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SHORT COMMUNICATION

Detection of West Nile and Usutu Viruses in Italian Free Areas: Entomological Surveillance in Piemonte and Liguria Regions, 2014

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Abstract

West Nile virus and Usutu virus have established in different parts of Italy over the past 10 years. Piemonte and Liguria Regions (Northwestern Italy) are known to be nonendemic areas, despite the presence of competent vectors and environmental conditions conducive to maintaining infection. This work evidences for the first time, through an entomological surveillance implemented on the basis of risk factor approach, the presence of West Nile and Usutu viruses in Piemonte and Liguria Regions (Northwestern Italy).

Key Words: Arbovirus(es)—*Culex pipiens*—Mosquito(es)—Real time RT-PCR—West Nile.

Introduction

In recent years, arboviruses have shown an increasing ability to spread beyond their original areas, as experienced by the repeated emergences of West Nile virus (WNV) and Usutu virus (USUV) in Europe (Nikolay, 2015). These emerging viruses are antigenically close and probably use the same biological cycle, sharing the same host and vectors (*Culex pipiens*). Simultaneous circulation and ecological affinity between the two viruses have been reported in several studies (Savini et al., 2011). The capability to early detect and respond to emerging vector-borne disease outbreaks, through rapid pathogen detection, is a key component for disease response. Mosquito-based surveillance might be considered a mainstay in most surveillance programs for arboviruses. It allows to early detect the presence of viruses and can constitute the foundation for a public health alert system (Calzolari et al., 2013).

In addition to the activities supported by the Ministry of Health at national level, mosquito-based surveillance has been implemented in Liguria and Piemonte Regions (Northwestern Italy) since 2011. Previous studies showed a very low prevalence of USUV in mosquitoes in Piemonte and the absence of WNV in both regions. Moreover, the presence of competent vectors in both regions proves that these areas are suitable for maintaining and spreading these viruses (Rizzo et al., 2014).

This work reports results of entomological surveillance carried out in 2014 in these two Italian regions.

Materials and Methods

Entomological surveillance was performed from July 1 to November 7, with fortnightly samplings using Centers for Disease Control and Prevention (CDC) carbon dioxide-baited traps working for a night, BG sentinel carbon dioxide-baited traps working for a 24 h period, and gravid traps activated for 24–48 h. Manual aspiration by a Prokopack© aspirator was employed in WNV-positive sites. Forty trap sites (34 in Piemonte and 6 in Liguria) were selected, according to risk-based factors (proximity to wetland zones, presence of

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animal hosts, and different land use and habitat features); georeferenced and one or a combination of two different kinds of traps were placed.

Collected mosquitoes were immediately refrigerated, counted, identified using standard taxonomic keys (Stojanovich and Scott, 1997), and pooled in 100 specimens maximum, according to species, trapping site, and date, and then stored at −80°C.

After homogenization in PBS, viral RNA was extracted from mosquito pools using RNeasy Mini kit (Qiagen) with an automated QIAcube protocol. Mosquitoes were analyzed by both Real Time RT-PCR distinctive for WNV Lineage 1 and Lineage 2 (Del Amo et al., 2013) and Real Time RT-PCR for USUV (Cavrini et al., 2011). On positive pools, two traditional RT-PCR for WNV (Lanciotti et al., 2000) and USUV (Bakonyi et al., 2004) were carried out. Amplicons of the expected size (408 and 425 bp, respectively) were sequenced using the Big Dye Terminator kit v 3.1 (Lifetecnologies) and run on an ABI3130 Genetic Analyzer (Applied Biosystems).

All WNV- and USUV-positive pools were sent for confirmation, sequencing, and determination of Lineage to the Centre for Animal Exotic Diseases in Teramo, which is the OIE and National Reference Laboratory for WND. Mosquito abundance and infection data have been used to calculate the Vector Index (VI) for *C. pipiens* using the PooledInfRate statistical software package (Biggerstaff, 2009; Kilpatrick and Pape, 2013).

