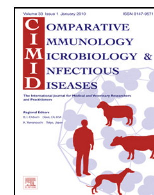
Comparative Immunology, Microbiology and Infectious Diseases 37 (2014) 131–141

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid



# Evaluation of a West Nile virus surveillance and early warning system in Greece, based on domestic pigeons

Serafeim C. Chaintoutis [a], Chrysostomos I. Dovas [a,*], Maria Papanastassopoulou [a], Sandra Gewehr [b], Kostas Danis [c], Cécile Beck [d], Sylvie Lecollinet [d], Vasilis Antalis [b], Stella Kalaitzopoulou [b], Takis Panagiotopoulos [c,e], Spiros Mourelatos [b], Stéphan Zientara [d], Orestis Papadopoulos [a]

[a] Laboratory of Microbiology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece
[b] Ecodevelopment S.A. – Environmental Applications, Filyro, 57010 Thessaloniki, Greece
[c] Department of Surveillance and Intervention, Hellenic Centre for Disease Control and Prevention, 15123 Athens, Greece
[d] European Reference Laboratory for Equine Diseases, UPEC, UMR 1161 Virology, INRA, ANSES, ENVA, 94704 Maisons-Alfort, France
[e] Department of Child Health, National School of Public Health, 11521 Athens, Greece

ARTICLE INFO

*Article history:*
Received 7 August 2013
Received in revised form 6 January 2014
Accepted 8 January 2014

*Keywords:*
West Nile virus
Surveillance
Early warning
Greece
Central Macedonia
Juvenile domestic pigeons
WNV point seroprevalence
Neutralizing antibodies
Incidence rates of human WNND
Usutu virus

ABSTRACT

In the summer of 2010 an epidemic of West Nile virus (WNV) occurred in Central Macedonia, Greece, with 197 human neuroinvasive disease (WNND) cases. In the following years the virus spread to new areas, with a total of 76 WNND cases in 2011, and 109 WNND cases in 2012 (14 and 12 WNND cases, respectively, in Central Macedonia). We established a surveillance system based on serological testing of domestic pigeons, using cELISA confirmed by serum neutralization test. In Central Macedonia, pigeon seroprevalence was 54% (95% CI: 49–59%) and 31% (95% CI: 24–37%) at the end of the 2010 and 2011 epidemic seasons, respectively. One serum was positive for neutralizing antibodies directed against Usutu virus. Pigeon WNV seroprevalence and incidence rates of human WNND after the 2010 epidemic were positively correlated ($\rho = 0.94$, at the regional unit level), while in 2011 the correlation ($\rho = 0.56$) was not statistically significant, possibly due to small number of human WNND cases recorded. To evaluate the efficacy of the system at alerting upon WNV enzootic circulation before the onset of human cases, we tested 270 pigeons in 2011 and 240 pigeons in 2012. In Central Macedonia, the first seroconversions in pigeons were recorded 44 and 47 days, respectively, before the first human WNND cases. Pigeon surveillance was used successfully for identification of areas with WNV enzootic transmission and for early warning. Timely diffusion of information to health authorities facilitated the implementation of preparedness plans to protect public health.

© 2014 Elsevier Ltd. All rights reserved.

## 1. Introduction

West Nile virus (WNV) is an RNA virus within the Japanese encephalitis virus group (genus *Flavivirus*, family *Flaviviridae*) [1]. WNV is transmitted by *Culex* mosquitoes in a cycle involving birds as amplifying hosts. Infected mosquitoes carry WNV in their salivary glands and infect susceptible bird species during blood-meal feeding [2]. A mosquito requires approximately 10–14 days after its initial blood meal to become infectious and to transmit WNV to other animals. This time interval is known as the extrinsic incubation period [3]. Spillover infections may occur

* Corresponding author. Tel.: +30 2310 999870; fax: +30 2310 999959.
*E-mail address:* dovas@vet.auth.gr (C.I. Dovas).

0147-9571/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cimid.2014.01.004

in mammals (including horses and humans), which are regarded as incidental or dead-end hosts, since they do not produce significant and long-lasting viremia, and do not contribute to the virus transmission cycle [2].

Antibodies against WNV have been present in domestic animals, as well as in ~1% of the humans in Greece since the 1960s [4–8]. Hemagglutination-inhibiting (HAI) antibodies against WNV were identified in sera obtained from sheep, goats, bovine, horses, mules, as well as in one rabbit, one hare and one pig. Furthermore, HAI antibodies against WNV were detected in sera obtained from pigeons, chickens, turkeys, a common snipe (*Capella gallinago*) and an Eurasian collared dove (*Streptopelia decaocto*) [5]. However, human WNV cases were not reported before 2010.

