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Quality control and data validation procedure in large-scale quantitative monitoring of mosquito density: the case of *Aedes albopictus* in Emilia-Romagna region, Italy

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ABSTRACT

The risk of local transmission of chikungunya, dengue, and Zika viruses from viremic travelers arriving from affected areas is a real possibility in southern Europe, due to the large presence of the vector *Aedes albopictus*. The first chikungunya outbreak in Europe occurred in the Emilia-Romagna region, Italy, in 2007, prompting the regional public health department to implement an *Ae. albopictus* monitoring and control plan. This paper presents the procedure developed for the quality control of the data collected by the regional ovitrap monitoring network. The quality control procedure consists of four successive steps; the first step is data acquisition and the second is an automatic data processing step, while skilled technicians according to specific procedures perform the third and fourth steps by checking the exact position of the ovitraps and by conducting human landing collections, respectively. The paper also presents the results of this quality control procedure applied to the data collected through the large-scale *Ae. albopictus* monitoring operation performed in Emilia-Romagna in 2015.

KEYWORDS

Aedes albopictus; chikungunya; dengue; Zika; monitoring network; risk assessment; quality control; data validation

1. Introduction

Aedes albopictus (Skuse) is an invasive mosquito species native to Eastern Asia, which has spread across many other regions [1–3] in recent decades, due to its ecological plasticity and to worldwide transports. This species is of public health concern as a vector of arboviruses such as chikungunya (CHIKV), dengue (DENV), and Zika (ZIKV) [4–6]. In Europe, *Ae. albopictus* was responsible for the outbreak of CHIKV in Northern Italy in 2007 [7] and in local transmissions of DENV in Southern France in 2010 and 2013, and in Croatia in 2010 [8–11]. In Emilia-Romagna, *Ae. albopictus* was detected for the first time in 1994 (Bellini unpublished data) and since then, despite its low active dispersal ability, it rapidly colonized all urban areas on the plains and low hills of the region, where it reached high population densities [12–14].

After the 2007 CHIKV outbreak, the regional Public Health service launched the 'Regional Plan for Monitoring and Control of Tiger Mosquito and Prevention of Chikungunya and Dengue'. The Plan includes actions such as management of the monitoring network, larval control, community participation campaign, adult control around imported CHIKV and DENV cases in order to prevent local transmission, education in primary schools and Municipality ordinances. The monitoring network is based on a system of ovitraps, presented in detail in previous papers [15,16] in which we demonstrated that egg density data, obtained by ovitrap-based monitoring, may provide a good estimate of the biting female density and may therefore be used to assess the epidemiological risk of outbreaks [17,18].

Monitoring by ovitraps has definite advantages, because they are low cost and the level of sensitivity is good, even technical labor operators can easily manage the traps at a reasonable cost. However, the results may be influenced by micro-environmental changes near the ovitrap, such as the cutting of vegetation, removal of breeding sites, local adulticide treatments, and even minimal displacement of the ovitrap can produce an effect on its attractiveness to females.

The development of a quality control methodology becomes necessary for two main reasons: 1. to standardize the management operations in placing, collecting, storing, and counting the eggs on the substrate so that the monitoring system always provides reliable data; 2. to highlight possible data alterations made on purpose to demonstrate the efficiency of operators and/or local administrations in keeping *Ae. albopictus* density under control.

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This paper presents the procedure for quality control of the *Ae. albopictus* monitoring system developed in Emilia-Romagna between 2008 and 2014, and the results achieved when this procedure was implemented in 2015.

2. Experimental section

2.1. Ovitrap network organization

Emilia-Romagna has about 4.43 million residents and includes 348 municipalities belonging to 9 provinces. The regional *Ae. albopictus* monitoring network (E-RMN_AA) was started in 2008 involving 64% of the municipalities, and was progressively extended, thus reaching 76% of the municipalities in 2015. Most of the municipalities not included in the E-RMN_AA were in the hilly area of the region, where *Ae. albopictus* population density is low, due to less favorable environmental conditions [15].

