Comparative field analyses of rapid analyte measurement platform and reverse transcriptase polymerase chain reaction assays for West Nile virus surveillance

Eric Williges[1]✉, Ary Farajollahi[1], Mark P. Nelder, and Randy Gaugler

Center for Vector Biology, Department of Entomology, Rutgers University, 180 Jones Avenue, New Brunswick, NJ 08901, U.S.A.

[1]Mercer County Mosquito Control, 300 Scotch Road, West Trenton, NJ 08628, U.S.A.

Received 14 July 2009; Accepted 21 September 2009

ABSTRACT: Rapid detection of West Nile virus (WNV) in mosquito pools is essential for predicting epizootics and epidemics. We compare the efficiency and sensitivity of the Rapid Analyte Measurement Platform (RAMP) to reverse transcriptase polymerase chain reaction (RT-PCR) from 2005 to 2008 from field mosquito populations in Mercer County, NJ. Overall, 316 pools tested negative and 115 pools tested positive for WNV. Eighty-nine pools tested positive using RAMP and all were confirmed by RT-PCR; 26 pools were WNV-negative using RAMP but positive using RT-PCR. False-positives from RAMP were not detected in our four-year study, indicating that RAMP is a reliable tool when used to augment existing RT-PCR-based WNV surveillance programs. Local mosquito control programs using RAMP will benefit from its ease of use, quick results, and lack of false positives but should understand the sensitivity of this test when compared to RT-PCR. Used with standard methods, RAMP will enhance existing mosquito control and WNV surveillance by providing rapid results and improved mosquito management decisions. ***Journal of Vector Ecology* 34 (2): 324-328. 2009**.

Keyword Index: Arbovirus, Culicidae, mosquito, RAMP, RT-PCR, West Nile virus.

INTRODUCTION

West Nile virus (WNV) (Flaviviridae: *Flavivirus*) is a mosquito-transmitted arbovirus maintained in an avian-mosquito transmission cycle, with humans, horses, and other vertebrates as dead-end hosts. In North America, WNV was first detected during the summer of 1999 in New York City; simultaneously, crow mortality and human-encephalitis cases were reported and not attributable to a known etiological agent (Lanciotti et al. 2000). Throughout its North American range, WNV is now endemic and continues to impact human, horse, and avian populations (United States Geological Survey 2009). In northeastern North America, *Culex* species, especially *Culex pipiens* L., are the primary enzootic vectors of WNV (Marra et al. 2004, Turell et al. 2005, Molaei et al. 2006). West Nile virus surveillance is now a primary focus of mosquito control operations throughout the continent. In New Jersey, WNV testing occurs via a statewide testing and reporting system overseen by the Division of Health and Human Services. Surveillance systems are relatively slow at responding to WNV presence in vector populations due to the considerable time needed to transport mosquito samples to a central location and time required for testing (i.e., reverse transcriptase polymerase chain reaction (RT-PCR)); however, new assays are readily available for quick testing for WNV and are continuously being developed. These novel assays need to be tested under field conditions as to their efficacy and utility in mosquito control and arbovirus surveillance programs.

Accompanying the introduction of WNV to North America was the quick development of systems for detecting WNV in field-collected mosquitoes and sentinel birds (Burkhalter et al. 2006). Some of these WNV-detection assays in mosquitoes include VecTest®, One-Tube RT-PCR® kits (TITAN®, Qiagen, Valencia, CA), and monoclonal-antibody capture ELISA. However, these assays are either expensive or have a low sensitivity compared to RT-PCR (Burkhalter et al. 2006). Surveillance efforts have concentrated on WNV-RNA detection in mosquito pools and dead birds using RT-PCR (Eidson et al. 2001, White et al. 2001). The Rapid Analyte Measurement Platform (RAMP) (Response Biomedical Corp, Vancouver, B.C.) system is a modified enzyme immunoassay test for WNV detection that does not require the extensive technical laboratory expertise or costly equipment inherent to RT-PCR. It utilizes WNV-specific antibodies conjugated to fluorescent latex particles. Upon mixing a homogenized sample with the conjugated antibody complex, a portion is added to the proprietary RAMP cartridge. As this sample migrates thru the cartridge, antigen-bound particles are immobilized at the detection zone. Additional control particles are immobilized at an internal control zone. After drying, the RAMP reader measures the amount of fluorescence emitted by particles at each zone and displays a result which is a relative value reflecting the ratio between the fluorescence values at the detection and internal control zones. Previous investigations into RAMP for WNV detection in mosquito pools and corvids have shown these tests to be very reliable for indicating the presence of WNV (i.e., >94%) compared to the gold-standard RT-PCR (Burkhalter et al. 2006, Padgett et al. 2006, Sutherland and Nasci 2007). While these studies have shown the utility of RAMP for WNV detection, they have not demonstrated its usefulness

