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Arboviruses in mosquitoes from Emilia-Romagna (July 18–August 10, 2023)

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Aim of the study

Viral diseases transmitted by mosquitoes are increasingly reported in recent years worldwide[1]. Between these viruses West Nile virus (WNV) is actively surveyed in the Emilia-Romagna region, with the principal aim to guarantee the health safety of blood donations. Along with WNV the related Usutu virus (USUV) is also monitored mainly to exclude cross-reactivity[2].
Other mosquito borne arboviruses circulating in Europe that may cause human diseases are not under surveillance.
With the aim to investigate arboviruses circulating in Emilia-Romagna, we take advantage to the Regional plan of arbovirus surveillance, to search for other arboviruses, in particular Tahyna virus (TAHV) and Sindbis virus (SINV) in mosquitoes collected in the lowland of the region.

Methods used

Mosquitoes were collected by 98 georeferenced traps evenly distributed in the lowland area of the Emilia-Romagna region, according to 11x11 km grid (Fig. 1). Attractive traps, baited with CO_2 were used overnight every two weeks. Collected mosquitoes in the period from July 18 to August 10, 2023, were identified at species level with morphological keys and pooled according to day and place of collection in monospecific pools, with a maximum of 200 females per pool (Tab. 1). Pools were ground in PBS with copper-plated beads, centrifuged and part of the supernatant were submitted to biomolecular analysis. Extracted samples were retro transcribed and tested with specific real time PCRs for WNV[3], USUV[4], TAHV[2], and SINV[5]; moreover, the pan-genus protocols plus sequencing was applied for orthoflaviviruses and orthobunyaviruses.

Results and conclusions

In the four week period, 44,035 mosquitoes, belonging to 10 species, were collected, grouped in 745 pools and tested. The most collected species were *Culex (Cx.) pipiens* (67.6 %, 29,744) followed by *Aedes (Ae.) caspius* (22.3 %, 9838), *Ae. vexans* (5.6%, 2484) and *Ae. albopictus* (4 %, 1780).
More abundantly detected viruses were WNV (51 pools) and USUV (16 pools). This result may be influenced by the organization of surveillance that is focused on the detection of these viruses.
The viruses were detected mainly in *Cx. pipiens* pools, 48/51 pools for WNV and all the 16 pools for USUV, confirming this species as their major vector. Three pools composed by other mosquito species, such as *Ae. albopictus*, *Anopheles (An.) plumbeus*, *An. maculipennis s.l.*, tested positive for WNV. This does not mean that these species are playing a concrete vector role, but it may indicate their potential contribution as a bridging vector, in particular for the Asian Tiger mosquitoes in which this role was already hypothesized.
No positive-SINV pools were collected, the lack of detection of that virus seem to indicate at least its limited diffusion, even if further investigations are necessary to evaluate its potential presence. The TAHV was detected in 7 mosquito pools, namely *Ae. caspius* (2), *Cx. pipiens* (2), *Ae. albopictus* (1), *Ae. vexans* (1), *An. maculipennis s.l.* (1). Interestingly *Ae. caspius*, in addition to being one of the species with two positive pools, showed also the lowest Ct values in real time PCR, indicating a major viral load compared to other mosquito species. The detection of TAHV deserves a more in deep investigation, to evaluate potential cases of human disease caused by this virus.

Table 1 – Number of mosquitoes tested by species and detected viruses.

Species	Number	%	Pools	TAHV+	WNV+	USUV+	SINV+
Aedes albopictus	1780	4.0	138	1	1	–	–
Aedes caspius	9838	22.3	170	2	–	–	–
Aedes cinereus	21	<0.1	2	–	–	–	–
Aedes geniculatus	4	<0.1	2		–	–	–
Aedes vexans	2484	5.6	100	1	–	–	–
Anopheles maculipennis s.s.	99	0.2	38	1	1	–	–
Anopheles plumbeus	17	<0.1	8	–	1	–	–
Coquillettidia richiardi	47	0.1	9	–	–	–	–
Culex pipiens	29,744	67.5	259	2	48	16	–
Culiseta longiareolata	1	<0.1	1	–	–	–	–
	44,035		727	7	51	16	

