Seasonal monitoring of Aedes albopictus*: practical applications and outcomes*

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Summary

The introduction of the Asian tiger mosquito *Aedes* (*Stegomya*) *albopictus* (Skuse, 1897) (Diptera: Culicidae) into temperate regions poses serious concerns for the risk of the spreading of arboviral epidemics, as confirmed by the chikungunya fever outbreak, in Italy. Integrated wide-sized ovitrap monitoring is a helpful tool in any good pest-control strategy. The *Ae. albopictus* population dynamics were monitored over a four-year period in the town of Pesaro (Marche, Italy), using 60 ovitraps. Twenty-day larvicide-based treatment cycles were implemented for the manholes of the urban area and also the inhabitants were involved in pest control relating to their own properties. The weekly median of eggs laid was reduced from 2008 to 2011, which indicated the good performance of the vector control and a reduction in the related epidemics risk. The monitoring system adopted is described here, and proposed as a cost-effective and available system for extensive vector surveillance.

Keywords

Ovitrap, *Aedes albopictus*, vector monitoring, arbovirus, pest control, mosquito, surveillance system.

Introduction

The diffusion of the Asian tiger mosquito *Aedes (Stegomya) albopictus* (Skuse, 1897) (Diptera: Culicidae) is increasing across Europe, as well as on other continents. Over the last two decades, its presence in Mediterranean areas has increased, as has its spread into the northern countries of the European Union, with new exotic species of mosquito also being introduced (2, 3, 11, 15, 29). In some countries, *Ae*. *albopictus* has displaced *Ae*. *aegypti* (Linnaeus, 1862). This poses concern for dengue and chikungunya epidemics, for the increased involvement of *Ae*. *albopictus* as a vector, and for the spread of arboviruses outside endemic areas (1, 4, 7, 9, 30). This situation has been confirmed by the recent outbreaks of dengue fever in France (8) and Croatia (5), and of chikungunya fever in Italy (10). These outbreaks also suggest the need to improve the surveillance and control of this arbovirus vector and pest, *Ae. Albopictus* (1). Indeed, it is known that a lack of mosquito control and related public health strategies can enhance the population density of *Ae. albopictus*, which can thus increase the risk of further epidemics (4, 11, 21, 28, 30).

In Italy, the Ministry of Health has recommended that the Public Health Services improve arbovirus surveillance. However, mosquito surveillance and control are often not very well practised and are based on little experience, because of the lack of national schemes for these activities (12). Consequently, every municipality is responsible for its own territory, as well as the population being responsible for their own properties, with only a few regions carrying out well-coordinated surveillance (4, 14, 24).

The distribution of *Ae*. *albopictus* is determined by several environmental variables (2, 15, 16), with the main parameters arising from previous studies being the mean temperature in January ($JanT_{mean}$), the annual mean temperature ($AnnT_{mean}$), and the annual precipitation. A $JanT_{mean}$ <0 °C affects the survival rate of the diapausing eggs during the winter period (17, 18). When the $AnnT_{mean}$ is >11 °C, this determines the areas that are suitable for adult survival (2, 18, 19, 20). Finally, an annual rainfall >500 mm is the threshold for habitats where mosquito populations can thrive (22).

In the regions along the Adriatic coast in Italy, the *Ae*. *albopictus* population trend is characterised by a peak in summer and the disappearance of the adults in winter (with the exceptions of most of the more southern latitudes). This trend helps to control the mosquito population early in spring (at the initial stage of infestation), which will also benefit from focused surveillance of human cases of arboviruses during the higher risk periods of epidemics (23, 12, 14). To achieve this goal, a regional scheme for chikungunya and dengue surveillance and *Ae*. *albopictus* monitoring was set-up in the Marche and Emilia Romagna regions of central-eastern Italy, with the involvement of the larger municipalities in both of these regions (1, 24). This scheme was carried out in the Marche region with very limited budget and resources. The monitoring system that was followed, which was not mandatory for all municipalities in the region, also included staff training (community workers, environmental officers, cleaners, volunteers of the regional Civil Protection Authorities). Information and practices were shared with the stakeholders (the local population, politicians and general practitioners).

This report describes the monitoring system adopted in the town of Pesaro (Marche, Italy), and it discusses the results of the survey through the years of 2008 to 2011.