In 2010, a WNV epidemic occurred in Greece, with 197 human cases that suffered from neurological signs (WNND) and 35 fatalities [9]. Two other epidemics occurred the following years, with 76 human WNND cases, as well as 8 fatalities in 2011 and 109 reported WNND cases in humans and 16 fatalities in 2012 [10,11]. A lineage 2 WNV strain named "Nea Santa-Greece-2010" was detected in pools of *Culex pipiens* mosquitoes [12–14], a donated human blood sample [15], a Belgian traveler returning from Greece [16], captive sentinel chickens [14,17,18] and an Eurasian magpie (*Pica pica*) [19], between 2010 and 2012.

Worldwide surveillance efforts to detect WNV before infection of incidental hosts (humans, horses) were based on collection and testing of adult mosquitoes and/or birds [20,21]. Four categories of birds have been used for WNV surveillance; dead wild birds, trapped wild birds, captive sentinel birds, or domestic sentinel birds [22]. In the USA the value of using WNV-infected dead birds as an indicator of increased WNV disease risk has been demonstrated in several studies [23–25]. However, in Europe, and especially in Greece, dead bird surveillance could not be applied, since abnormal bird mortalities were not observed [19,26].

Sentinel birds can be used to detect the presence of WNV in a geographical location. An ideal sentinel bird is a species that is susceptible to infection, is resistant to disease, develops a detectable immune response rapidly, is maintained easily, presents negligible health risks to handlers, does not contribute to local pathogen transmission cycles and seroconverts to the target pathogen prior to the onset of disease outbreaks in the community [22]. Chickens and pigeons develop low-level WNV viremia [27] and are maintained easily in captivity, making them ideal sentinel species [28]. Sentinel chicken – based WNV surveillance systems have provided evidence of WNV transmission several weeks before the occurrence of human cases [29]. Moreover, pigeons have already been identified as potential sentinel birds [22]. Consequently, active surveillance of domestic birds has been used successfully in America and Europe for their early warning capacity [30–35].

The present study was initiated in Greece at the end of the 2010 WNV epidemic. It comprised two sub-studies with the following objectives: (a) to determine the geographical spread of WNV, immediately after the 2010 and 2011 epidemic seasons, using WNV antibodies in juvenile domestic pigeons (*Columba livia domestica*) as indicators of WNV circulation, (b) to assess the correlation between the WNV point seroprevalence in pigeons and the incidence rates of human WNND cases (first study), and (c) to evaluate the early warning capacity of a pigeon surveillance system, e.g. its capacity to provide data on WNV circulation prior to the onset of human cases in Central Macedonia, during the 2011 and 2012 epidemic seasons (second study).

## 2. Materials and methods

### 2.1. First study: WNV point seroprevalence in juvenile pigeons after the 2010 and 2011 epidemics, and correlation with incidence rates of human WNND

#### 2.1.1. Sampling in domestic pigeons

Two samplings were performed in juvenile domestic pigeons, in order to determine the spread of WNV in selected areas in Greece after the end of the 2010 and 2011 epidemics, respectively (Fig. 1). The first sampling took place between October 2010 and February 2011 and 655 pigeons (131 pigeon pens, 5 pigeons per pen) were sampled. During this period, 430 sera were obtained from pigeons in Central Macedonia (the area worst affected during the 2010 epidemics), specifically from the regional units (prefectures; geographical areas equivalent to Nomenclature of Territorial Units for Statistics – NUTS level 3 areas) of Imathia, Pella, Kilkis and Thessaloniki. In addition, 110 sera were obtained from Eastern Macedonia (Kavala) and Thrace (Xanthi), 65 from Thessaly (Larissa and Magnissia) and 50 from Attica. The second sampling took place in October 2011, where 210 pigeons were sampled (42 pigeon pens, 5 pigeons per pen). This sampling was limited to Central Macedonia.

A two-stage stratified cluster sampling methodology was adapted in order to select a random sample of pigeons. The study area was divided into two strata: urban and rural areas. In the first stage, pens (clusters) were randomly selected from the study area using probability proportional to the human population size of the urban or rural areas. In the second stage, 5 pigeons were included in the study per selected pen. The pigeons were ringed and the coordinates of each pen were recorded using Global Positioning Systems (GPS) technology. All samples were obtained from pigeons over 45 days old, to ensure that the detected antibodies did not result from passive transfer of maternal IgY [36]. Given that there were no data on WNV circulation in pigeons prior to the 2010 epidemic, all pigeons that were sampled during October 2010–February 2011 period were younger than 12 months old, in order to exclude infections before 2010. In 2012, all sampled pigeons were either younger than 5 months old, or seronegative during the previous sampling period.

Blood samples were drawn with sterile syringes from the brachial vein. The volume of blood extracted did not exceed 500 μl. Blood was collected in 1.5 ml microcentrifuge tubes, allowed to clot and the tubes were centrifuged (21,000 × g, 5 min, 4 °C). Sera were transferred to clear microcentrifuge tubes and stored at −80 °C until they were assayed.

#### 2.1.2. Serological testing

All sera ($n$ = 865) were tested for the presence of antibodies against WNV envelope protein (E), using

Download English Version:

https://daneshyari.com/en/article/2428241

Download Persian Version:

https://daneshyari.com/article/2428241

Daneshyari.com