

AMCARF Project Status Report

This report will be used to communicate the progress, successes, and challenges of your AMCARF Funded project. The report will be used by the Review Committee to assess overall productivity of the project and its continued alignment with the AMCARF objectives. It will also be used to make future prioritizations of research and development efforts.

The report should not exceed 8 pages of text. Please include any additional attachments with key figures that may highlight critical findings. Use the following headings to build your report:

Individual filling out this form: Kristy Burkhalter

Email: ktb3@cdc.gov

Report Type:

<input checked="" type="checkbox"/>	Report Type	Report Deadline
	Progress Report for all <u>2018</u> awardees (including multi-year)	Monday, July 16, 2018
	Progress Report for <u>2018</u> awardees of multi-year projects requesting an additional year of funding	Monday, September 17, 2018
<input checked="" type="checkbox"/>	Annual Report for all <u>2018</u> awardees (including multi-year)	Friday, December 14, 2018

Project Title: Field evaluation of arbovirus detection using honey cards in California

AMCARF project number: 2018-02

Project Cost: \$7878.40

Project Leader: Kristy Burkhalter

Collaborators: (Include cooperating laboratories and AMCARF supported personnel and percent effort)

Placer Mosquito and Vector Control District (Jake Hartle, Tim Garner (seasonal to trap/sort; supported 50% by AMCARF funds), Joel Buettner, Phil Spinks)

Project Objectives: (Bullets as stated in the approved proposal. Add new or modified objectives.)

- Evaluate the utility of the honey card method to replace or supplement traditional pool-based arbovirus surveillance.
- Determine an appropriate algorithm for using honey card data for operational use.
- Field comparison of real-time RT-PCR and commercially available real time RT loop mediated amplification (LAMP) assays for WNV, SLE and WEE RNA detection in mosquito pools and honey cards.

Total Project Progress:

Key Research Accomplishments: (Bulleted list of accomplishments from this project)

- Site visit completed – protocols developed and finalized
- Mosquito and honey card collections and testing for WNV- completed

Reportable Outcomes:

Describe major outputs including for example papers, inventions filed and patents issued, or new mosquito control guidance or practices.

Describe how the project has advanced the field of mosquito control scientifically.

List Objectives and specifically describe progress tied to objectives and deliverables in the approved proposal.

Methods:

Card prep & trapping:

Prior to the launch of the field project I visited Placer MVCD to optimize protocols as described in the mid-project status report. Our selected method of preparing the cards (Fig 1) was used for the entirety of the project and worked very well. Briefly, 1 mL of a 50:50 honey:water mixture (dyed blue) was applied via plastic pipette to the bottom of a small plastic bag housing 1 FTA card to allow the mixture to wick up and saturate the card. A pre-cut hole in the side of the bag exposed ~80% of the card for mosquito feeding. The (dry) cards and bags can be prepared in advance. Immediately prior to departure from the lab to set the traps, the honey mixture is added to the bags and taped to the inside of a Fay cup. By the time traps were placed in the field, the honey mixture had saturated the card, and the cards remained moist throughout the night until collected the next day.

Six Fay-Prince traps were set at 2 sites (3 traps per site, 1 night per week) that were chosen due to their historical WNV activity. Traps were set and retrieved according to PMVCD standard procedures, and mosquitoes and cards were placed into coolers with dry ice for transport back to the lab. The plastic bags containing honey cards were placed into individual plastic bags and mosquitoes are identified and pooled as described below; all samples were stored at -80C until shipped to CDC for testing.

Mosquito pool and honey card testing:

The following method provided the best recovery of RNA from the cards during pre-trial optimization: each card was placed flat in a 35x10mm petri dish or 6-well cell culture plate, and 400 ul of Rapid RNA extraction reagent (Invitrogen) was added to saturate the entire card. After a ~20 minute incubation, the card was placed in a 10 ml syringe and the liquid was squeezed into a 1.7 mL tube. Any excess liquid that remained in the dish was aspirated and added to the tube as well. An aliquot of honey card liquid was removed for RNA extraction (Qiagen Viral RNA minikit), according to the manufacturer's protocol and tested with real time RT-PCR. Two real-time RT-PCR detection kits were used to test the cards and compared (Qiagen Quantitect Probe RT-PCR kit and Qiagen Quantifast Pathogen RT-PCR kit).

