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First report on entomological field activities for the surveillance of West Nile disease in Italy

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Summary

West Nile virus (WNV) is neuropathogenic for birds, horses and humans and is maintained in natural cycles between birds and mosquitoes, particularly the *Culex* genus; horses and humans are considered to be incidental hosts. A surveillance plan was implemented in Italy in 1998, following a limited outbreak of WNV equine encephalomyelitis and a WNV outbreak in France very close to the Italian border. This plan to assess the risks of the virus being introduced again included entomological surveillance performed in 15 study areas considered 'at risk' of WNV introduction in the country. Entomological surveys conducted in Italy from 2003 to 2007 resulted in the capture of a total of 28 798 mosquitoes, of which there were 14 765 adults and 14 033 larvae belonging to 22 species. According to the literature, eight of the species identified have been found to be naturally infected with WNV or were successfully infected in the laboratory in some parts of Europe and in the United States, namely: *Aedes albopictus* (Skuse, 1897) (= *Stegomiya albopicta*), *Aedes vexans* (Meigen, 1830), *Anopheles maculipennis* Meigen, 1818, *Coquillettidia richiardii* (Ficalbi, 1889), *Culex modestus* Ficalbi, 1889, *Culex pipiens* Linnaeus, 1758, *Culex theileri* Theobald, 1903 and *Ochlerotatus caspius* (Pallas, 1771) (= *Aedes caspius*).

Keywords

Culex pipiens, Italy, Monitoring, Mosquito, West Nile, Surveillance, Virus.

Introduction

West Nile virus (WNV) is a mosquito-borne virus that belongs to the family *Flaviviridae* and is a member of the Japanese encephalitis complex. WNV is neuropathogenic for birds, horses and humans (59) and is maintained in natural cycles between birds and mosquitoes, particularly those of the *Culex* genus. Horses and humans are considered to be incidental hosts (3). WNV is widely distributed in Africa, the Middle East, Eurasia and, was introduced into North America more recently (23, 28, 44). Human and animal infections were not documented in the western hemisphere until the 1999 outbreak that occurred in the New York City metropolitan area. In 2003, WNV activity was reported in 46 states and caused illness in over 9 800 people. Since its detection in the United States in 1999, WNV has spread across 47 mainland states (65), resulting in a total of 27 598 cases with 1 086 deaths in humans (7). WNV occurred in Canada in 2001 (20) and reached the countries of the Caribbean and Central America in 2003 (8, 43). It has also been circulating in several countries in continental Europe and in the Mediterranean Basin. Recent outbreaks causing human encephalitis occurred in Algeria in 1994 (39), in Romania between 1996 and 2000 (62), in the

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Czech Republic in 1997 (39), in Russia in 1999 (26) and in Israel in 2000 (67). Epizootics of the disease in horses occurred in Morocco in 1996 (39), in Italy in 1998 (2) and in France in 2000 (39). One of the peculiarities of this flavivirus is that it can be transmitted by different genera and several species of mosquitoes. To date, the list of mosquitoes in which WNV has been isolated includes at least 75 species, even if additional mosquito species have been found positive in the United States (West Nile virus isolated, West Nile RNA detected or West Nile antigen detected using a variety of diagnostic tests) (5, 6). WNV is enzootic primarily amongst birds (27) and appears less frequently in mammals, with equines and humans considered to be dead-end hosts (13). *Culex pipiens* is generally regarded to be the most competent vector of WNV in Europe and was possibly involved in the Cerbaie-Fucecchio outbreak (2) and in Romania, where it acted as both enzootic and epizootic vector (55). Nevertheless, to define the possible role of *Cx. pipiens* in the maintenance and transmission of arboviruses, the bionomics of the various mosquito biotypes need to be understood (45, 66).

This virus has also been detected in other haematophagous arthropods. Amongst others, WNV has been detected in the biting midge *Culicoides sonorensis* Wirth & Jones (Diptera: Ceratopogonidae) in the United States (41) and in 10 species of ticks (21) from six genera, namely: *Argas*, *Ornithodoros* (Ixodida: Argasidae), *Amblyomma*, *Dermacentor*, *Hyalomma*, *Rhipicephalus* (Ixodida: Ixodidae) (22, 37). However, the precise role of these and other haematophagous arthropods in the transmission of WNV requires further investigation.

