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ON-SITE REAL-TIME WEST NILE VIRUS SURVEILLANCE TO IMPROVE THE REACTION TIME OF ADULTICIDE MOSQUITO CONTROL MANAGEMENT

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Abstract One of the major groups of mosquito-borne diseases is Flaviviruses with prominent human pathogens, such as West Nile virus (WNV), dengue, Zika viruses. Nowadays, WNV is appearing more and more in several countries, thanks in part to climate change and other factors. WNV is endemic in parts of Europe, the Middle East, West Asia, Australia, Africa, and across the American continent. The primary vector for WNV is *Culex pipiens*, and some *Aedes* and *Anopheles* species may be competent vectors as well. Proper, accurate, and comprehensive vector control, surveillance, and laboratory diagnostics are important not only in WNV endemic countries but other places as well. Currently, most data on the presence of the virus comes from event-based surveillance. Here we demonstrate a protocol that can be used to detect the presence of certain viruses on-site within a short timeframe, permitting mosquito control activities for outbreak prevention. With the mobile laboratory we present, it is possible to detect the virus directly from mosquitoes within 3-4 hours, on the spot. This method provides an opportunity to process freshly caught mosquitoes in the trunk of a car under variable field conditions. This process contributes greatly to the increasing use of targeted, environmentally friendly, biological mosquito control but most importantly it facilitates the fine-tuning of outbreak prevention mosquito control activities.

Key words Mosquito-borne pathogens, surveillance, prevention, *Aedes albopictus*.

INTRODUCTION

West Nile virus (WNV) belongs to the genus Flaviviruses, it is one of the most common viruses transmitted by mosquitoes in Europe. In nature, the virus is maintained in mosquito populations through vertical transmission (adults to eggs) and circulates between mosquitoes and birds which serve as natural reservoir hosts. The virus occasionally infects humans and other mammals, like horses, which serve as dead-end hosts in the transmission (Lustig et al., 2018; Zana et al., 2020). Approximately 80% of people who are infected will not show any symptoms, however in some rare cases, the virus can cause serious or fatal neurological disease in humans. The primary vectors of WNV are species belonging to the mosquito genus Culex, in Europe, mainly Culex pipiens (Linnaeus, 1785) is responsible for WNV transmission. However, other species such as the exotic invader Aedes albopictus (Skuse, 1894) and members of Anopheles genus (Meigen, 1818) may also be a competent vector for WNV transmission (Fortuna et al., 2015; Kemenesi et al., 2014; Mancini et al., 2017; Maquart et al., 2016; Nir et al., 1968; Shocket et al., 2020) therefore investigation of a broader scale of mosquito species is continuously needed. Unfortunately, there is no human vaccine against WNV, thus surveillance of the virus and the maintenance of mosquito populations is particularly important. More and more monitoring programs are being set up throughout Europe but most of the data still comes from event-based (human cases) surveillance (Bakonyi and Haussing, 2020; Young et al., 2021; Gossner et al., 2017) . However, it is possible to detect the virus directly from mosquitoes before human cases occur, these laboratory processes take a

long time from the sample field collection to the molecular identification of the virus. This turnover time makes it difficult to integrate surveillance data into mosquito control activities. For all these reasons, our primary goal was to develop a mobile-laboratory protocol that allows the in-situ detection of the virus from mosquitoes. This method can greatly reduce the time of action, significantly supporting integrated mosquito management efforts, and raises the opportunity to suppress the targeted mosquito population to reduce upcoming human or animal case numbers, giving a direct mode of action before outbreaks flourish.

MATERIALS AND METHODS

Sample collection

Mosquito trapping was carried out in Valencia and Andalusia Regions, Spain, 2021. To test the applicability of our protocol we visited 23 previously selected sites based on reported WNV cases in the ongoing season (humans and horses) and based on the high abundance of mosquitoes measured in previous years. Mosquito collection at these sites was performed using standard trapping methods and trap types, namely BG-Sentinel (combined with lure and CO2), BG-Mosquitaire (with lure), CDC-Light trap (with yeast as CO2 attractant) (Figure 1). After collection from traps mosquitoes were euthanized by freezing using portable cool boxes, then they were visually separated by sex and genera (Anopheles, Aedes, or Culex) and female mosquitoes were grouped by sampling site and date of collection for WNV testing in separate pools with a max of 20 individuals per tube.