Results and Discussion

In 2014 surveillance activities, a total of 20,382 mosquitoes were sampled (19,122 by attractive traps and 1260 by manual aspiration). The most abundant species detected was *C. pipiens*, mainly collected by CDC traps in Piemonte and gravid traps in Liguria, followed by *Aedes albopictus* largely collected by BG sentinel traps (Table 1). The collected mosquitoes were sorted in 914 pools and tested. WNV Lineage 2 was detected in three pools of *C. pipiens*. Two pools were collected in Piemonte by CDC traps (Alessandria province) on August 27 (weekly VI = 0.158 confidence interval, CI 95% = 0.010–0.783) and September 10 (weekly VI = 0.216, CI 95% = 0.015–1.085). The third WNV-positive pool was collected in Liguria by gravid traps at Genoa airport on September 29 (weekly VI = 0.272, CI 95% = 0.016–1.646). The obtained RNA sequences of the three positive samples were submitted to GenBank under the accession numbers KT877358, KT877359, and KT877360. The weekly VI calculated for *C. pipiens* was very low, supporting a low risk of human WNV infection (Bolling et al., 2009). USUV was detected in two pools of *C. pipiens*: the first collected in Piemonte in Alessandria province by CDC traps on August 27 and the second in Liguria in La Spezia province by gravid traps on September 23.

This is the first report of WNV in both regions. After confirmation, control measures against WNV and its vector were quickly implemented, as provided by national legislation and by local disinfestations protocols (adulticiding and larviciding programs). Serological tests (ELISA IgM–Ingenasa) were performed on sera from sentinel equines randomly sampled within a 4 km radius from the positive mosquito pools collection sites. IgM antibodies were detected in a serum sample collected from a sentinel healthy horse living near the first positive mosquito collection site, suggesting a recent WNV contact.

WNV has established an endemic cycle in different areas of Italy through native wild birds and local mosquitoes,

Table 1. Mosquito Species Collected in Piemonte and Liguria: Number of Individuals Per Species Caught by Different Traps (2014)

	Liguria Region		*Piemonte Region*				
Mosquito species	*BG-Sentinel trap*	*Gravid trap*	*Aspirator*	*BG-Sentinel trap*	*CDC trap*	*Gravid trap*	*Total by mosquito species*
Culex pipiens	979	1409	196	719	6715	739	10,757
Culex sp.	41	31	0	0	0	0	72
Culex territans	1	0	16	0	0	0	17
Culex theileri	1	0	0	0	0	0	1
Aedes albopictus	2419	317	9	910	229	185	4069
Aedes sp.	63	2	0	0	0	0	65
Aedes vexans	0	0	1	12	620	1	634
Anopheles maculipennis	0	0	0	0	1	0	1
Anopheles maculipennis s.l.	1	0	1035	35	275	267	1613
Anopheles plumbeus	1	1	0	4	8	1	15
Culex hortensis	0	1	3	0	0	0	4
Culex impudicus	0	21	0	0	0	0	21
Culex mimeticus	0	1	0	0	0	0	1
Culex modestus	0	0	0	0	38	0	38
Culiseta annulata	0	2	0	3	0	0	5
Culiseta longiareolata	5	12	0	0	0	0	17
Culiseta sp.	0	1	0	0	2	0	3
Culiseta subochrea	0	0	0	0	3	0	3
Indeterminate	0	0	0	0	3	0	3
Ochlerotatus caspius	6	0	0	412	2482	23	2923
Ochlerotatus geniculatus	0	0	0	24	90	5	119
Ochlerotatus sp.	1	0	0	0	0	0	1
Total by trap	3518	1798	1260	2119	10,466	1221	20,382

which allowed the virus to survive the winter season. WNV Lineage 2 emerged in Italy in 2011 (Savini et al., 2012), the year after detection in Greece and it has been the main Lineage circulating in Italy in 2013 and 2014. It was responsible for numerous equine and human cases.

USUV was reported in Piemonte since 2011 (Rizzo et al., 2014). After the first detection, it was found every year in field-collected mosquitoes (*C. pipiens*) in the eastern part of Piemonte, confirming the establishment of this virus in this area. No USUV-positive mosquito pools had been previously reported in Liguria. Even if USUV is characterized by a lower pathogenicity than WNV, it should receive more consideration particularly in areas where it cocirculates with WNV. Neuroinvasive human cases were recently observed in patients with either normal (Santini et al., 2015) or impaired immune system (Pecorari et al., 2009).

The mosquito surveillance system gives the opportunity to have data on mosquito population composition. The conjunction of different methods for mosquito trapping provides information on host-seeking, blood-feeding, and resting behavior of mosquitoes, useful for a better knowledge of the vector population and to address virological investigation.

Such standardized and consistent surveillance efforts provided the basis for the evaluation of trends in vector activity and for the implementation of surveillance activities that allowed the detection for the first time of WNV in Piemonte and Liguria and USUV in Liguria. Control measures were then quickly applied in close collaboration with local veterinary and human health services.

Author Disclosure Statement

No competing financial interests exist.

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