A human epidemic of WNV has been ongoing in the United States since 1999, while limited outbreaks have occurred sporadically in southern Europe following the introduction of the virus by infected birds migrating from Africa with the subsequent involvement of local mosquito populations (40).

In Italy, a limited focus of WNV equine encephalomyelitis involved 14 race horses at nine localities in four provinces of the Toscana Region (central Italy) in 1998. Diagnosis was performed using the complement fixation (CF) test at the Italian National Centre for Exotic Diseases (CESME, *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'* in Teramo). Subsequently, WNV was isolated and identified by molecular analysis at the Pasteur Institute in Paris in December 1998 from two of the Italian horses presenting signs of encephalitis (2). This event, together with a more recent outbreak of WNV that occurred very close to the Italian border in France (38) and the extensive presence in the country of another potential vector, *Ae. albopictus* (50, 54), encouraged the Italian government to implement a surveillance plan to assess the risk of the virus being introduced yet again. Since 2002, the Italian Ministry of Health has approved a national plan based on serological and entomological surveys (involving sentinel chickens and equines in the first case and mosquitoes in the second) by promulgating three ministerial orders and a ministerial decree since 2007 November (30, 31, 32, 33).

This surveillance plan is implemented by the CESME in Teramo and is co-ordinated by the network of *Istituti Zooprofilattici Sperimentali* located across Italy (which all belong to the Italian National Health/Veterinary Service) and by the Unit of Vector Control of the *Istituto Superiore di Sanità* for entomological expertise.

The authors present the results of the entomological survey that lasted five years and covered the entire country. These results are compared to information from the literature.

The results of this entomological inquiry represent part of the ongoing surveillance system that is focused on improving the understanding of the ecology of WNV in Italy.

Materials and methods

Study areas

A total of 15 study areas considered 'at risk' for WNV introduction in Italy were selected for longitudinal surveillance (Table I; Fig. 1) on the basis of features considered most suitable for virus circulation, such as the simultaneous presence of humans, stock farms with horses, migratory birds and mosquitoes. These areas were selected and defined in accordance with standardised criteria developed by the Important Bird Areas (IBA) European Programme (16) and in collaboration with the Italian National Institute for Wildlife (INFS) in Bologna, namely: areas regularly inhabited by 20 000 or more water birds or by 1% of the individuals in a population of one species or subspecies of water birds (12). Study areas were georeferenced and included in a list of the Italian wetlands used for a waterfowl winter census. Italy was divided into a grid of square units of 400 km^2 (20 km each side) by using geographic information systems (GIS). The extent of the 'at risk' areas included all the squares (comprising the monitoring sites) separated by a 20 km buffer drawn around the centroids (15).

Entomological collection procedures

The collection of adult mosquitoes and larvae was performed by the staff of the *Istituti Zooprofilattici Sperimentali* involved in the Italian national plan of WNV surveillance in accordance with standard procedures developed by the *Istituto Superiore di Sanità*. In each study area, monthly mosquito catches were scheduled from November to March, while from April (early spring) to October (late autumn) catches were conducted fortnightly, for a total of about 16 surveys per year for five years (2003-2007). Adult mosquitoes were collected using Centers for Disease Control (CDC) light traps and by aspiration in resting sites (mainly animal shelters); all samples were stored in dry conditions. A total of 28 light traps were used, two for each study area; these were placed in shaded positions that were sheltered from wind and rain at a distance of about 1.5 m from the ground. They were operated from dusk to the early morning. In addition, one trap was placed near an animal shelter and another near the henhouse where 'sentinel' chickens were stocked for the purposes of the serological surveillance. 'Sentinel' refers to cases when an animal (most often a chicken) is kept in a known location (e.g. chicken coop) and its blood is sampled routinely (usually every week or two) and tested for the presence of WNV. If the test reveals a positive result, this is an indication that virus is circulating in the area under study (7). As many resting adult mosquitoes as possible were collected from the walls of stock shelters in the morning using electric aspirators.

