

MOSQUITO-BASED ARBOVIRAL SURVEY IN WESTERN LOMBARDY, ITALY: 2009–2013

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Abstract A mosquito-based arbovirus surveillance was settled in “Parco Lombardo delle Valli del Ticino” and in neighbouring sites in Milano province (Lombardy). Nine sites were monitored between 2009 and 2013 sampling 82,106 mosquitoes, of which 68.6 % belonged to *Culex pipiens* L. species. Mosquito were sorted in pools according to species, data and place of collection and submitted to biomolecular analysis for detection of flaviviruses and orthobunyaviruses. Usutu virus was detected in three different pools of *Cx. pipiens*, Batai virus was detected in a pool of *Anopheles maculipennis s. l.* and in two pools of *Cx. pipiens*, and Tahyna virus in a pool of *Aedes vexans* (Meigen) and in one of *Ochlerotatus caspius* (Pallas). Obtained data give useful information on mosquito community in surveyed area and demonstrate the ability of this surveillance system to detect arboviruses circulating in mosquitoes. **Key words** Arbovirus, surveillance, *Culex pipiens*, *Anopheles maculipennis*, *Ochlerotatus caspius*, Usutu virus, Tahyna virus, Batai virus

INTRODUCTION

Burden of arthropod-borne viruses (arboviruses) has been steadily increasing in recent years, creating health problems worldwide. Also in Italy diseases caused by these viruses have been increasingly reported, with relevant outbreaks, like the Chikungunya epidemic in Castiglione (Emilia-Romagna), and the 69 human cases of West Nile disease reported to the end of September in 2013 (ECDC data). Additionally, other arboviruses have been already detected in Italy, like Batai and Tahyna viruses (Nicoletti et al., 2008; Huhtamo et al., 2013), or seem to be recently introduced, like USUV. The pathogenic potential of these viruses is not well characterized and probably is underestimated (Gratz, 2006), and they cause diseases often considered neglected.

Health surveillance programs, also based on vectors screening, were established in Italy for monitoring presence of potentially pathogenic arboviruses. Results and characteristics of a entomological surveillance program adopted in western Lombardy, between 2009 and 2013, are described herein.

MATERIALS AND METHODS

From 2009 to 2011, mosquitoes were trapped in the “Parco Lombardo della Valle del Ticino” (Lombardy region), a 91,000 ha Regional Natural Park that protects the Italian stretch of Ticino River, an area that includes a variety of natural, agricultural and urban sites, although in one of the

most densely populated areas of Italy. Stations (5 in 2009, of which one monitored also in 2010-2011) were located in 5 natural areas characterized by riparian vegetation, in close proximity to urban and agricultural environments, with a high number of rice fields. In 2012 and 2013 mosquito monitoring was conducted in a riding school located in a rural area, surrounded by rice fields, in Parona municipality (PV). Moreover in 2012 urban parks were monitored in the city of Milan (2), and Cusago Municipality (3). Mosquitoes were trapped using modified CDC traps baited with carbon dioxide (Bellini et al., 2002), and a BG sentinel plus carbon dioxide in 2012 in Cusago and Milan. Sites were monitored weekly from June to the end of September. The modified CDC traps worked one night per week from approximately 5:00 PM to 9:00 AM. BG sentinel traps worked during the day, from approximately 10:00 AM to 20:00 PM.

Collected mosquitoes were identified using morphological characteristics according to classification keys (Schaffner et al., 2001; Severini et al., 2009) than pooled by date of collection, location, and species (with a maximum number of 200 individuals per pool), ground and submitted to biomolecular analysis (Calzolari et al., 2010). For simultaneous detection of arboviruses, pools were tested by screening PCRs for detection of orthobunyaviruses (Kuno et al., 1996) and flaviviruses (Scaramozzino et al., 2001). Amplicons obtained by positive PCRs were sequenced by an automated fluorescence-based technique following the manufacturer's instructions (ABI-PRISM 3130 Genetic Analyzer, Applied Biosystems, Foster City, CA). These sequences were employed as tools for the identification of viruses by performing a BLAST search in the GenBank database.

RESULTS AND DISCUSSION

Mosquito Collection

Using CO₂-baited traps, from 2009 to 2013, a total of 82,106 mosquitoes were collected; more than 68% were *Culex pipiens* L. Other species abundantly sampled were *Aedes vexans* (Meigen) and *Ochlerotatus caspius* (Pallas), both in about 11%. Also a relevant number of anopheline mosquitoes were collected (more than 8%), the largest number of which belonged to *Anopheles maculipennis* complex. This complex includes different species in which adult females are morphologically indistinguishable, but have different competence for Malaria parasite transmission (Severini et al., 2009). The knowledge of species composition of this complex is important to assess the possible risk of reintroduction of the parasite. Specimens of other eight species, including the Asian Tiger Mosquito, *Aedes (Stegomyia) albopictus* (Skuse), were collected under the 1% of the total for species.

Virus Detections

Three different viruses were detected during the survey, the flavivirus USUV and the two orthobunyaviruses BATV and TAHV.

Usutu virus was detected in three different pools of *Cx. pipiens*, in 2009 and 2011. This virus was first confirmed in Europe in Vienna, in 2001, and it seems to spread to neighbouring countries from here (Pfeffer and Dobler, 2010), but it was retrospectively detected in birds sampled in 1996 in Italy (Weissenböck et al., 2013). USUV was abundantly detected in mosquitoes in Pianura Padana since 2009 (Calzolari et al., 2010). The main vectors of USUV are ornithophilic mosquitoes, mainly of the genus *Culex*; wild birds are principal reservoirs. The medical importance of USUV is not fully understood, but this virus appears to be pathogenic to humans (Pfeffer and Dobler, 2010).

Batai virus was detected in a pool of *Anopheles maculipennis s. l.* (in 2009) and in two pool of *Cx. pipiens* (one in 2009 and one in 2011). This virus, mainly spread by species *An. maculipennis*

and *Anopheles claviger* (Meigen) in Europe, is also present in Asia and Africa (Gratz, 2006; Hubálek, 2008). Several serological surveys conducted in Europe showed a low prevalence of antibodies to BATV in humans and a higher prevalence of antibodies in other mammals (Lundström, 1999). BATV was recently isolated from mosquitoes in Piemonte (Huhtamo, et al., 2013). This virus has not been associated with clinical disease, only mild influenza-like symptoms were associated with seroconversion to BATV in humans (Gratz, 2006).

Tahyna virus was detected in a pool of *Ae. vexans* (in 2009) and in one of *Oc. caspius* (in 2010). This virus has been mainly isolated from *Ae. vexans* but was also detected in *Oc. caspius* and in other mosquitoes (Gratz, 2006; Hubálek, 2008). Several serological surveys and viral isolations reported the widespread occurrence of TAHV in humans and other mammals in many European countries (Lundström, 1999), but this virus is also present in Africa and Asia (Gratz, 2006; Hubálek, 2008). TAHV infection in humans causes influenza-like symptoms and, in some cases meningoencephalitis and atypical pneumonia (Gratz, 2006). The detection of viral RNA in mosquitoes, confirmed the presence of this virus in northern Italy, as reported previously (Nicoletti et al., 2008).

This positive PCR detection demonstrates that wide range of arboviruses involved in human and animal diseases could be detected by entomological survey. The described survey allowed the detection of neglected virus, for which human cases are probably underestimated, pointing out important epidemiological data on temporal and geographic diffusion of these viruses, in addition to the description of mosquitoes present in the monitored area. This work demonstrates that entomological surveillance can provide useful data in assessing the risk of arbovirus spreading.

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