Materials and methods

The monitoring system presented here was carried out in the Pesaro municipality (43°91'20"N; 23°91'57"E), in the north of the Marche region (central-eastern Italy), at 11 m a.s.l., with a population of 95,011 inhabitants, and a total urban area of ca. 12,658 ha (6).

The monitoring was achieved through the use of ovitraps. Each ovitrap consisted of a black plastic pot of 400 ml that was filled with dechlorinated water that contained a strip of masonite (12.5 × 2.5 cm in size), where the *Ae. albopictus* laid their eggs. A total of 60 ovitraps were distributed homogeneously in the urban area. Each trap was placed at a minimum distance of 500 m from the next one. The positions of ovitraps were previously planned using the geographic information system. The ovitrap density was decided upon according to the Emilia Romagna guidelines, as reported for municipalities with urbanised areas between 3,001 ha and 5,000 ha and without previous monitoring data (24, 25). The ovitraps were placed at ground level by trained technicians, preferably on grass, hidden and in the shade, so the *Ae*. *albopictus* can rest and lay their eggs. The ovitraps were left in these same positions through the whole period of the survey. On a weekly basis, and usually on Wednesdays, the masonite strips were collected and delivered to the laboratory. The ovitraps were then filled up again, with the masonite strip was replaced (26).

Identification and counting of the eggs were performed in the laboratory, under stereomicroscopy at 40× magnification (Olympus SZX7, Japan).

Over the four years of the survey (2008-2011), the ovitraps were activated for a period of 20 weeks each year, during the vectorial season. This occurred from week 20 to week 40 of each year (late May to early October). The total number of eggs per ovitrap was published on-line in a weekly bulletin (17). The median number of total eggs harvested weekly from the ovitraps was used for the descriptive analysis. The median was used because the data do not fit a normal distribution.

Analysis of the seasonal population dynamics of *Ae*. *albopictus* was performed using the ovitrap index, as the number of positive ovitraps/ the number of active ovitraps ×100. These are reported as weekly box-and-whisker plots.

The presence of annual trends was tested for by using the Cuzick test, a non-parametric test for trends across ordered groups, which is an extension of the Wilcoxon rank-sum test (13, 27, 28). The Cuzick test was used to evaluate the trends in the median levels of eggs laid across the four years considered. The statistical analyses were performed with the Stata 11.1 software, and the data have also been reported using the Map Info Professional software, version 7.5 (data not shown).

Each ovitrap was identified by its own barcode, which also coded for the coordinates and elevation of each site monitored. The week of sampling and the number of eggs were the data produced by the laboratory, and this information was automatically included in a database. Excel files of the weekly monitoring data were sent to the municipalities.

The meteorological data were obtained from a local weather station: the Meteorological Observatory “Valerio”, Pesaro. These data included the daily temperatures (T_{min}, T_{max}) and the rainfall through the four years of 2008 to 2011.

Results

The percentage of active ovitraps over the four years of monitoring ranged from a minimum of 92% to a maximum of 100%. The median numbers of eggs and the selected percentiles per year are detailed in Table I.

Table I. Median egg numbers per year, with selected percentiles (P).

Year	Eggs per year, with percentiles (P)						
	Maximum	**Minimum**	**P25**	**P50**	**P75**	**P98**	**P99**
2008	980	0	0	40	107	407	535
2009	547	0	0	8	51	289	325
2010	330	0	0	0	4	75	150
2011	320	0	0	0	3	145	205

Figure 1 illustrates the weekly box-and-whisker plots over the whole of the monitoring season through each of the four years (as weeks 20 to 44). Figure 2 shows the ovitrap indices calculated for the same periods.