Larval collections were performed on the same sites for each study area when feasible, not all

Table I
Name, location and identification of the 15 study areas selected for the national West Nile virus surveillance programme in Italy

Region	Location (Province)	Identification of location
Abruzzo	Mouth of Vomano River (Teramo)	A
Basilicata	South Giuliano lake (Matera)	B
Calabria	Mouth of Neto River (Crotone)	C
Campania	Serre Persano (Salerno)	D
Emilia Romagna	Comacchio Valleys – Bando natural Reserve (Ferrara)	E
Friuli Venezia-Giulia	Grado and Marano Lagoon (Gorizia)	F
Lazio	Sabaudia Lake (Latina)	G
Marche	Sentina (Ancona)	H
Molise	Mouth of Biferno river (Campobasso)	I
Puglia	Manfredonia (Foggia)	L
Sardegna	S'Ena Arrubia pond (Oristano)	M
Sicilia	Vendicari coastal ponds (Siracusa)	N
Toscana*	Fucecchio marshes (Pistoia)	*
Umbria	Trasimeno Lake (Perugia)	O
Veneto	Averto Valley (Venezia)	P

* Entomological surveillance in the Fucecchio marshes was performed independently of the national programme, as part of a regional West Nile virus surveillance programme funded by the Region of Tuscany (51)

Figure 1
Location of the study areas in Italy (yellow dots)

the mosquito species are distributed equally as adults and larvae, so some species are more common as adults and rare as larvae and vice versa; collecting larvae is often useful to provide more detailed information on the species composition of the local mosquito fauna, especially in the case of species that are more common as larvae than as adults. Temporary and stable, artificial and natural potential larval breeding sites were visited fortnightly near the location of the light traps and larvae were collected by dipping and were preserved in 70% ethanol. For identification, larvae and adult mosquitoes were submitted to the CESME and to the *Istituto Superiore di Sanità* as follows: specimens of larvae were identified based on morphological characteristics as defined for Italian mosquitoes by Romi *et al.* (50) and by the *Istituto Superiore di Sanità* identification keys for Italian mosquitoes. For species identification of early stages, only fourth instar larvae were considered.

Results

Entomological surveys conducted in Italy from 2003 to 2007 have resulted in the capture of a total of 28 798 mosquitoes, of which 14 765 were adults and 14 033 were larvae. Among the adult specimens, 13 060 were suitable for species identification, whereas 1 705 were identified at genus level only because they were damaged. Among the larvae, 14 001 were suitable for species identification, whereas 32 were identified at genus level because of unsuitable storage. Overall, 22 mosquito species were identified, 4 of which belonged to the subfamily *Anophelinae* (one genus only) and eighteen to the subfamily *Culicinae* (six genera). A list of the species collected is presented in Table II, while the abundance of species of over 20 specimens is shown in Figures 2 and 3. Three species represented 84.06% of the entire adult sample, namely: *Ochlerotatus caspius* (36.37%, *n* = 5 370), *Cx. pipiens* (36.9%, *n* = 5 449) and

Table II
Mosquito species collected in the study areas of the national West Nile virus surveillance programme between 2003 and 2007
(relative frequencies of species out of the total number of specimens are given in brackets)

