in the Netherlands (SOVON) we have seen an increase of local residents and bird enthusiasts. These volunteers have been instrumental in collecting bird samples at our sentinel sites in the Blijdorp Zoo and the Essenburgpark during our bi-weekly sampling and testing of wild birds, as well as for bird observations (counting) around our Rotterdam tidal parks field sites (WP5).

Furthermore, seeking public engagement, in 2024 we launched a flyer campaign with QR codes and social media promotion to invite residents from urban areas near our study sites in Rotterdam to participate through the Mosquito Alert citizen science system. As described further below, Mosquito Alert facilitates public engagement in vector mosquito surveillance and this can potentially be used to guide pathogen sampling in the future.

Figure 7. Flyer campaign for Mosquito Alert App in Rotterdam during 2024.

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3. Mosquito Alert: Harnessing Citizen Science for Vector Surveillance

Mosquito Alert is a citizen science system that enables members of the public to participate in vector mosquito surveillance using a simple mobile phone application linked to a powerful set of tools for making their contributions useful for public health management, research, and education (1–3). Participants can report adult vector mosquitoes as well as their breeding sites. They can also report mosquito bites, indicating the part of their body on which they were bittern and the approximate time of day, both of which are useful for inferring mosquito species. Reports of adult mosquitoes include photographs which are classified by species by volunteer entomologists through an online Mosquito Alert platform called the Digital Entolab (1). The system also increasingly employs an automated image recognition system for providing quicker feedback to participants and alerting relevant stakeholders when vector species of interest are spotted in areas where they were previously not known to be present (1,4,5).

The Mosquito Alert system provides information directly to local, regional, and national public health agencies so that they can take action based on validated information provided by participants. Over the past decade it has been used to detect the spread of key vector species across Europe, triggering public health responses (2,5–7). These early warnings of new vector presence could make the system useful for guiding pathogen sampling decisions. In addition, data from Mosquito Alert is used to model vector population distributions in space and time. These models can help make decisions about where human-mosquito interaction risks are greatest, or simply where vector population density appears to be highest – both of which could be useful in planning pathogen surveillance.

3.1 Recruiting Participants

Citizen science is powerful, in part, because of its scalability. Whereas traditional vector surveillance relies heavily on labor intensive and costly trapping by entomologists and field workers – often requiring time lags for trapping periods (e.g. 1 week) and then lab identification of catches – citizen science offers the possibility of obtaining information about vector species anywhere that there are people present to report them (2). But this requires recruiting these people to participate. One of the main challenges in any citizen science approach to vector surveillance is simply this recruitment and engagement process (8).

In the case of Mosquito Alert, there has been a clear increase in participation since the system was first launched in 2014. This has been driven partly by improvements in the dissemination process and improvements in the app design and functionality. It has also been driven by geographic expansion, from the system's initial focus on Spain into many other countries. That has been done through translating the app into multiple languages and, most importantly, collaborating with teams of interested scientists, public health stakeholders and others who take responsibility for disseminating the system, validating photographs through the Digital Entolab, and making sure the information is useful for local public health authorities (5). This process has been expanded through IDAlert, focusing on study sites in Spain, the Netherlands, Greece, Germany, and Bangladesh.

One way of measuring participation is by the number of times people have "registered" with Mosquito Alert. Registration is defined as transmitting consent to participate in the project, which is done through the Mosquito Alert app when the participant first install is. (Once a person installs the app, they must provide informed consent before they are able to use it, and this consent is transmitted to the Mosquito Alert server.) The number of registrations is not an exact measure of the number of participants, since some people may end up registering multiple times. This happens if they install the app, consent, then delete it and install and consent again, or when an app upgrade has required a change in the anonymous participant identifiers. With those caveats in mind, Mosquito Alert has registered a total of 464,393 participant registration to date since it launched in 2014. Figures 1 and 2 show the time series of daily and cumulative registrations to date.