In the urban localities covering an area ≥600 ha, the ovitrap number was determined using the Taylor Equation (1) [16], while in localities covering an area <600 ha the number of ovitraps was in the range of 1–20, according to the availability of financial resources,

$$N = [Z_{\alpha/2}/D]^2 * a * m^{b-2} \quad (1)$$

where Z is the Standard Normal Distribution Value for a defined probability level [19], D is the desired precision level, a and b are the Taylor coefficients, and m the average of eggs/ovitrap/two weeks.

In 2008, the number of ovitraps was calculated using the Taylor equation for the precision level of $D = 0.2$ in the cities of Rimini, Cesena, and Forlì (where an outbreak of CHIKV occurred in 2007), while a precision level of $D = 0.3$ was chosen for the other towns with an urban surface area ≥600 ha [16].

In 2009, we standardized the level of precision to $D = 0.3$ in all the towns and cities, and we reduced the total number of ovitraps in Rimini, Cesena, and Forlì from 387 to 195. GIS software (Geographic Information Systems, ESRI ArcView 3.3, Redlands, California) was used to divide the monitored urban areas into quadrants, each one with one station in a green shaded area [16]. The procedure adopted for reducing the number of ovitraps in 2009 was the following:

- the number of ovitraps in each town was calculated using $D = 0.3$ in the Taylor equation;
- the definition of grid cell size was based on the number of ovitraps;
- a random elimination of ovitraps was done, to leave only one ovitrap per grid cell.

In 2008 and 2009 the ovitrap (CAA7) consisted of a black plastic pot with a capacity of 400 ml (upper diameter 8 cm), filled to 2/3 of its height with 285 ml of dechlorinated water. A 12.5 × 2.5 cm Masonite strip was fixed to the ovitrap with a metal clip as egg deposition substrate.

To reduce management costs, a new type of ovitrap (CAA14) was used from 2010, which allowed fortnightly sampling. The new ovitrap was a 1.4 L black plastic container (Figure 1) holding 800 mL of *Bacillus thuringiensis* var. *israelensis* solution (1 mL of Vectobac 12AS / ovitrap) (Valent BioSciences, Sumitomo) and a strip of masonite (15 × 2.5 cm) as egg deposition substrate, which was replaced every two weeks [20]. The egg collection capacity of the two types of ovitrap was compared in a 16-week field trial conducted using 29 ovitraps type CAA7 and 29 ovitraps type CAA14. The resultant correlation was $R^2 = 0.79$; $F(1, 7) = 26.33$ and $p < 0.002$ (Figure 2), thus allowing use of the entire set of data collected in the period 2008–2015.

Use of the new ovitraps positively influenced the precision level D, which was less than 0.25 in some areas (mainly because of the reduced variability in the number of eggs collected over a two-week period versus a one-week period). Consequently, from 2010 we progressively standardized the precision level to $D = 0.25$ in all towns with an urban surface area ≥600 ha, according to the following procedure:

- elimination of ovitraps that presented a high number of missing data for various reasons;

Figure 1. Ovitrap used in the Emilia-Romagna monitoring network since 2010 (CAA14).

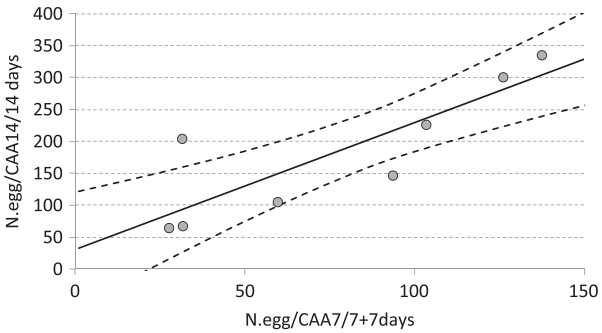


Figure 2. Correlation between the numbers of eggs collected in two weeks by the ovitrap CAA14 (managed every two weeks) and the ovitrap CAA7 (managed every week).

- among ovitraps situated too close to each other (<100 m), elimination of ovitraps whose data deviated more from the mean seasonal trend of the specific locality.

To summarize, the E-RMN_AA evolved from 2785 ovitraps in 2008, which were weekly serviced and distributed across a cumulative urban area of 1088.9 km^2 (one ovitrap/0.39 km^2), to 2641 ovitraps in 2015, that were serviced every 14 d and distributed across a cumulative urban area of 1120.3 km^2 (one ovitrap/0.42 km^2).