Genus and species	Identification of location*	No. ♀♀	No. ♂♂	Total no. of adults (%)	Total no. of larvae (%)
Aedes (*Stegomyia*) *albopictus* (Skuse, 1897)	A, F, G	16	–	16 (0.11)	32 (0.2)
Aedes (*Aedimorphus*) *vexans* (Meigen, 1830)	G	5	–	5 (0.03)	–
Anopheles (*Anopheles*) *algeriensis* Theobald, 1903	M	1	–	1 (0.01)	–
Anopheles (*Anopheles*) *claviger* (Meigen, 1804)	C, F, L	65	–	65 (0.44)	2 (0.00)
Anopheles (*Anopheles*) *maculipennis* Meigen, 1830	A, D, E, F, H, O, P	1 489	103	1 592 (15.1)	139 (0.99)
Anopheles (*Anopheles*) *plumbeus* Stephens, 1828	F	171	–	171 (1,16)	–
Coquillettidia (*Coquillettidia*) *richiardii* (Ficalbi, 1889)	G	42	3	45 (0.30)	–
Culex (*Neoculex*) *impudicus* (Ficalbi, 1890)	C, F	1	–	1 (0.01)	65 (0.5)
Culex (*Culex*) *pipiens* Linnaeus, 1758	A, B, C, D, E, F, G, H, I, L, M, N, O, P	4 955	494	5 449 (36.9)	12 525 (89.3)
Culex (*Maillotia*) *hortensis* Ficalbi, 1889	D, E, O	8	1	9 (0.06)	208 (1.5)
Culex (*Barraudius*) *modestus* Ficalbi, 1889	O	–	–	–	1 (0.00)
Culex sp.	A, D, E, F, I, L	586	9	595 (1.32)	6 (0.04)
Culex (*Culex*) *theileri* Theobald, 1903	G, H, L, M	9	4	13 (0.09)	15 (0.11)
Culiseta (*Culiseta*) *annulata* (Schrank, 1776)	D, F, G, L, M, P	81	7	88 (0.66)	86 (0.61)
Culiseta (*Culicella*) *litorea* (Shute, 1928)	G	–	–	–	6 (0.04)
Culiseta (*Allotheobaldia*) *longiareolata* (Macquart, 1838)	A, C, D, I, L, M, O	21	19	40 (0.27)	249 (1.77)
Culiseta sp.	D	4	1	5 (0.03)	2 (0.01)
Ochlerotatus (*Ochlerotatus*) *caspius* (Pallas, 1771)	A, C, E, F, G, H, L, M, N, P	5 343	27	5 370 (36.37)	208 (1.48)
Ochlerotatus (*Ochlerotatus*) *detritus* (Haliday, 1833)	F, G, L, M	155	2	157 (1.6)	214 (1.62)
Ochlerotatus (*Ochlerotatus*) *rusticus* (Rossi, 1790)	F, G, O	25	–	25 (0.17)	19 (0.14)
Ochlerotatus (*Ochlerotatus*) *dorsalis* (Meigen, 1830)	G, H	1	2	3 (0.02)	–
Ochlerotatus (*Finlaya*) *geniculatus* (Olivier, 1791)	G, M	1	2	3 (0.02)	–
Ochlerotatus (*Finlaya*) *zammitii* (Olivier, 1791)	N	–	–	–	219 (1.56)
Ochlerotatus sp.	E, F, G, L, M, P	1 101	4	1 105 (7.48)	24 (0.17)
Uranotaenia (*Uranotaenia*) *unguiculata* (Edwards, 1913)	E, H, P	3	4	7 (0.05)	13 (0.09)
Total		14 083	682	14 765	14 033

* See Table I for the identification of each location

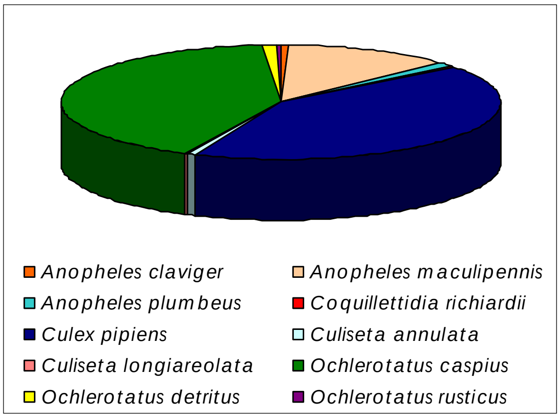

Figure 2
Mosquito species identification in Italy, 2007: adults

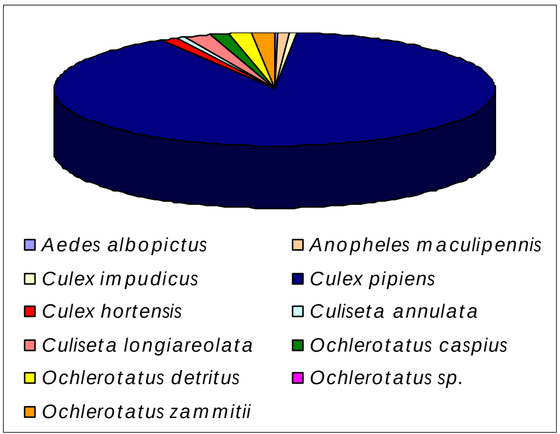

Figure 3
Mosquito species identification in Italy, 2007: larvae

An. maculipennis (10.78%, *n* = 1 592). Among the larvae (Table II) *Cx. pipiens* was the most prevalent species, representing 89.3% of the sample (*n* = 12 525).