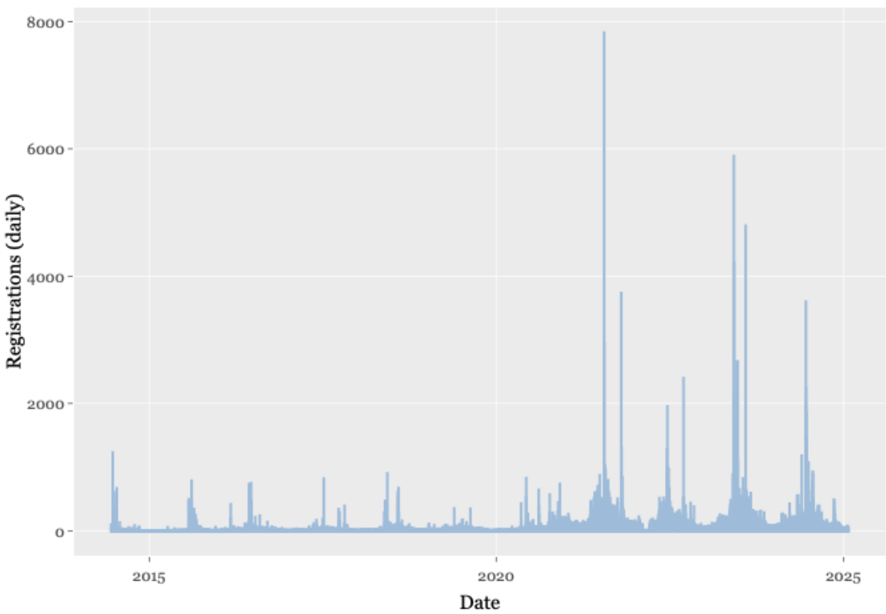

Figure 1: Daily Mosquito Alert registrations worldwide, 2014-present.

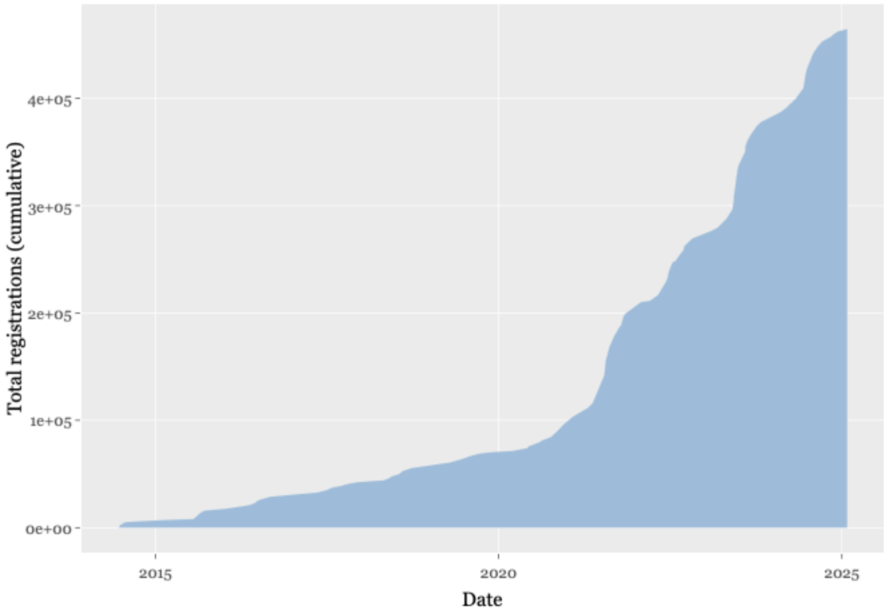

Figure 2: Cumulative Mosquito Alert registrations worldwide, 2014-present.

Simply registering to participate, of course, does not guarantee that people will actually report mosquitoes. Indeed, only a fraction of those who register ever transmit a report through the system. Still, that fraction has been enough to provide valuable insights into vector mosquito spreading and dynamics. To date Mosquito Alert has received a total of 257,240 reports from 82,655 participants in 192 countries. Of these, 138,534 (53.85%) are reports of adult mosquitoes, 25,733 (10%) are reports of mosquito breeding sites, and 92,973 (36.14%) are reports of mosquito bites. Mosquito Alert has received a total of 126,435 reports containing photographs, representing 76.97% of the total adult and site reports combined (these are the report types to which photographs can be attached). Of these, 100,229 are adult reports (72.35% of the total adult reports) and 25015 are site reports (97.21% of the total site reports). Of the 100,229 adult reports with photos, 90,929 (90.72%) have been validated through the Digital Entolab system to date. The latest statistics on participation are available at https://labs.mosquitoalert.com/participation/.