when employed for WNV surveillance in natural mosquito populations conducted by mosquito control operators. The growing availability and use of RAMP technology among mosquito control programs provided the rationale for comparing RAMP and RT-PCR to determine their efficacy for WNV detection in field-caught mosquitoes. Through a longitudinal study at the county level, we demonstrate that dual use of RAMP and RT-PCR provides faster data collection and decreases response time for mosquito control programs, limiting public health threats posed by WNV-infectious mosquito populations.

MATERIALS AND METHODS

Mosquitoes were collected daily using gravid and carbon dioxide-baited traps as part of Mercer County Mosquito Control's vector surveillance program. Traps were placed throughout the county from early May until late October of each year from 2005 to 2008. All traps were placed in the field at approximately 12:00 EDST and collected the following morning at approximately 10:00 EDST; mosquitoes were transported on dry ice and stored at -70° C. Mosquito pools (≤ 50 females) emphasized *Culex* species (Culex *pipiens* L. and *Culex restuans* Theobald) because they are the primary WNV amplification vectors in the northeastern U.S. and have the highest prevalence of WNV infections (Andreadis et al. 2004). Mosquito pools were all assayed for WNV by RAMP (positive results if reading ≥ 30) following manufacturer recommendations and then again by RT-PCR using established protocols (Lanciotti et al. 2000, Farajollahi et al. 2005). Infection rates (IR) were calculated using the Microsoft® Office Excel plug-in, PooledInfRate, available from the CDC (Biggerstaff 2006), which allowed for weekly calculations of bias-corrected maximum likelihood estimations (MLE). Values were calculated in two series: (1) using data collected from the RAMP assays without RT-PCR confirmation, and (2) using data from RT-PCR confirmation of RAMP assays.

RESULTS

We assayed 431 mosquito pools for WNV using both RAMP and RT-PCR for each pool. Overall, 316 pools tested WNV-negative and 115 pools tested WNV-positive using both systems. Eighty-nine pools tested positive using RAMP and all were confirmed by RT-PCR. An additional 26 pools were WNV-negative using RAMP but WNV-positive using RT-PCR. The time required to obtain results from mosquito assays was on average two to five h for RAMP and seven to ten days for RT-PCR. The extended time for RT-PCR testing is primarily due to the shipment of specimens to a central state laboratory and a fixed weekly schedule for testing those samples.