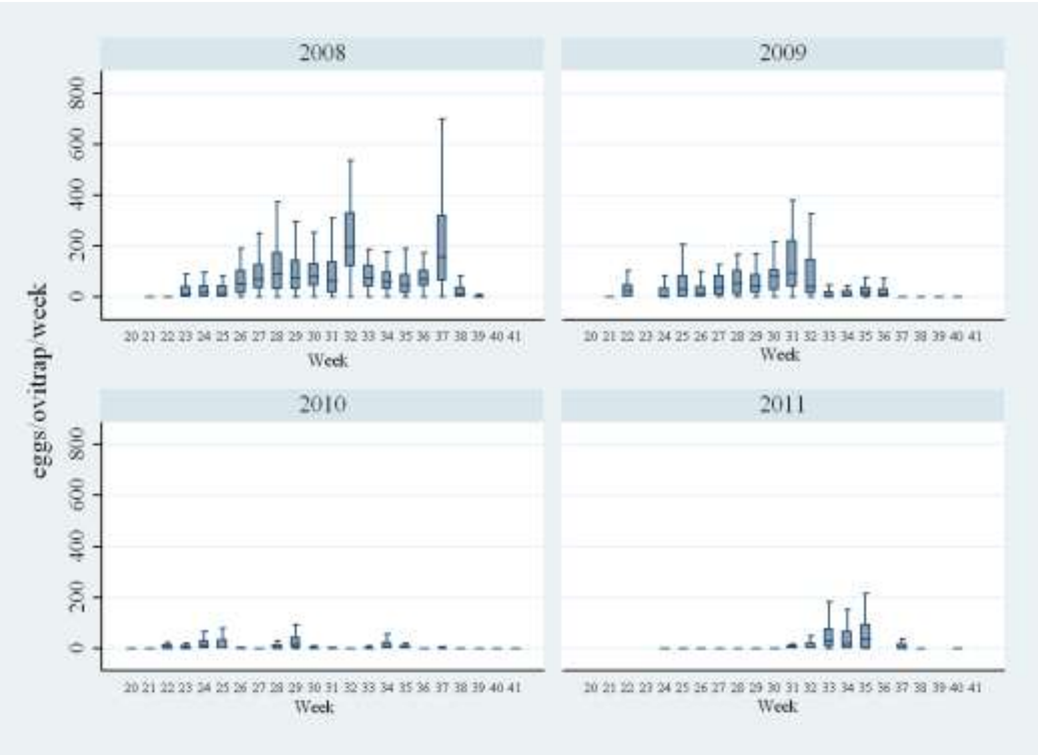

Figure 1. Box-and-whisker plots of the seasonal population dynamic based on egg density for the years 2008 to 2011.

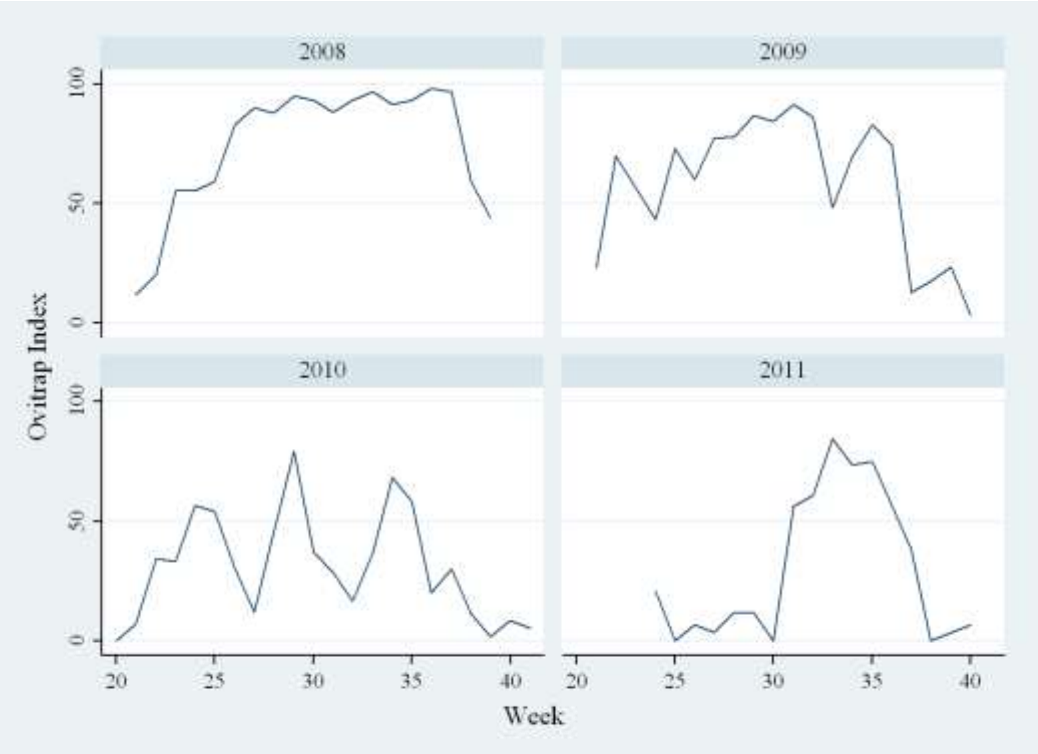

Figure 2. Ovitrap index trends for the years 2008 to 2011.