3.2 Accounting for Sampling Bias

In addition to recruitment, making a citizen science system like Mosquito Alert useful for vector or pathogen surveillance and research also requires accounting for sampling bias. Sampling bias arises from the uneven distribution of participants and of participant attention/surveillance across geographic space and across time. There are simply some locations and some times of day, of the week, or of the year in which there are more participants or in which participants are more likely to notice and report a mosquito if it is present. This can be more or less important depending on how the system is being used. For simply detecting the presence of vector species in new areas, any detection is useful whether or not it was more likely to happen in one given area than in another due to higher levels of sampling. Even in that case, however, it is important to be aware of the locations and times in which low sampling is occurring so that one does not assume a lack of detections is due to a lack of vector presence. More complicated, however, is the case of modeling vector distributions – something that could be valuable for making pathogen sampling decisions. Here, it is very important to account for sampling effort to reduce bias from model estimates.

In the case of Mosquito Alert, this is done in two ways. First, the app itself includes optional anonymous background tracking to collect information about approximately where participants are located each day. Specifically, the app detects the device's location 5 times per day, it masks this location on a grid of 0.025 degrees longitude and latitude, referred to as sampling cells, and it transmits this information to the system's server with a randomly generated user UUID. The server processes this information using a model of participant reporting propensity that shows propensity to drop off over time after participants initially install the app. The server automatically aggregates the total number of participants and their reporting propensities for each sampling cell day as a cumulative probability of at least one reporting being sent from the cell on each given day, and as an expected number of participants reporting from that cell on each given day. Figure 3 shows the distribution of total sampling effort in these cells from 2014 to present (combined).