In 2005, RT-PCR provided the first WNV-positive

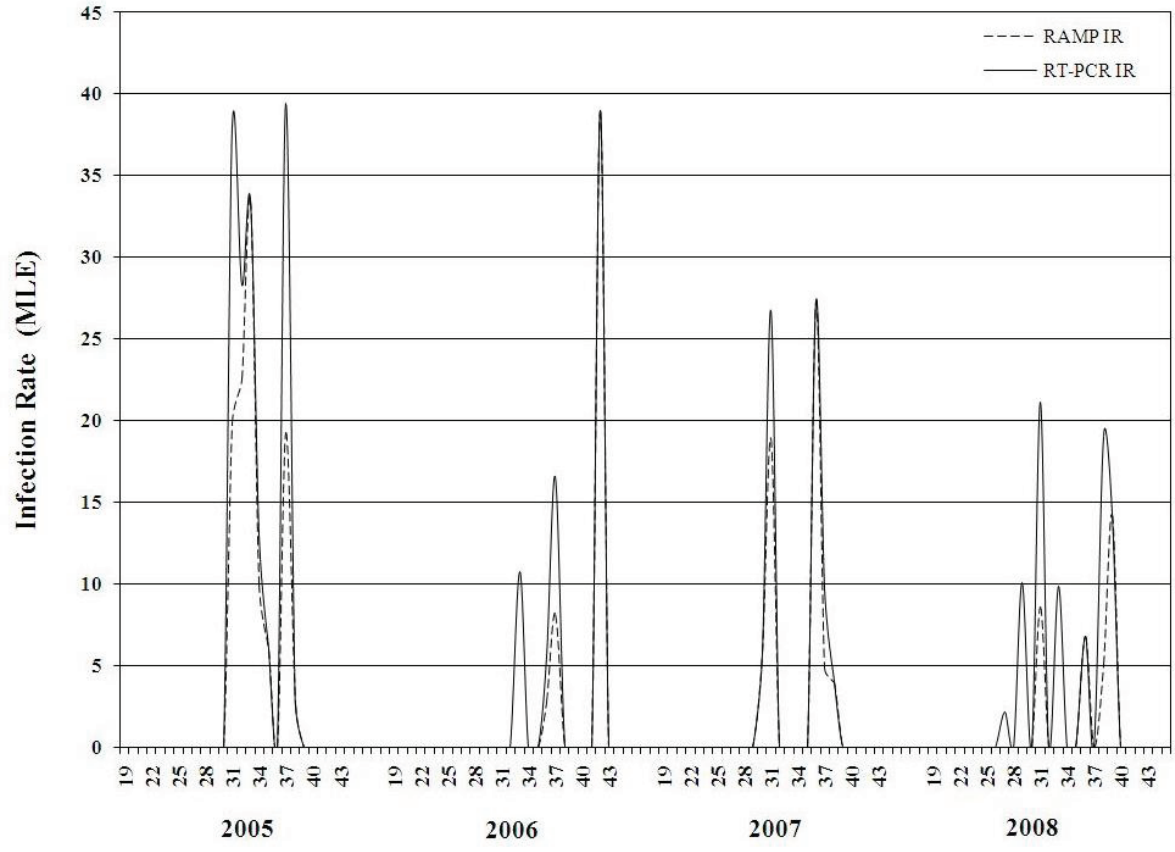

Figure 1. West Nile virus detection from mosquito pools tested by RAMP and confirmed with RT-PCR (Mercer County, NJ, 2005-2008). Infection rates were calculated per 1000 mosquitoes by using a bias-corrected maximum likelihood estimate (MLE).

pool during epiweek 31 with a infection rate (IR) peak of 39.4 during week 37 and a final positive pool in epiweek 38 (Figure 1). The first positive pool in 2005 using RAMP was also during epiweek 31 with an IR peak of 33.4 during epiweek 33 and a final positive pool in epiweek 38. In 2006, using RT-PCR the first positive pool was detected during epiweek 33 with an IR peak of 39 during epiweek 42 and the final positive pool in epiweek 42. The first positive pool in 2006 using RAMP was detected during epiweek 36 with IR peak of 39 during epiweek 42 and the final positive pool in epiweek 42. The first WNV-positive pool in 2007 using RT-PCR was in epiweek 30 with an IR peak of 27.33 in epiweek 36 and a final positive pool in epiweek 38. The first WNV-positive pool in 2007 using RAMP was in epiweek 30 with an IR peak of 27.3 in epiweek 36 and a final positive result coming in epiweek 38. Data from 2008 show the first RT-PCR positive during epiweek 27 with an IR peak of 21.1 in epiweek 31 and a final positive pool in epiweek 39. Results obtained using RAMP during 2008 show the first positive pool in epiweek 31 with an IR peak of 14.2 in epiweek 39 and the final positive pool in epiweek 39.

DISCUSSION

Using RAMP, we detected WNV in 94% of pools when compared to RT-PCR during our assays of field-caught mosquitoes. These results are consistent with laboratory studies in which RAMP assays detected 96% of WNV-positive pools when compared to RT-PCR assays (Burkhalter et al. 2006). Other studies have investigated the relationship between pool size and the presence of plaque forming units (PFU), with 100% of WNV-positive pools using RAMP when compared to RT-PCR with pool sizes of 50-200 mosquitoes with 3.3 to 4.0 $\log_{10}$ PFU/ml (Sutherland and Nasci 2007). Similarly, although WNV detection was performed using avian oral swabs, RAMP detected greater than 95% of WNV-positive pools when compared to RT-PCR (Padgett et al. 2006). Our study is novel, using field-caught mosquitoes and represented a long-term analysis of RAMP and RT-PCR use in an operational mosquito surveillance and control program.

An important consideration for mosquito control programs is the mutual sensitivity and accuracy of RAMP and RT-PCR; careful assessment and interpretation of these outcomes are important when making effective mosquito management decisions. We provide four WNV presence-absence scenarios that are possible when verifying RAMP results with RT-PCR. Two scenarios exist when both assays agree: both are either positive or negative (Figure 2). A third scenario is a negative RAMP and a positive RT-PCR result, indicating the lower sensitivity of RAMP when compared to RT-PCR (Burkhalter et al. 2006). The final scenario is that RAMP is positive and RT-PCR is negative, an undesirable outcome, indicating a false positive with the RAMP assay. False-positives from RAMP were not detected in our four-year study and our rate of false negatives was 6%, demonstrating that RAMP is a reliable tool when used to augment existing RT-PCR-based, WNV surveillance programs.