In the first year of monitoring (2008) the seasonal dynamics analysis (Figures 1, 2) showed three peaks of egg density: the first during the week 28, in mid-July (median number of eggs/ ovitrap/ week, 91; ovitrap index, 87%); the second for week 32, in mid-August (median number of eggs/ ovitrap/ week, 195; ovitrap index, 93%); and the third for week 37, in mid-September (median number of eggs/ ovitrap/ week, 155; ovitrap index, 96%). Considering the whole observation period, the weekly ovitrap index was >50% and the median number of eggs exceeded 50 per week from week 26 to week 37 (Figures 1, 2).

In the second year of monitoring (2009), there were again three peaks seen: the first peak of eggs was detected during week 25, in mid-to-late June (median number of eggs/ ovitrap/ week, 31; ovitrap index, 72%); the second peak for week 28, in mid-July (median number of eggs/ ovitrap/ week, 52; ovitrap index, 77%), and the third peak for week 31, in early August (median number of eggs/ vitrap/ week, 93; ovitrap index, 91%) (Figures 1, 2).

In the last two years of monitoring (2010, 2011), the data showed greatly lowered seasonal trends for the median numbers of eggs (Figures 1, 2). In 2010, three small peaks were again detected: the first in week 25, in mid-to-late June (median number of eggs/ ovitrap/ week, 4; ovitrap index, 54%); the second in week 29, in mid-to-late July (median number of eggs/ ovitrap/ week, 16; ovitrap index, 79%), and the third in week 34, towards the end of August (median number of eggs/ ovitrap/ week, 7; ovitrap index, 68%). Finally, in 2011, the single peak for the median numbers of eggs was seen for week 35, early in September (median number of eggs/ ovitrap/ week, 37; ovitrap index, 75%). Although they showed low median numbers of eggs, the ovitrap indices for 2010 and 2011 remained at relatively high levels (as ovitrap index >50%) for weeks 34 and 35, and for weeks 31 to 35, respectively (Figures 1, 2).

The seasonal population trends of the *Ae*. *albopictus* dynamics from 2008 to 2011 are shown in Figure 3. The significant of the progressive reduction in the number of eggs from 2008 to 2011 was verified by the Cuzick test for the trend (p <0.0001).

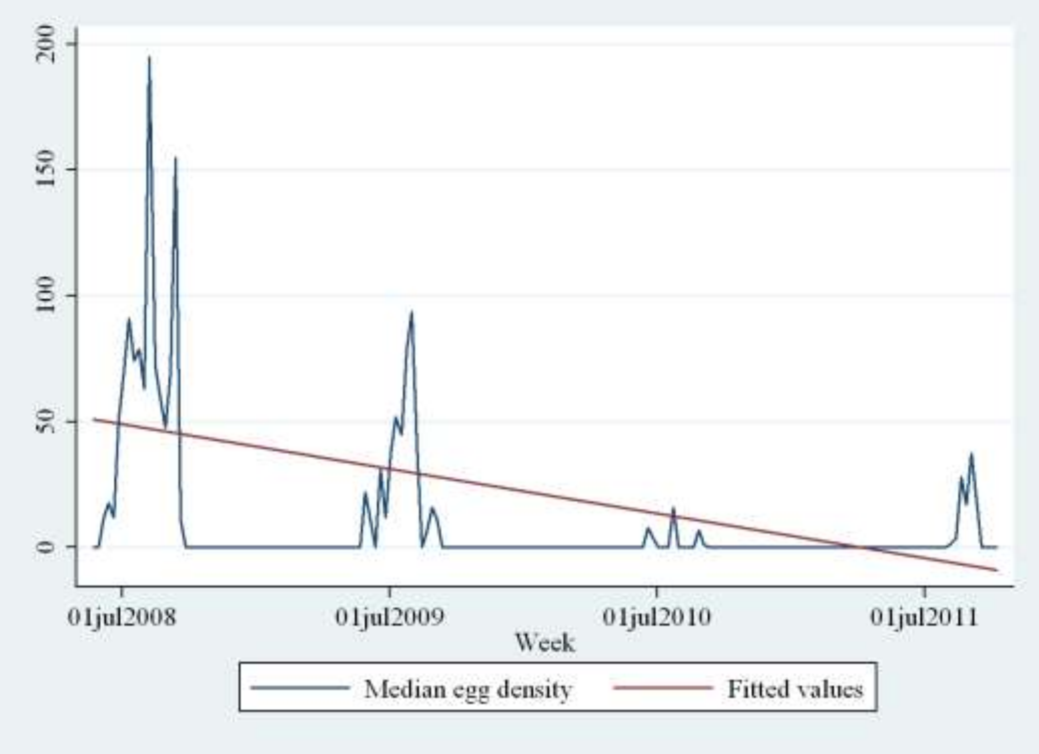

Figure 3. Trend in the weekly median number of eggs for the years 2008 to 2011.