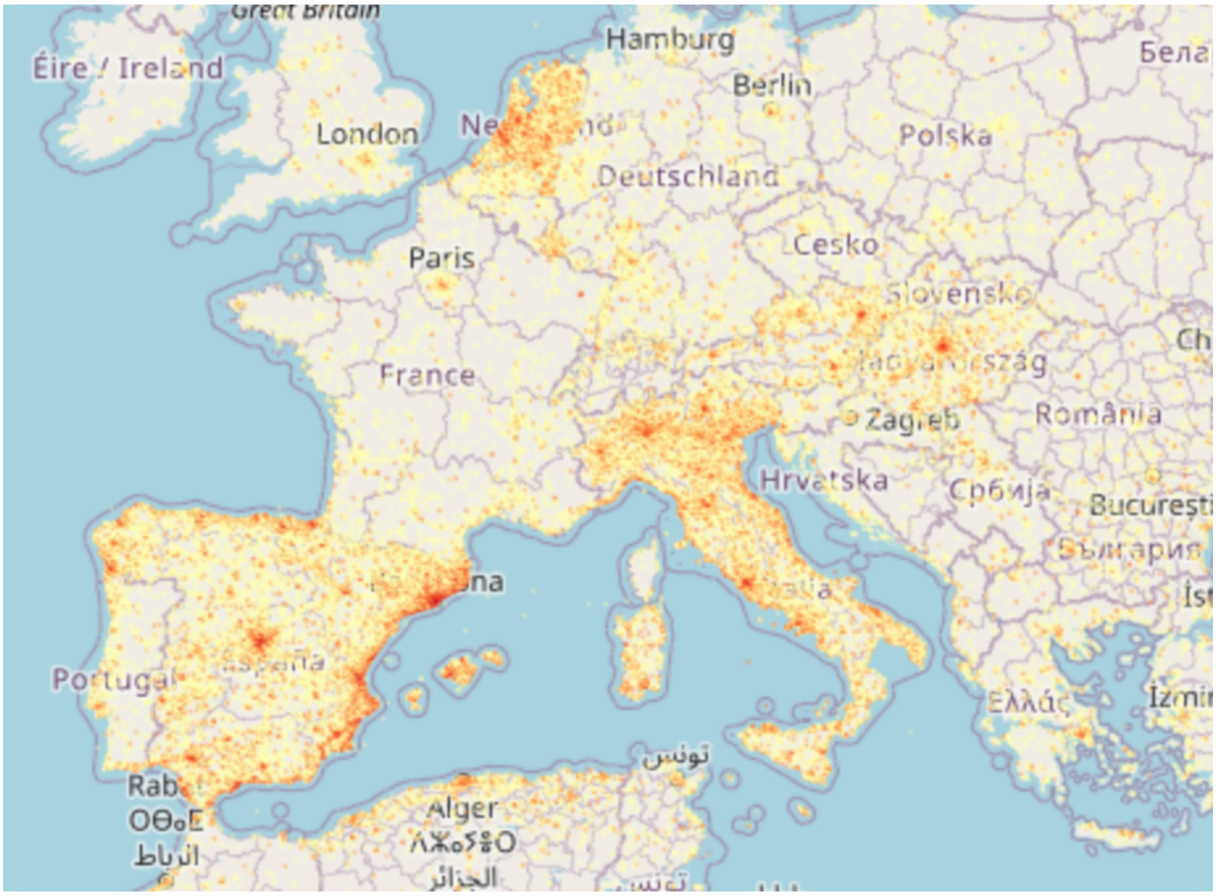

Figure 3: Mosquito Alert sampling effort based on aggregated, anonymous, optional background tracks. Higher effort indicated shown by darker red cells.

This approach to sampling effort estimation has proved useful for reducing sampling bias in large scale models of vector mosquito distribution (2). For smaller scale decision-making, however, one concern could be over variation in sampling effort below the scale of the 0.25 degree sampling cells. This was recently explored for Barcelona using ground-truth data on mosquito presence from the Barcelona Public Health Agency to estimate the probability of Mosquito Alert reporting as a function of census tract socio-economic characteristics (9). The method developed in that work is now being used to improve estimates of *Ae. albopictus* population distribution at high resolution in Barcelona. Figure 4 shows estimates of the total sampling effort in each of Barcelona's census tracts for 2023, represented as the probability of at least one report being sent from the tract if the species were present there.

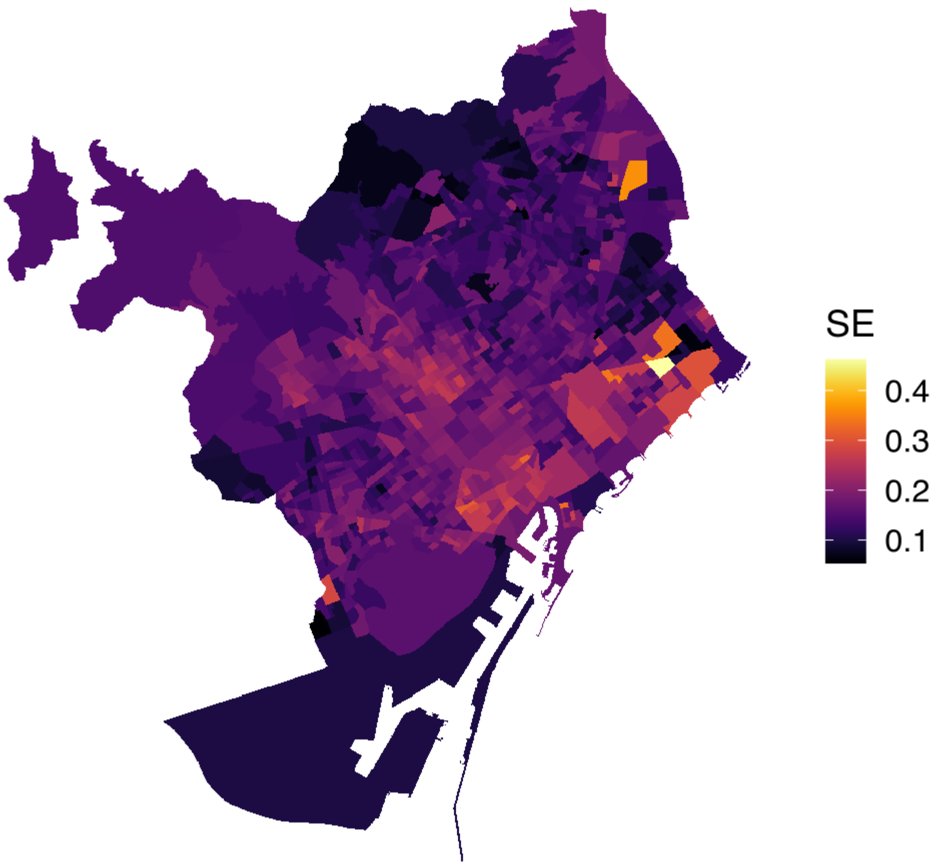

Figure 4: Estimated sampling effort at the census tract level in Barcelona for 2023.