Figure 1. Mosquito sample collection with CDC Light trap (on the left) and BG-Sentinel (on the right).

Virus screening

For the extraction of viral nucleic acid, samples were first homogenized manually (quartz sand and 500µl PBS buffer were added to each pool) using plastic sticks. Total RNA was extracted using Beckman Coulter RNAdvance Viral XP; RNAdvance Viral XP 1.5 mL Tube Protocol (Beckman Coulter, Inc. CA, USA), following the manufacturer’s protocol. Compared to generally used RNA extraction protocols, this kit does not require any centrifugation steps, contains only a few types of reagents, and is suitable for field use supplemented with a magnetic rack (Thermo Fisher Scientific, MagJET Separation Rack) **(**Figure 2**).**

Figure 2. WNV detection protocol from mosquito specimens under field conditions.

Samples were tested for WNV by real-time (RT)-PCR with previously publishes primers and probes. The RT-PCR was performed on MyGo Mini S Real-Time PCR Instrument (IT-IS Life Science Ltd.) using the Brilliant III Ultra-Fast QPCR Master Mix (Agilent Technologies, CA, USA). The diagnostic test was designed to detect both the genetic lineages 1 and 2 of WNV, using the primers WN10533-10552 (AAG TTG AGT AGA CGG TGC TG) and WN10625-10606 (AGA CGG TTC TGA GGG CTT AC) targeting a 92 bp region in the 3´noncoding region (10,533–10,625). The probe WN10560-10579 (CTC AAC CCC AGG AGG ACT GG) was labeled with FAM. The total RT-PCR master mix was 15 µL containing primers at a concentration 1µM and probe at a concentration of 0,25 µM. The thermal cycling program consisted of 10 min at 50 °C for reverse transcription, 3 min at 95°C for denaturation, and 45 cycles of 10 sec at 95°C and 25 sec at 60°C for amplification (Tang et al., 2006). The PCR program took approximately 2 hours. The full protocol for in-situ WNV detection, from emptying the traps to PCR results, takes about 3-4 hours with the above-mentioned conditions, depending on the number of investigated mosquitoes. After PCR results destructive data analysis was performed.

RESULTS AND DISCUSSION

During our field visit altogether 353 mosquito individuals belonging to 7 species were collected and tested. *Cx. pipiens* (n=207), *Culex perexiguus* (n=13), *Ae. albopictus* (n=39), *Aedes caspius* (n=32), *Aedes detritus* (n=30), *Anopheles atroparvus.* (n=28), *Culiseta longiareolata* (n=4). A total of 54 pools were combined and processed for WNV testing and 2 pools were positive, which combined 5 mosquitoes. (2 *Cx. pipiens* and 3 *An. atroparvus* collected from Andalusia, Coria village). WNV cases have been reported in Andalusia during the last couple of years, indicating this region as a WNV hot-spot. In another area of our trapping, we found no evidence for active WNV circulation. Although members of the genus *Anopheles* are not primary vectors of WNV, several literature data support our result (Kemenesi et al., 2014, Mancini et al., 2017, Maquart et al., 2016, Nir et al., 1968). To date, several studies have described the possibility of detecting WNV rapidly, but all of them required laboratory conditions. These have helped virus surveillance by detecting the virus from human samples or by processing mosquitoes that have been collected over several years. (González-Reiche et al., 2010; Lanciotti et al., 2000; Szentpáli-Gavallér et al., 2014) . To our best knowledge this is the first in-situ surveillance system for WNV, which can directly aid targeted mosquito control efforts and outbreak preparadness.

CONCLUSIONS

In the present paper, we introduce a mobile lab protocol that allows us to detect mosquito-borne viruses of human health significance from mosquitoes on the spot, in a very short time, before there were human infection cases in the given area. This method can be integrated into most of the currently ongoing vector-borne virus surveillance activities as well as the biggest advantage, it can be complemented with rapid-response in mosquito control actions and can be optimized for other viruses transmitted by mosquitoes.

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