As micro-environmental conditions such as vegetation coverage and species composition, shadow degree during the whole day, wind exposition, protection from animal drinking greatly influence ovitrap attractiveness to egg-laying females, thus affecting the number of eggs collected from the ovitrap, and, in turn, the reliability of the monitoring data, operators were requested to take particular care with ovitrap positioning.

2.2. Quality control procedure

In 2015 the E-RMN_AA included 25 urban areas ≥600 ha (Table 1), where the number of ovitraps was planned based on a statistical basis and may therefore be used for data analysis at the city level (SC), and 240 urban areas <600 ha, where the number of ovitraps was not planned based on a statistical basis (PC). The total number of ovitraps (SC+PC municipalities = 265) allowed data analysis at the provincial and regional levels (Table 2).

The quality control procedure included a number of consecutive steps of data control: the first was activated automatically while the second step required specific actions. Individual ovitrap data (SC+PC) provided the data analysis at the provincial and regional levels (first step validation), while the SC data were processed as mean values per city and provided the data analysis at the municipality level (second, third, and fourth step validation).

The procedure sequence is as follows (Figure 3):

(1) individual ovitrap data (SC+PC) fortnightly collected, 9–10 collections per season in the period May–October (raw data);
(2) first validation step of the ovitrap data (SC+PC) which feed the database (DB) for the analysis at the provincial and regional levels;
(3) second (automatic), third, and fourth validation steps (non automatic) of the data of SC cities (urban area >600 ha) which satisfied the first validation step;
(4) data of SC cities not validated, discarded from the database;
(5) validated data of SC cities included in the historical series for analysis at the municipality level.

2.2.1. Data collection and first validation step

The raw data provided every 14 d by the regional reference centers responsible for egg counting (Regional Agency for Environmental Protection and University of Ferrara) undergo the first validation step. Only data obtained from ovitraps in regular conditions at the time of inspection were used (e.g. ovitraps without water at the time of inspection, or not placed in the correct position, or even missing, were not included in the database).

2.2.2. Second validation step

The system automatically checks the data produced by the SC cities validated at the first step, and uses two simple algorithms:

Table 1. Data used for analyses at the city level in 2015. Only the data from urban areas over 600 ha (SC) were used (where the number and position of the ovitraps were based on a statistical approach).

City	Mean altitude (m a.s.l.)	No. residents (ISTAT 2011)	Surface (km^2)	No. ovitraps
Bologna	54	374,944	140.7	110
Carpi	26	67,203	131.1	26
Casalecchio di Reno	61	35,287	17.4	40
Castel Maggiore	29	17,100	30.9	20
Cervia	5	28,542	82.2	50
Cesena	44	95,525	249.5	57
Cesenatico	2	24,956	45.1	33
Comacchio	0	23,168	283.8	31
Correggio	31	24,501	77.8	35
Faenza	35	56,922	215.7	50
Ferrara	9	134,464	404.4	79
Forlì	34	116,208	228.2	44
Formigine	82	33,091	46.8	22
Guastalla	25	15,020	52.6	20
Imola	47	68,019	205.0	50
Lugo	12	32,684	116.9	45
Mirandola	18	24,163	137.0	44
Modena	34	181,807	183.2	48
Parma	57	182,389	260.8	60
Piacenza	61	101,778	118.5	40
Ravenna	4	155,997	652.9	81
Reggio E.	58	165,503	231.6	40
Riccione	12	35,233	17.1	50
Rimini	5	140,137	134.5	60
San Giovanni in P.	21	26,679	114.4	22
Total	30.64	2,161,320	4,178.1	1,157

Table 2. Number of ovitraps (SC+PC) used for the analyses at the provincial and regional levels in 2015.