The RAMP system should be supplemental and not a replacement for RT-PCR testing in mosquito surveillance programs. In 2006, RT-PCR confirmation detected the first WNV-positive result in epiweek 33. If RAMP assays were the sole method of WNV detection the first WNV-positive result would not have been detected until three weeks later during epiweek 36. In 2008, RT-PCR confirmation detected the first WNV-positive pool in epiweek 27, whereas RAMP did not detect a WNV-positive result until epiweek 31, a month later. In these situations, a solitary assay system would compromise control strategies, creating an inaccurate representation of WNV in the county. Mercer County deployed the RAMP system for WNV testing because of its ability to provide focused testing on specific species and localities where WNV activity has been detected. Our testing focused on areas with prior detection of WNV-positive mosquitoes, allowing us to respond quickly to local-transmission foci. Utilizing RAMP has allowed us to test local mosquito populations in these focal areas while continuing to expand our state-sponsored RT-PCR testing to new WNV foci. The targeting of specific foci and vector species are important factors in our program, especially considering that local mosquito control programs are economically limited for pools they can test weekly using state-funded programs. Using RAMP to supplement WNV detection in mosquito populations can aid local mosquito control programs in providing an accurate view of WNV activity.

Maximum likelihood estimations of infection rates

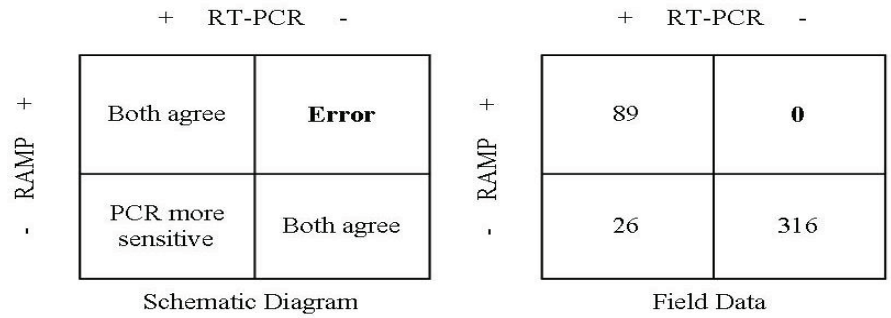

Figure 2. Schematic representation of field-collected data from RAMP-tested and RT-PCR-confirmed mosquitoes in Mercer County, NJ (2005-2008).

differed between the two assay systems and these differences should be considered when making mosquito control decisions. The RT-PCR IR values increased before RAMP IR values each year and the first RT-PCR-positive results were detected before positive RAMP assays in two of the four years. The early detection of WNV in mosquito populations is advantageous for effective and prompt control. Furthermore, the IR-value-peaks using RT-PCR were typically greater than those found using the RAMP system alone, due to the higher sensitivity of RT-PCR testing. The IR values are important to consider because they indicate the increased sensitivity of RT-PCR and the ability to detect early season pools, whereas RAMP results are concentrated in a shorter period during peak WNV presence in mosquito populations. If a mosquito control program conducts an adulticide application based on IR thresholds, then the IR values for RAMP samples should be lower than RT-PCR samples (given similar methodologies). We recommend RT-PCR during the early season when WNV prevalence in mosquito populations is relatively low and supplementing RT-PCR with RAMP when WNV prevalence is relatively high in mosquito populations and when mosquito control programs need quick decisions for adulticide applications.

Used in tandem with existing methods, RAMP can enhance detection of WNV and improve existing mosquito control programs by providing fast WNV-assay results and mosquito management decisions. During an active mosquito season, when virus levels reach their greatest thresholds, quick results from tested mosquito pools become imperative. Standard pooling of specimens and submission for testing may take upwards of ten days from submission to retrieval of results through RT-PCR testing at external health departments. Using RAMP, we have greatly reduced the wait period to around two to five h, primarily during peak activity when informed and timely control decisions become crucial for public health safety. Our testing has shown that RAMP is a valuable tool to augment RT-PCR testing of mosquito pools. When assaying mosquito species historically positive for WNV and targeting previously identified foci of WNV activity, RAMP has improved our surveillance measures. Future work should determine the efficacy of RAMP when testing known and emerging bridge vectors for WNV, along with attempts at quantifying levels of virus necessary in mosquito populations for RAMP to be most effective. The introduction of WNV into North America has lead to an increase of research and implementation of novel control strategies. The development of new WNV-detection systems must be evaluated by testing at local mosquito control programs in order to assess their utility. While no single method can suffice in the face of mosquito-borne arboviruses, augmentation with multiple efficient and rapid methods will improve surveillance and control of mosquitoes for public health.