3.3 Modeling Vector Population Distributions

Based on these corrections, we have been building and improving models of vector mosquitos from Mosquito Alert data at different scales. Figure 5 shows model estimates of the probability of encountering *Ae. albopictus* in each of the municipalities in the region surrounding Barcelona on a specific day in 2024 (10 September). Figure 6 shows this probability across Barcelona at 20 m resolution for all of 2023. In both cases, we account for sampling effort and for the information the actual geographic distribution of the species as well as for daily temperature and relative humidity, and for land cover.

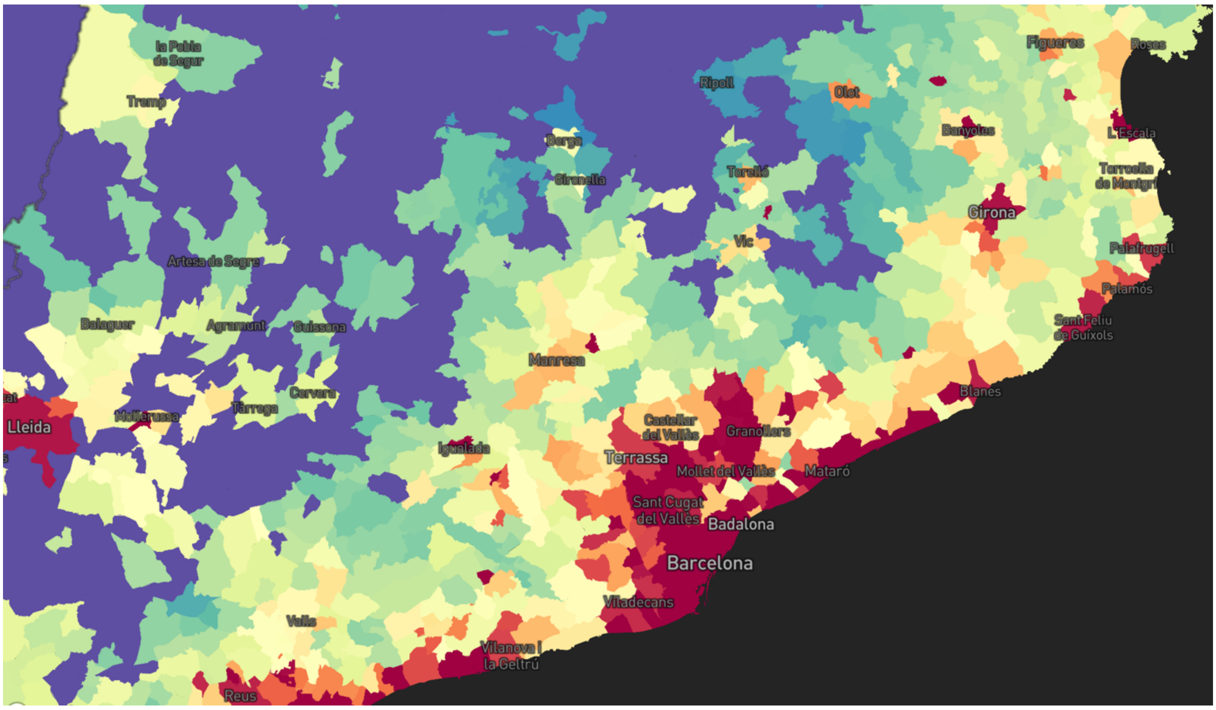

Figure 5: Municipality-level estimates of *Ae. albopictus* encounter probabilities across the region surrounding Barcelona on 10 September 2024 based on Mosquito Alert data modelled with land cover and weather variables and accounting for sampling bias. Reds indicate higher probability; blues lower probability.

These models offer important insights into the distribution of *Ae. albopictus* that can be used not only to make decisions about vector control, but to also plan pathogen sampling. *Ae. albopictus* is considered to be established in all of the broad areas shown in both figures, but there is clearly variation in its population density. The map in Figure 5 suggests municipalities that might be usefully targeted for pathogen sampling, and that in Figure 6 offers guidance as to where within one of those municipalities (Barcelona) this sampling could be focused based on (a) probability of finding the vectors, and/or (b) risk of human-vector encounters.