Discussion

Among the species collected, four are known to feed on birds and humans, namely: *Anopheles plumbeus* Stephens, 1828, *Culiseta annulata* (Schrank, 1776), *Culiseta litorea* (Shute, 1928) and *Ochlerotatus detritus* (Haliday, 1833) (= *Aedes detritus*) (47) and therefore could be considered as potential 'bridge vectors' (Table III); six species, *Anopheles algeriensis* Theobald, 1903, *Anopheles claviger* (Meigen, 1804), *Ochlerotatus rusticus* (Rossi, 1790) (= *Aedes rusticus*) (47), *Ochlerotatus dorsalis* (Meigen, 1830) (= *Aedes dorsalis*) (47), *Ochlerotatus geniculatus* (Olivier, 1791) (= *Aedes geniculatus*) (47) and *Ochlerotatus zammitii* (Olivier, 1791) (= *Aedes zammitii*) (47) are known to bite humans but are considered infrequent avian feeders (50) and consequently their occurrence could represent a low risk of transmission of WNV to humans. According to the literature, eight of the remaining species have been found to be naturally infected with WNV or successfully infected in the laboratory in certain areas of Europe and in the United States, namely: *Ae. albopictus*, *Ae. vexans*, *An. maculipennis*, *Coquillettidia richiardii*, *Cx. modestus*, *Cx. pipiens*, *Cx. theileri* and *Oc. caspius* (4, 14, 21, 24, 25). A brief description of the bionomics of the species that can play different roles in the epidemiology of the WNV disease is discussed below.

Ae. albopictus, the 'Asian tiger mosquito' has recently become established in Western Europe (it originates from South-East Asia). It was first reported in Italy in 1990 (53) and has spread across the country, mainly dispersed by the internal trade of used tyres that harbour its eggs (10, 11, 34, 49). The species has recently revealed its ability to adapt very rapidly to the local climate of colonised areas: females of some populations occur in central and southern Italy and continue their trophic activity throughout the winter without interruption (61). *Ae. albopictus* females have shown competence for over 20 arboviruses, including dengue, West Nile and yellow fever (35). Apart from its nuisance as a biting insect, the adaptability and behaviour of *Ae. albopictus* makes it suitable as a bridge vector of WNV. Moreover, this virus has occasionally been isolated from the same species in the United States, but not elsewhere (29) and the mosquito has proved to be a competent vector in the laboratory (63).

The distribution of *Ae. vexans* is considered to extend from the west of the Palearctic Region to Kazakhstan in the east (17) and is present in the Afrotropical, Nearctic and Neotropical Regions (42). The species is an aggressive human biter.

In Italy, *An. maculipennis* occurs as a complex of seven species, namely: *An. maculipennis*, *An. labranchiae* Falleroni, 1926, *An. atroparvus*

Table III
Mosquito species collected during surveillance in the study areas and their potential role in West Nile virus transmission according to the literature
(9, 21, 24, 46, 50, 56, 57, 58)

Genus and species	Involved in West Nile virus transmission elsewhere*	Bird-biting**	Human-biting**	Possible bridge vector: bird and human-biting**
Aedes (Stegomyia) albopictus (Skuse, 1894)	X	X	X	–
Aedes (Aedimorphus) vexans (Meigen, 1830)	X	–	X	–
Anopheles algeriensis (*Anopheles*) Theobald, 1903	–	–	X	–
Anopheles (Anopheles) claviger Meigen, 1804	–	–	X	–
Anopheles (Anopheles) maculipennis Meigen, 1818	X	–	X	–
Anopheles (Anopheles) plumbeus Stephens, 1828	–	X	X	X
Coquillettidia (Coquillettidia) richiardii (Ficalbi, 1889)	X	X	X	X
Culex (Neoculex) impudicus Ficalbi, 1890	–	X	–	–
Culex modestus (*Barraudius*) Ficalbi, 1889	X	X	X	X
Culex (Culex) pipiens Linnaeus, 1758	X	X	X	X
Culex (Maillotia) hortensis Ficalbi, 1889	–	–	–	–
Culex (Culex) theileri Theobald, 1903	X	–	X	–
Culiseta litorea (*Culicella*) (Shute, 1928)	–	X	–	–
Culiseta (Culiseta) annulata (Shrank, 1776)	–	X	X	X
Culiseta (Allotheobaldia) longiareolata (Macquart, 1838)	–	–	–	–
Ochlerotatus (Ochlerotatus) caspius (Pallas, 1771)	X	–	X	–
Ochlerotatus (Ochlerotatus) detritus (Haliday, 1833)	–	X	X	X
Ochlerotatus(Ochlerotatus) dorsalis (Meigen, 1830)	X	–	X	–
Ochlerotatus (Finlaya) geniculatus (Olivier, 1791)	–	–	X	–
Ochlerotatus (Rusticoides) rusticus (Rossi, 1790)	–	–	X	–
Ochlerotatus zammitii (*Finlaya*) (Olivier, 1791)	–	–	X	–
Uranotaenia (Pseudoficalbia) unguiculata (Edwards, 1913)	–	–	–	–