Meteorological data were collected and analysed to provide the means for the January and annual temperatures and the total annual rainfall (Table II). The $JanT_{mean}$ through these four years was always >0 °C, with $JanT_{mean}$ from 3.1 °C (2010) to 5.8 °C (2008). Similarly, the $AnnT_{mean}$ are considerably higher than 11 °C, varying from 17.8 °C (2010) to 19.1 °C (2008). The rainfall from 2008 to 2010 was also a lot higher than the minimum of 500 mm: from 664 mm (2011) to 1,203 mm (2010). The mean temperatures and the rainfall measured during the monitoring period are briefly reported in figure 4.

Table II. Mean temperatures and rainfall for the years 2008 to 2011.

Year	$JanT_{mean}$ (°C)	$AnnT_{mean}$ (°C)	Rainfall (mm)
2008	5.8	19.1	864
2009	4.9	19.0	787
2010	3.1	17.8	1,203
2011	4.0	18.8	664

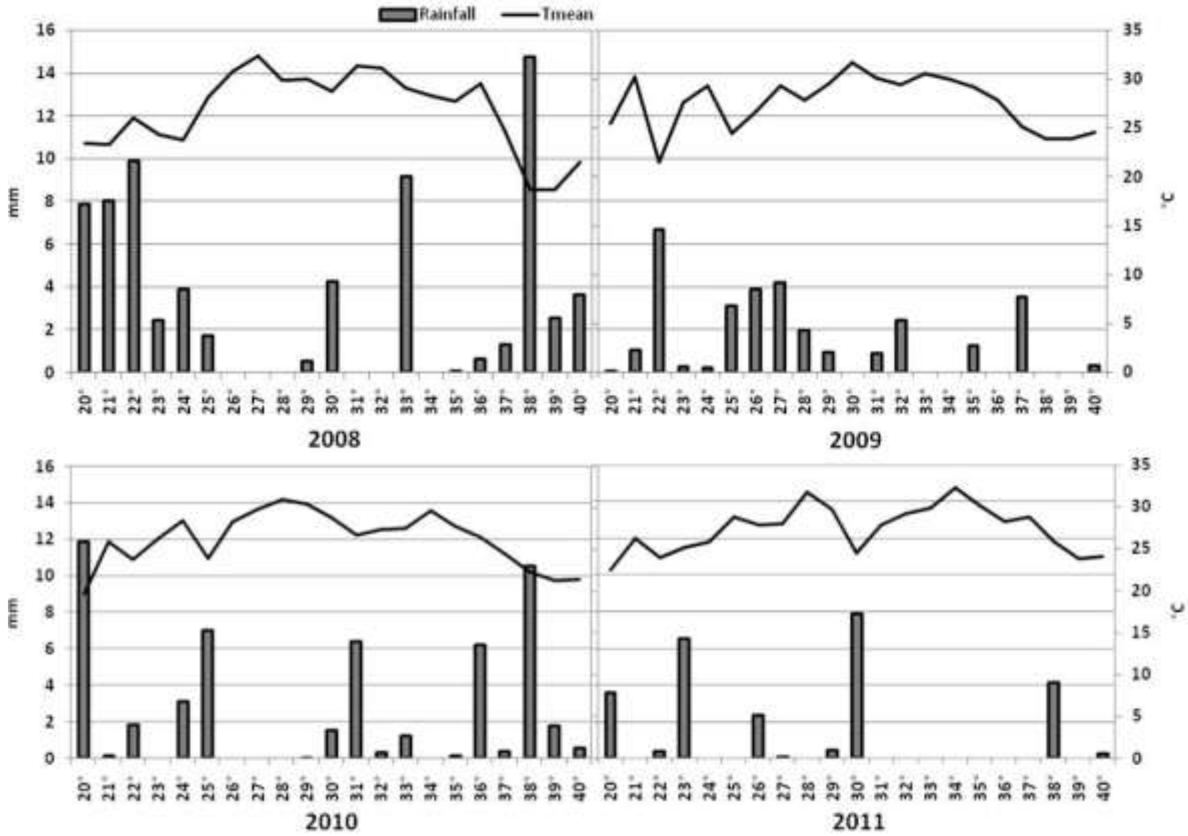

Figure 4. Mean weekly temperatures (line graph) and rainfall (histogram) from week 20 to week 40 for the years 2008 to 2011.