Mosquito pools were homogenized according to standard CDC procedures. Pools were extracted for viral RNA using a Qiagen BioRobot Universal, and tested for WNV using the Qiagen Quantitect Probe RT-PCR kit. All presumptive positive mosquito pools or cards were re-extracted and retested by real time RT-PCR for confirmation.

Data collection:

Each week, mosquito collection data were reported to CDC by PMVCD. In addition to the traditional collection data recorded (sex, species, site, etc), mosquitoes that fed on the cards (indicated by any amount of blue in the abdomen or thorax, Fig 2) were noted in the spreadsheet, and pooled/tested individually to aid in statistical analysis at the end of the field trial. Only WNV/SLE/WEE vector mosquitoes are pooled by PMVCD for testing; however, non-vector species data were recorded (including noting blue mosquitoes) for informational purposes.

Results:

Mosquitoes and cards were collected from epi weeks 27 – 38 for a total of 67 trap nights (accounting for a few trap failures). A total of 18,254 female *Cx. pipiens* and *Cx. tarsalis* were pooled into 1,347 pools (including blue mosquitoes pooled individually) for arbovirus screening; all have been tested for WNV. If the mosquitoes had been pooled traditionally (i.e, into pools of 1-50 regardless of feeding status), the total number of pools would have been 467. The analysis below is based on “traditional” pooling.

Of the 67 traps set, 32 traps (~48%) produced at least one positive WNV pool (n = 75 positive pools total). Each trap contained one FTA card; 12/67 traps (~18%) produced a positive WNV card. These 12 positive-card traps contained 183 “traditionally” pooled mosquito pools, which would have resulted in a 39% (183/467) reduction in mosquito pool testing if these pools were excluded from testing based on the card results. A less conservative approach would be to exclude pools from an entire site if any card in any of the multiple traps set at that site were positive. In this scenario, pool testing would have been reduced by 229 pools, or 49%. No positive cards were detected from traps that did not contain at least one positive mosquito.

Only 18% (12/67) traps produced positive cards, however these 12 traps contained 39% of the total pools tested throughout the course of the field trial. Statistical analysis and scenario modeling is in progress at the time of this report to determine an algorithm for testing cards vs. testing pools, and whether an agency would be better suited testing the pools from traps producing small pool numbers, or screening cards first when pool numbers are higher and chances of detecting a positive card are higher. Regardless, the absence of mosquito identification, pooling, and processing makes card screening a more time- and cost-efficient surveillance method regardless of the number of pools in a trap.

The 12 positive honey card results were obtained using the Qiagen Quantifast Pathogen RT-PCR kit. Only 5 of the 12 WNV+ cards were detectable using the Quantitect Probe kit (this is unsurprising as similar sensitivity results were seen during pre-field optimization of card extraction and testing methods). These results highlight the need for the most sensitive and reliable RNA detection assays available for maximum detection of viral RNA from the cards.

Because we did detect positive cards, it was interesting to note that feeding rates were fairly low (although comparable to other studies). Feeding rates of vector mosquitoes in most of the traps (n = 44 traps) were less than 11% (Fig 3). Even more surprising, of the 12 traps that produced positive cards, only 3 of those traps contained positive blue mosquitoes. The other 9 positive cards came from traps that did not contain positive blue mosquitoes, although they did contain positive (nonfed) mosquito pools. This could be explained in a couple of ways. First, if the mosquitoes did feed but took such a small honey meal that it was difficult to visualize, some “blue” mosquitoes may have been included in unfed pools inadvertently. A more likely explanation, however, is that positive mosquitoes deposited enough detectable viral RNA through probing or excretion onto the cards.

Progress Assessment:

List Milestones and assign a color. Be honest and critical of your work and solutions for overcoming challenges. If not completed list % completed.