* References 21 and 24
** References 9, 46, 50, 56, 57 and 58

Van Thiel, 1927, *An. sacharovi* Favre, 1903, *An. messeae* Falleroni, 1926, *An. melanoon* Hackett 1934 and *An. subalpinus* Hackett and Lewis 1935. The distribution of *An. maculipennis* as a single polytypic species occurs in Europe, North Africa and north Asia (17).

An. plumbeus is a Palearctic species that it is very common in Italy; suitable breeding habitats are available everywhere (50). *An. plumbeus* is often found with *Ochlerotatus geniculatus* (Olivier), *Oc. berlandi* Seguy and *Orthopodomyia pulcripalpis* (Rondani) in the holes of barks of trees in urban parks (50).

Cq. richiardii has a Mediterranean distribution, extending as far east as the Chernovtsy Region of Russia (17). In Italy, the species is relatively rare due to the absence of the typical larval habitats but its presence has been reported on the mainland as well as on the larger islands (50). *Cq. richiardii* is a univoltine species with larvae developing slowly from one year to the next, surviving under leaves and in mud during the winter. Females feed both indoors and outdoors on birds and mammals, including humans (56).

Cx. modestus is widespread in Europe and Central Asia (17); in Italy it occurs along the northern coastal plains (50). Breeding sites in rural environments include permanent or semi-permanent constructions that retain water, such as irrigation canals or drainage systems; larvae can also develop in slightly saline water and can be found with *Cx. pipiens*.

Larvae are sampled from the late spring to early autumn but peak in July and August. Females feed so aggressively on humans that they were considered one of the most annoying mosquitoes, together with *Oc. caspius* and *Ae. vexans* along the northern coastal plains of Italy (50).

Cx. pipiens occurs everywhere and in the Palearctic Region consists of two biological forms, namely: the urban form of *Cx. pipiens molestus* Forskål (65) and the rural form, *Cx. pipiens pipiens* (50). Female *Cx. pipiens pipiens* are mostly bird-biting, they hibernate from the autumn to the spring, are anautogenous (they require blood-meal for egg development) and eurygamous (they need open spaces for mating). Breeding sites consist of various types of pools and water containers. On the other hand, the female *Cx. pipiens molestus* biotypes feed on humans, they are mainly autogenous (they produce eggs without the first blood-meal) and stenogamous (they are capable of mating in limited spaces), and continuous generations are created throughout the year, without diapause. These two forms often overlap over extensive areas and morphological differences can be taken back to the different siphonal index even if this feature is visible by means of biometric analysis of very large amounts of larvae (52). In regard to the biochemical aspects, the electrophoretic analyses of several enzymatic loci enable identification of adults (64).

Cx. theileri is not distributed continuously, as it occurs in Canary Islands, the Mediterranean Basin, Yemen, south-east Africa and India (17); its presence in Italy is limited to the southern regions and to Sardinia and Sicily. Larvae of *Cx. theileri* develop in rice fields, ponds and swampy areas. Early stages occur from the spring to autumn and the species reach maximum adult density in the summer. The species pass the winter as adults and females feed mainly at dusk, often on humans (50).

Culiseta annulata is widespread in Europe and is also observed in Anatolia and North Africa; it is very common in Italy (50). This species that has nocturnal activity is mainly ornithophylic although it can feed on humans and domestic animals. Larvae and adults can overwinter over a very short period of diapause, but usually females may lay eggs and produce a generation during the coldest months of the winter (50).

Oc. caspius is distributed extensively along the coasts of Europe and the Mediterranean (17). In Italy, it occurs mainly along the coastal plains (50). The species prevails in flooded fields or salt marshes that are suitable for the early stages of development (48); it also occurs in places where water is artificially retained, such as rice fields and canals (50). Its main habitats are river valleys where it is predominant among mosquitoes that attack humans and animals (17).