Acknowledgments

We thank Bruce Wolf and Pat Bryant (NJDHSS Virology Laboratory) for confirmation of RAMP samples by RT-PCR. We thank Bob Kent and Claudia O'Malley (NJDEP Office of Mosquito Control Coordination) for coordinating testing efforts and technical assistance. This is New Jersey Agricultural Experiment Station publication D-08-08292-13-09 supported by State funds with partial support from the New Jersey State Mosquito Control Commission.

REFERENCES CITED

Andreadis, T.G., J.F. Anderson, C.R. Vossbrinck, and A.J. Main. 2004. Epidemiology of West Nile virus in Connecticut, USA: a five year analysis of mosquito data 1999-2003. Vector-Borne Zoonot. Dis. 4: 360-378.

Biggerstaff, B.J. 2006. PooledInfRate, Version 3.0: a Microsoft Excel® Add-In to compute prevalence estimates from pooled samples. Centers for Disease Control and Prevention, Fort Collins, CO.

Burkhalter, K.L., R. Lindsay, R. Anderson, A. Dibernardo, W. Fong, and R.S. Nasci. 2006. Evaluation of commercial assays for detecting West Nile virus antigen. J. Am. Mosq. Contr. Assoc 22: 64-69.

Eidson, M., N. Komar, F. Sorhage, R. Nelson, T. Talbot, F. Mostashari, R. McLean, and West Nile Virus Avian Mortality Surveillance Group. 2001. Crow deaths as a sentinel surveillance system for West Nile Virus in the Northeastern United States, 1999. Emerg. Infect. Dis. 7: 615-620.

Farajollahi, A., W.J. Crans, P. Bryant, B. Wolf, K. Burkhalter, M. Godsey, S.E. Aspen, and R.S. Nasci. 2005. Detection of West Nile viral RNA from an overwintering pool of *Culex pipiens pipiens* (Diptera: Culicidae) in New Jersey, 2003. J. Med. Entomol. 42: 490-494.

Lanciotti, R.S., A.J. Kerst, R.S. Nasci , M.S. Godsey, C.J. Mitchell, H.M. Savage, N. Komar, and N.A. Panella. 2000. Rapid detection of West Nile virus from human clinical specimens, field collected mosquitoes, and avian samples by a TaqMan RT-PCR assay. J. Clin. Microbiol. 38: 4066-4071.

Marra, P.P., S. Griffing, C. Caffrey, A.M. Kilpatrick, R. McLean, C. Vrand, E. Saito, A.P. Dupuis, L. Kramer, and R. Novak. 2004. West Nile virus and wildlife. Bioscience 54: 393-402.

Molaei, G., T.A. Andreadis, P.M. Armstrong, J.F. Anderson, and C.R. Vossvrinck. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, northeastern United States. Emerg. Infect. Dis. 12: 468-474.

Padgett, K.A., B. Cahoon-Young, R. Carney, L. Woods, D. Read, S. Husted, and V. Kramer. 2006. Field and laboratory evaluation of diagnostic assays for detection West Nile virus in oropharyngeal swabs from California wild birds. Vector-Borne Zoonot. Dis. 6: 183-191.

Silver, J.B. 2008. *Mosquito Ecology: Field Sampling Methods*. Springer-Verlag, New York.

Sutherland, G.L. and R.S. Nasci. 2007. Detection of West Nile virus in large pools of mosquitoes. J. Am. Mosq. Contr. Assoc. 24: 389-395.

Turell, M.J., D.J. Dohm, M.R. Sardelis, M.L. O'Guinn, T.G. Andreadis, and J.A. Blow. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. J. Med. Entomol. 42: 57-62.

United States Geological Survey. Disease Maps. [Internet]. Reston, Virginia: US Geological Survey [accessed 24 Sept 2008]. http://diseasemaps.usgs.gov.

White D.J., L.D. Kramer, P.B. Backenson, G. Lukacik, G. Johnson, J. Oliver, J.J. Howard, R.G. Means, M. Eidson, I. Gotham, V. Kulasekera, S. Campbell, Arbovirus Research Laboratory, and Statewide West Nile Virus Response Teams. 2001. Mosquito surveillance and polymerase chain reaction detection of West Nile virus, New York State. Emerg. Infect. Dis. 7: 643-649.