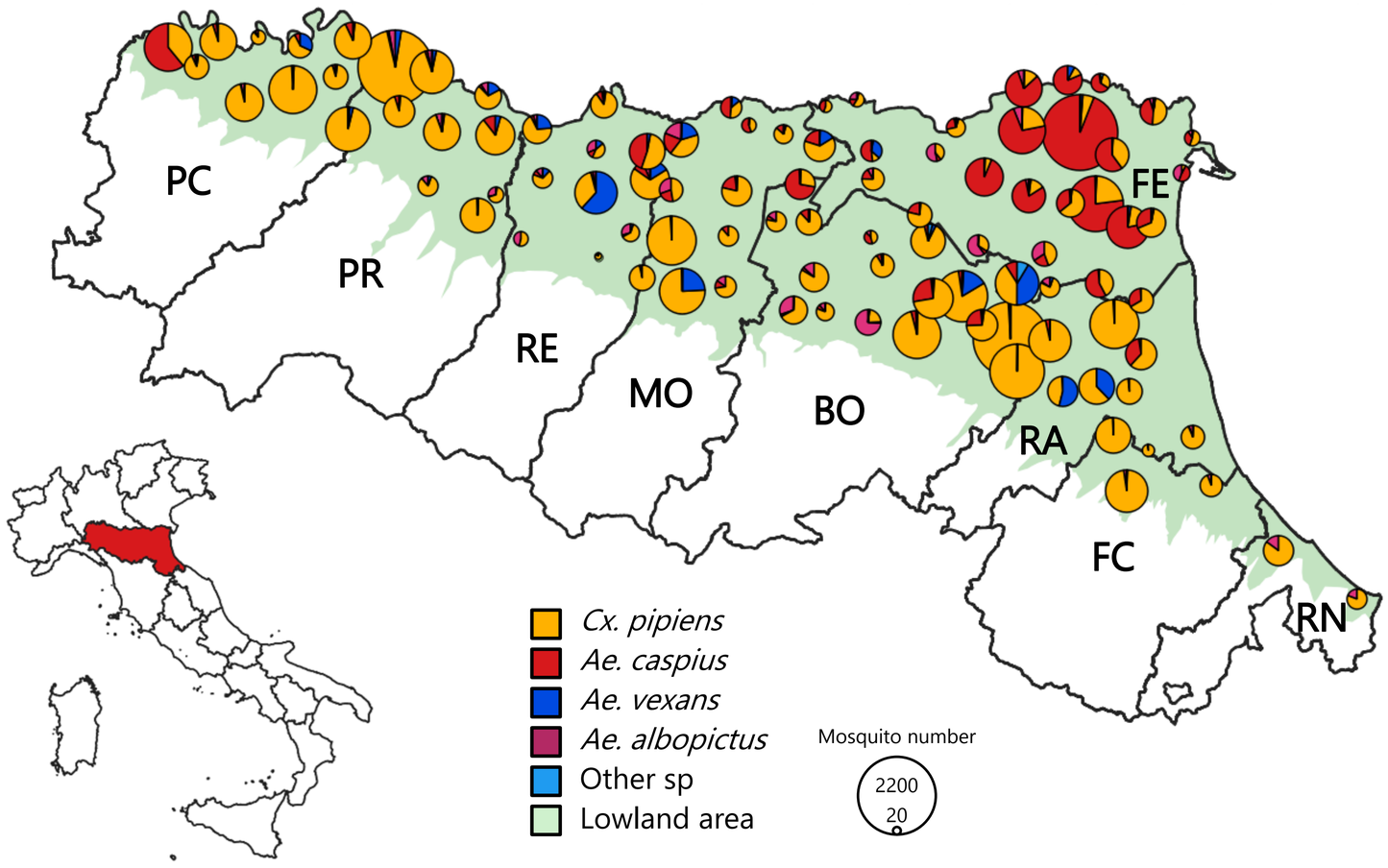

Figure 2 - Localization of sampling sites on the Emilia-Romagna map showing the sampled mosquitoes (circles proportional to the sampled specimens) and the location of Emilia-Romagna (in red) on a map of Italy. Provinces: PC: Piacenza, PR: Parma; RE: Reggio; MO: Modena; BO: Bologna; FE: Ferrara; RA: Ravenna, FC: Forlì-Cesena, RN: Rimini.