Oc. detritus is a widespread species in the Palearctic Region and usually occurs along coastal plains (50). Larvae overwinter at different stages of development and the first adults emerge in the spring and bite humans persistently: adult mosquitoes of this species are mainly anthropophilic (48).

The predominance of adult *Oc. caspius*, *Cx. pipiens* and *An. maculipennis* is possibly due to their prevalence in wetlands and peridomestic environments, especially in the presence of cattle and poultry and also due to the intrinsic selectivity of the CDC light traps. As an example that is representative of the annual seasonal dynamic of these populations from all the study areas, Figure 4 shows the monthly abundance of the three species and the total number of specimens collected in all the study areas in 2007. Moreover, to illustrate the situation in different climatic zones across Italy, three examples of seasonal abundance of the same species are given in Figures 5, 6 and 7, where we considered the study area of Friuli Venezia-Giulia, Puglia and Lazio Regions to be representative of the north, south and central areas of Italy, respectively. The trends revealed by the Figures are mostly influenced by climatic conditions, above all by temperature, rainfall and relative humidity that determine the increase or the decline in presence of one or more species.

Oc. caspius, most abundant along the north-eastern Adriatic coastal plains, mainly in the Friuli Venezia-Giulia Region, as shown in

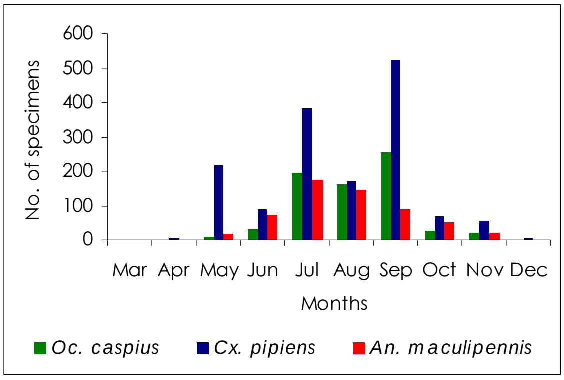

Figure 4
Seasonal dynamics of *Ochlerotatus caspius*, *Culex pipiens* and *Anopheles maculipennis* populations from all study areas, March to December 2007

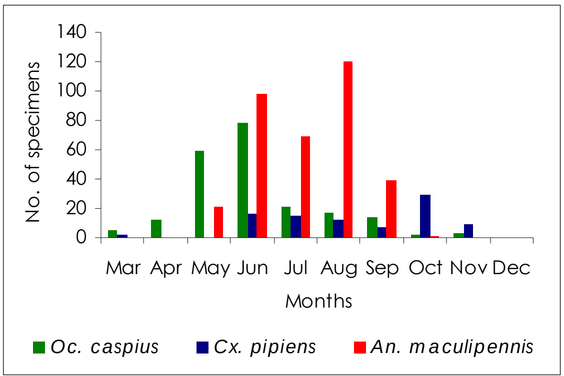

Figure 5
Seasonal dynamics of *Ochlerotatus caspius*, *Culex pipiens* and *Anopheles maculipennis* populations from the study area of the Friuli Venezia-Giulia Region (north-east Italy), March to December 2007

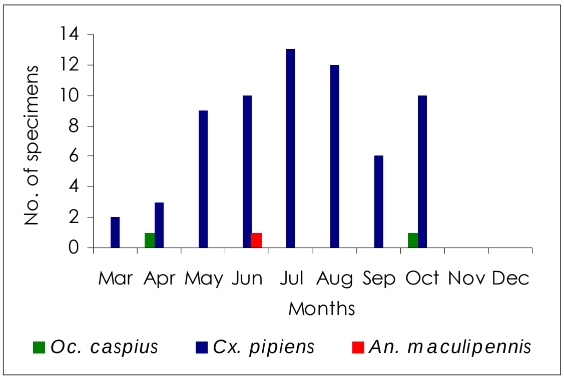

Figure 6
Seasonal dynamics of *Ochlerotatus caspius*, *Culex pipiens* and *Anopheles maculipennis* populations from the study area of the Puglia Region (southern Italy), March to December 2007