Province	Mean altitude (m a.s.l.)	No. residents (ISTAT 2011)	No. ovitraps
Piacenza	229	285,922	179
Parma	289	433,154	168
Reggio E.	227	519,458	222
Modena	320	688,286	383
Bologna	194	976,175	474
Ferrara	5	357,980	247
Ravenna	36	385,729	356
Forlì-Cesena	201	388,019	363
Rimini	277	321,457	250
Total	218	4,356,180	2,641

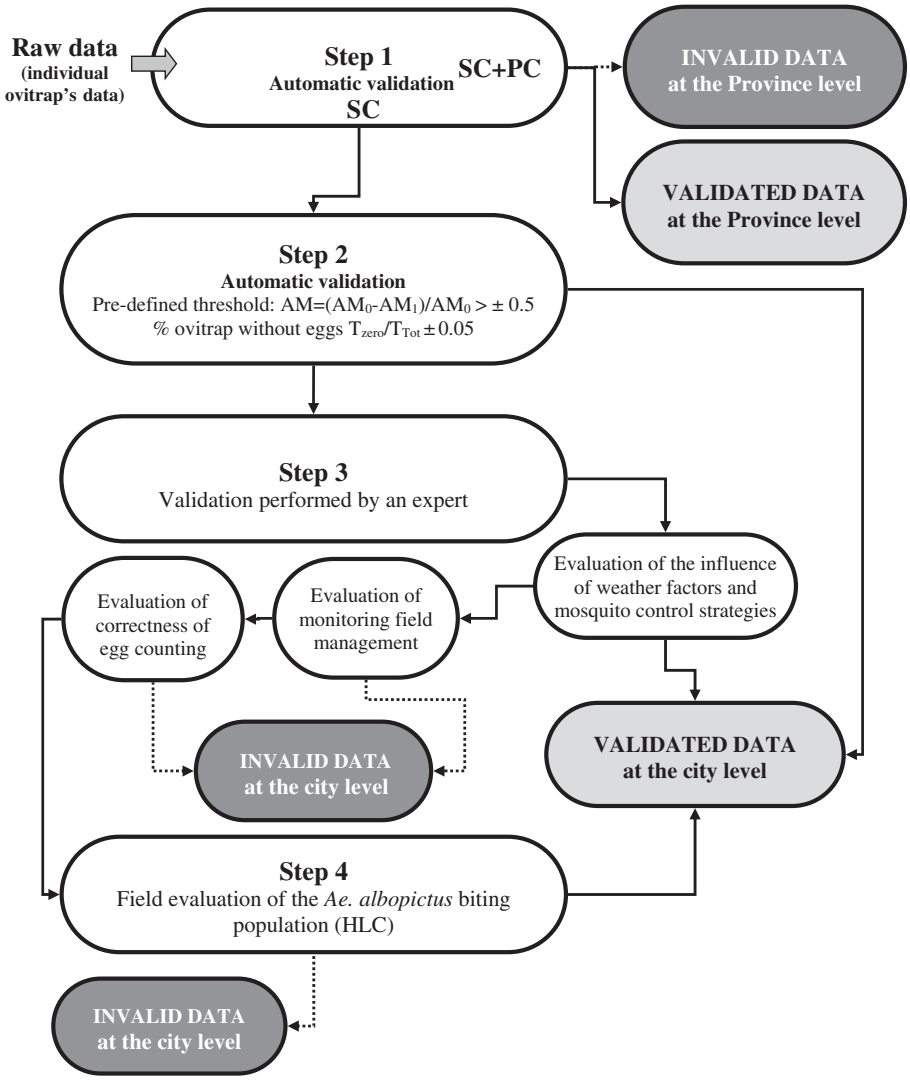


Figure 3. Procedure for the quality control of *Aedes albopictus* ovitrap-based monitoring data in Emilia-Romagna.

(1) the percentage of ovitraps without eggs;
(2) the variation between the mean number of eggs/ovitrap/two weeks recorded in the SC city and the regional mean.

The system considers the data as valid if:

(1) the percentage of ovitraps without eggs is below the pre-established threshold of 5% during weeks 27–37 in urban areas with an elevation below 400 m asl. In this period, *Ae. albopictus* population is increasing and ovitraps with no eggs are to be considered a very rare event (Figure 4). If the number of negative ovitraps as a percentage of the total number of ovitraps in a city is higher than 5% for two consecutive collections, the system requires the data to undergo further validation steps before being included in the database;
(2) the ratio between the city and the regional mean numbers of eggs/ovitrap/per two weeks must be within a certain range and should not exceed an established threshold during consecutive years. The climatic and environmental conditions were similar in the SC cities and we assume that also the relationship between local and regional mean egg density is quite constant over the years.