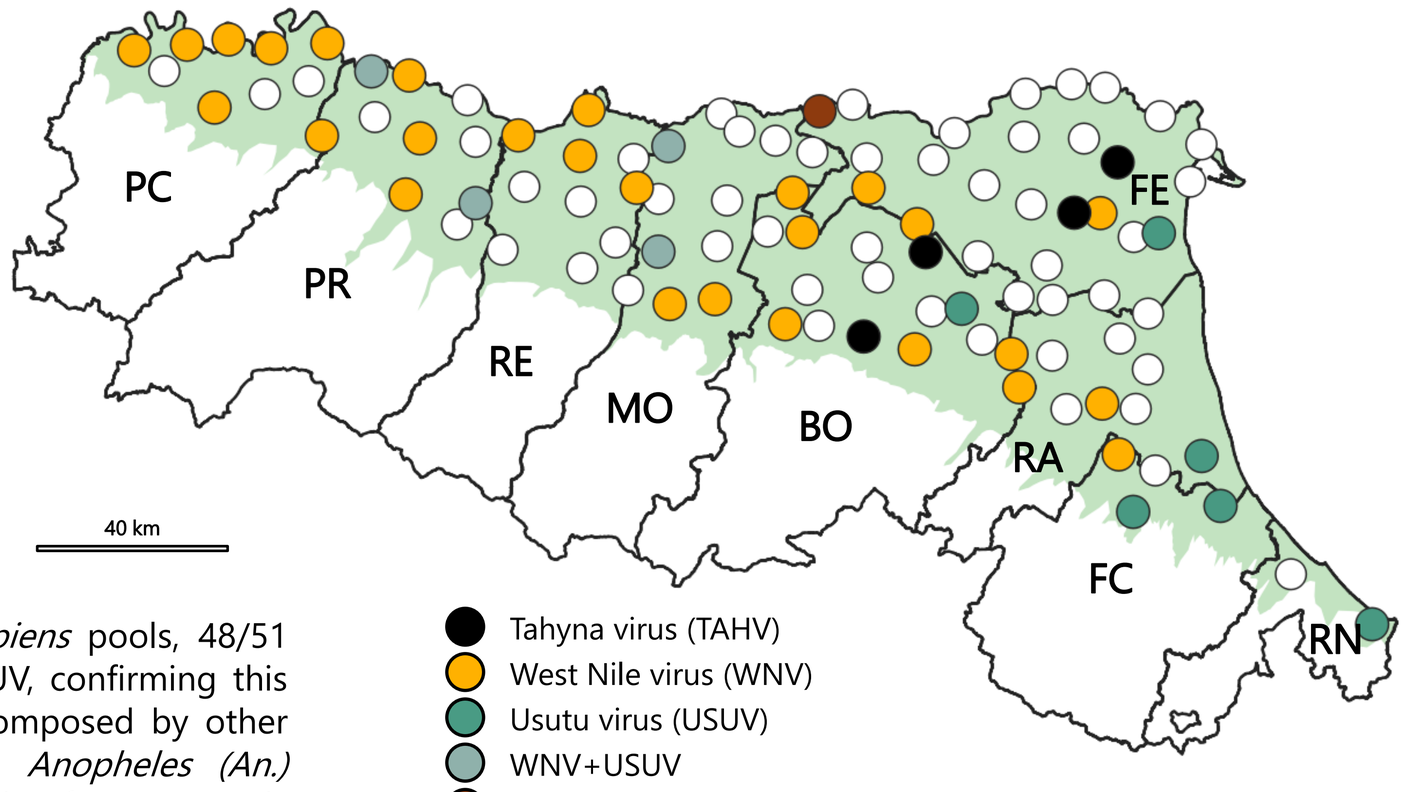

Figura 2. Map of sampled sites in ER with reference to the detections of mosquitoes positive poos. Surveyed area (lowland) in green.

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