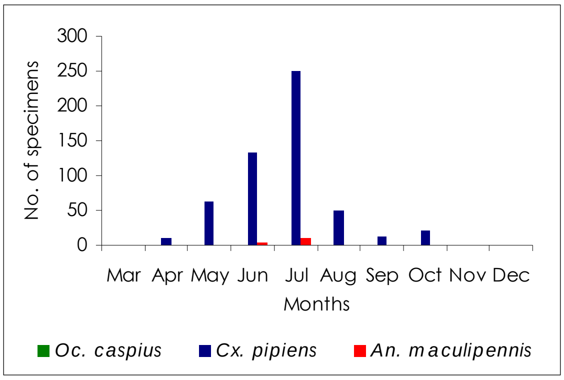

Figure 7
Seasonal dynamics of *Ochlerotatus caspius*, *Culex pipiens* and *Anopheles maculipennis* populations from the study area of the Lazio Region (central Italy), March to December 2007

Figure 5, and in the Emilia Romagna Region seems no longer to be very common in the Lazio Region where it was absent from the adult specimens in 2007 (Fig. 7). This species is typical of coastal habitats, such as brackish marshes, and is considered capable of playing a role as a vector of WNV. This statement is supported by its ability to transmit the virus, based on the previous detection of WNV in specimens of this species occurring elsewhere (21). In Italy, even if this species is known to mostly feed on mammals and humans (Table III), it can fill the role of a bridge vector based on its focal abundance on farms and in tourist areas.

Cx. pipiens, generally regarded as the most competent vector of WNV in Europe (55), was present in all the study areas, showing the usual seasonal thermophilic trend with a rise in the spring and the peak of abundance in July-August (Figs 4, 5, 6 and 7). Most of the larvae collected during the study period were obtained from this species which is considered to be the most abundant in wetlands. *Cx. pipiens* has a broader host range, including many bird species (1), so it could amplify the volume of WNV circulating in the late spring and early summer (51). Moreover, *Cx. pipiens* can play the role of bridge vector (3), transmitting the virus from birds to horses and/or humans (Table III). Despite information reported in the literature, the members of the *An. maculipennis sensu latu* complex are known to usually feed on mammals, including humans, rather than on birds (29), most of our specimens were collected in poultry shelters which led us to consider them as a potential bridge vector, and also in the light of the recent detection of naturally WNV-infected specimens that have occurred elsewhere (21). Among members of the complex, three isolates of the virus have been recovered in Portugal and Ukraine (21). In the example reported in 2007, *An. maculipennis sensu latu* occurred only in June and July in the study area of the Lazio Region, in June in the Puglia Region (Fig. 6), whereas it is widespread in the Friuli Venezia-Giulia Region study areas, probably because of the different features of the cattle shelters where these mosquitoes are usually found.

An. plumbeus is relatively common on farms, in small villages and in urban parks where it feeds on both birds and humans (60). Although it presents the characteristics of a potential bridge vector, the virus has never been detected in this species.

Oc. detritus was relatively rare among the mosquitoes collected during our study but it may be considered as one of the potential bridge vectors because its behaviour does not differ much from that of *Oc. caspius.* To date, no WNV has been isolated from this species (21).

In the United States, WNV has been detected in *Ae. vexans* (19), but this species did not occur frequently in the study areas although it is common in Italy (50).

Cq. richiardii is known to feed on both birds and humans and some authors consider it to be one of the principal WNV vectors in Europe after *Cx. pipiens* and *Cx. modestus* (18, 24, 55). However, its involvement has not been confirmed in the more recent literature. In Italy, this species is relatively rare (as reflected by our findings) and probably could not fulfil the role of bridge vector on its own.

Our activity did not include some ornitho-philic mosquito species that are possibly involved in bird-to-bird transmission, e.g. *Culex* (*Barraudius*) *modestus* Ficalbi that was implicated as the primary enzootic vector during the 1962 outbreak of WNV in France (36). We collected only one specimen, despite the fact that this species is quite common in Italy. This data was probably missed because of the particular breeding sites of this species (permanent replacements of fresh water in rural areas of the coastal plains of the northern Italy) and the selective activity of the CDC traps.

Conclusions

In conclusion, national and international surveillance for WNV transmission will be important to monitor the spread of this virus. Further knowledge on the ecologic aspects of WNV transmission could provide additional tools that would be valuable to determine those geographic areas that are at greater risk from the disease.

The mosquito population has never been screened before in Italy and could offer valuable information on the occurrence of potential WNV vectors. In this perspective, the surveillance of Italian mosquitoes needs to continue to ensure that we have a more comprehensive understanding of the bionomics of the possible WNV vectors and to provide a clear picture of global WNV monitoring activities.

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