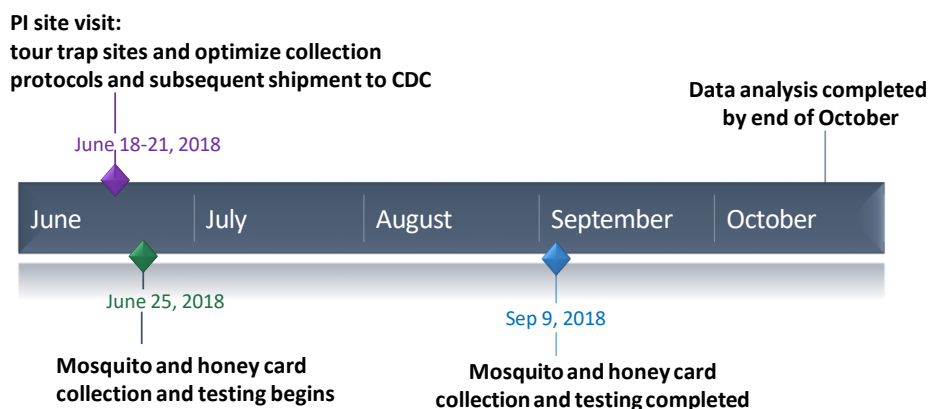
Green = on or ahead of schedule or successfully completed

Amber = slight delay but will meet all deliverables 1-6 months (specify) late

Red = major obstacles with a delay of more than 6 months, risk that key portions of the project will not be completed

Black = project was abandoned.

The following timeline and milestones were outlined in the original proposal:



All pools and cards have been tested for WNV

Pools and cards need to be tested for SLE and WEE; as well as cards screened for WNV via RT-LAMP. This will be completed by Feb 2019.

Plans for the following year: N/A

Conclusion:

This field project demonstrated that implementing honey card testing in a typical US surveillance program can supplement (but not replace) pool-based testing, and that honey card surveillance has a number of flexible applications. Honey cards can be screened while pools from the corresponding trap are sorted and identified (to determine vector abundance), and results from the cards can be returned before the mosquito sorting is complete. If the card is negative, the pools would have to be screened. If positive, however, operational decisions can be made sooner, and the pools can be discarded or retained for later testing in the week or during the off-season, to preserve historical pool-based testing data. Another application is to place honey cards in traps that typically collect more mosquitoes than can be reasonably tested by an agency.

Through this project we determined that sensitive nucleic acid detection assays are critical for the success of detecting viral RNA from honey cards, and agencies would need to optimize their testing protocols prior to the implementation of honey card surveillance. In addition, optimizing the card preparation and placement avoided some of the potential pitfalls we anticipated, including the card drying out (none of them did) and the mosquitoes not feeding on the card given only one night to feed (feeding rates were low, but not absent). Future work with this method will include testing cards placed in different types of traps, different geographical areas (with different vectors), and possibly using different sources of sugar that may prompt better feeding rates and even better card detection results.

Overall, we were very satisfied with the results of the trial. As mentioned above in the milestones sections, a few remaining items need to be completed, including screening all of the samples for SLEV and WEEV, screening the cards for WNV using a commercial RT-LAMP assay (although based on the real time RT-PCR results and the lower sensitivity of the RT-LAMP assay, it is highly unlikely this assay will be suitable for this method), and completing the statistical analysis. However, these will be completed by the 2019 AMCA meeting and included in the planned manuscript.

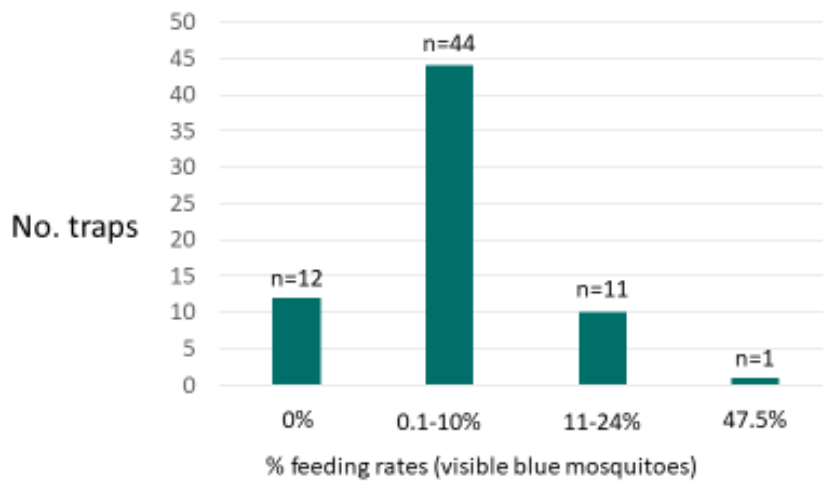
Supporting Data:

Fig 1. Honey card preparation.



Fig 2. Visualization of mosquitoes that have fed on honey cards.





Feeding rates of female vectors, by trap
Total traps = 67

Fig 3.