Discussion

As indicated above, the median number of egg per week showed a significant decreasing trend over the years from 2008 to 2011. During the second year of monitoring, the median number of eggs remained at lower levels than for the previous year, 2008, and especially during the period of the maximum population density. However, although in the second year of monitoring the peaks were lower, the ovitrap index and median number of eggs remained high from week 25 to week 32 (ovitrap index >50%; median number of eggs >50). In the other two years (2010 and 2011) very low egg densities were found, with the median number of eggs rarely exceeding 50.

The ovitrap index is related to the levels of diffusion of *Ae*. *albopictus* in the period of maximum population development, and not to the numbers of eggs harvested in the ovitraps, which are instead related to the population abundance. This pattern is due to the high sensitivity of these ovitraps, as it is known that each ovitrap can capture at least one egg even if only a few females are present.

Nevertheless, there was a decreasing trend in median eggs per week from 2008 to 2011 (although the ovitrap indices remained high in the period of maximum development). Furthermore, on the basis of preliminary assessments, there were no significant differences in the climatic parameters to justify such a massive reduction in the infestation in the years of surveillance. These data suggest excellent control of this pest.

This mosquito control was achieved using a diflubenzuron-based larvicide product over 8 cycles, of about 20 days each, over the 24 weeks each year during the vectorial season, so from May to October. Every week, the treatment of one district of the town of Pesaro was carried out, covering the whole total of 18,000 manholes for each cycle.

Treatments to kill the adult mosquitoes with a pyriproxyfen-based product were suggested only if social events were planned that were close to the population communities, and only if they could be confirmed to be a particular nuisance for the population by an inspection. The Public Health Office authorized all of these treatments.

The pest control strategy also included education of the population, particularly providing information to prevent, discover and eliminate mosquito breeding sites. Larvicide products for the treatment of inextinguishable breeding sites were also distributed to the population through reduced prices in local pharmacies (about 1,200 tablets/year of *Bacillus thuringensis israelensis* spores); these were also free to schools, agencies, and other partners. Information was set up throughout all meetings, with fact sheets and a toll free number established. Finally, a staff of technicians was available for visits upon request, and an entomologist provided sampling for larvicidal efficacy in manholes.

Conclusions

As suggested in the literature, the January and annual temperatures and the annual precipitation are the most important limiting climatic factors for the mosquito breeding cycles. Over these four years of investigation, the $JanT_{mean}$, $AnnT_{mean}$ and annual precipitation were always above the thresholds: >0 °C, >11 °C, >500 mm, respectively. Therefore, we can assume that in the Pesaro area the survival rate of the diapausing eggs during the winter period was not influenced by the winter temperatures. Moreover, looking at the $AnnT_{mean}$ and the rainfall, we can state that this area is very suitable for *Ae. Albopictus* to thrive. Thus, the climatic factors were not significant in limiting potential seasonal increase and spread of the local Pesaro *Ae. Albopictus* population.

In our experience, monitoring is a fundamental tool for effective mosquito control. This system based on ovitraps, as described herein, is economic and effective, and allowed the measuring of the main factors relating to the *Ae. albopictus* population: the abundance and diffusion. These parameters are very important for the evaluation of seasonal activities during any effective pest control system.

To ensure the reliability of pest control systems, control and monitoring should be performed by different interested parties. If both monitoring and pest control are well performed and managed, the risk of arbovirosis is also reduced and improved responses are possible in the case of any outbreak. However, it is still necessary to establish this level of organization in the major urban areas of the Italian peninsula, where mosquito infestation is often largely out of control, with the consequent high risk of epidemics.

This concern is probably due to a lack of knowledge of the risks associated with emerging arboviruses, and the lack of specific expertise among municipal technicians. The task of public health scientists (e.g. entomologists, surgeons, epidemiologists) is therefore to accelerate the timetable for the implementation of existing techniques and to design innovative techniques for mosquito monitoring and control. They also need to disclose the use of and spread the knowledge of such methods among politicians and administrators.

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