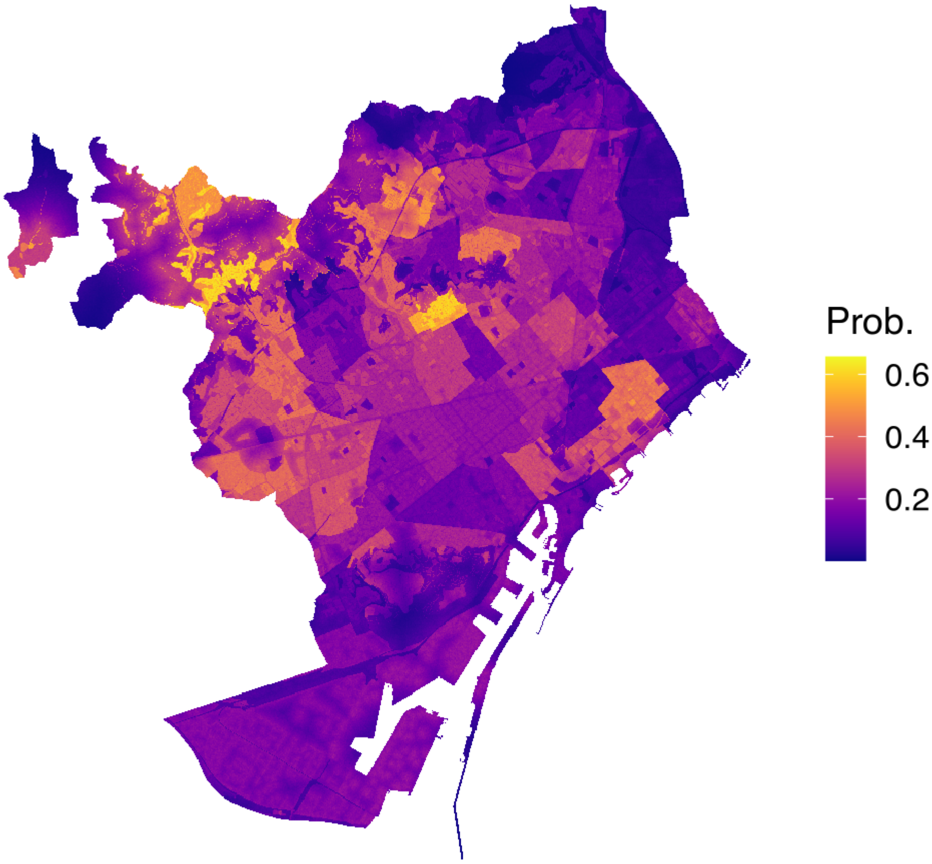

Figure 6: Probability of *Ae. albopictus* being encountered in Barcelona during 2023 at 20 m resolution, based on Mosquito Alert data modelled with weather and landcover variables, and accounting for sampling bias.

3.4 Limitations

There are a number of limitations that should be considered in evaluating the potential for citizen science vector surveillance to play a useful role in pathogen sampling. Some are the specific challenges already discussed: recruitment and sampling bias. Although we have found ways of meeting these challenges that appear to be working well in Barcelona, and to some extent throughout Spain and across Europe, there are important ways in which they continue to be of concern. Recruitment has been highly uneven, even across the countries in which substantial efforts have been focused, and engagement tends to drop off quickly even once people initially volunteer. Similarly, sampling effort estimates in Spain and, at high resolution in Barcelona, appear to be helping to correct for sampling bias based on comparisons between Mosquito Alert estimates and data from other sources. However, there is a lot of uncertainty in these models, cross validation with other data sources is difficult because there few true ground-truth sources of information on vector distribution, and the complexity of human behavior means that what works well at one point in time or in one area, may not work in others.

All of this suggests that citizen science should never be relied on as the sole tool for surveillance. It can be extremely useful but only when used in combination with other tools, including traditional vector surveillance. This holds true also for pathogen sampling. Moreover, the analysis of potential use for pathogen sampling presented in this section is still at an early stage and should be complimented with actual field tests.

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4. Conclusions

This report has described the testing of a comprehensive system in the Netherlands that uses FTA cards to integrate rapid arbovirus detection, response, and management with capacity-building among citizen scientists. This dual approach not only accelerates outbreak responses but also empowers local communities to contribute to public health initiatives. We have also shown how citizen science is being used for mosquito vector surveillance at different scales through the Mosquito Alert system. The extension of this type of surveillance for use in pathogen sampling appears possible, although we have also identified a number of limitations that should be considered, particularly related to participant recruitment and sampling bias. Citizen science has huge potential for improving early warning systems but this potential lies in its use in combination with other tools, including traditional vector surveillance.