The equation informing the automatic validation system is:

$$AM = \left(AM_0 - AM_1\right) / AM_0 > \pm 0.5 \qquad (2)$$

where AM is the coefficient of quality validation calculated by the system;

$AM_0 = Mc_0/Mr_0$ (Mc_0 is the mean number eggs/ovitrap in the city registered at time t_0 and Mr_0 is the mean No. eggs/ovitrap in the region at time t_0). To calculate Mr_0 we considered the average of valid data for the previous three years (t_0).

$AM_1 = Mc_1/Mr_1$ (Mc_1 is the mean number of eggs/ovitrap/two weeks in the city obtained at time t_1 and Mr_1 is the mean number of eggs/ovitrap/two weeks in the region at time t_1).

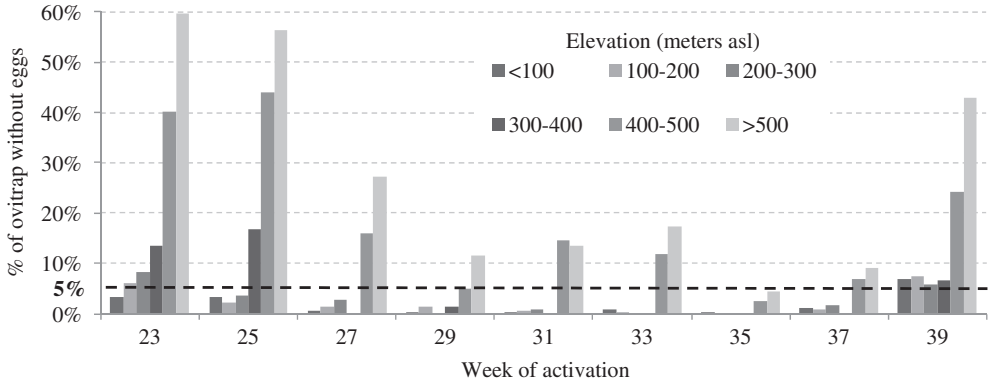


Figure 4. Emilia-Romagna ovitrap monitoring network (2015): percentage of ovitraps without eggs according to their altitude.

AM is positive when the number of eggs is higher than the expected and vice versa AM is negative when the number of eggs is less than the expected.

If the AM value calculated by the system is ≥ ±0.5 for two consecutive collections in the period July-August-September, the Mc_1 value (current mean ovitrap data) is considered temporarily invalid. In this case, the system requires the data to undergo further validation steps before being included in the database.

Ovitrap data collected in areas whose monitoring output had not been validated in the previous year undergo a specific procedure: if they are found to be invalid at the second step they are immediately excluded from the database without further processing; if they produce valid data, they must undergo the fourth step in the course of the year before being reintroduced into the database.

2.2.3. Third validation step

Data considered invalid at the second step undergo a third step of validation. A skilled technician, following a specific procedure, conducts the third step manually:

(1) analysis of local weather conditions, which may be responsible for the data anomaly (e.g. prolonged bad weather) or important changes in mosquito control strategy. If the weather or control activities can explain the data anomaly, the data are considered valid and included in the database; if neither of these reasons is found, data must undergo further validations;

(2) field inspection to check for correct ovitrap management. This includes checking the exact ovitrap position, the quantity of water in the ovitrap and the correct positioning of the masonite strip in the ovitrap. If the inspection shows incorrect field management, the data are considered not valid and excluded from the database. If the inspection shows correct field management, the data must undergo further validation;

(3) double-checking of the egg count by independent technicians on random samples of masonite strips. The egg re-count must take into account loss or damage of the eggs in the first count. If the double check shows significant errors (relative error >10%) in the egg count, the data are considered invalid and excluded from the database. If the double check shows a correct egg count, the data must undergo a further validation step.

2.2.4. Fourth validation step

The last step is performed only when the previous steps produce evidence of invalid data. In this step, human landing collections (HLC) are used to check the correlation between the number of biting females and the number of eggs in the specific city. In fact, previous studies have shown that a good correlation exists between these two population indices [18]. The HLC sessions are organized on the same grid used to position the ovitraps. A technician identifies a suitable position in each grid cell to conduct a 15 min HLC session, usually in a public green area. Five technicians using manual aspirators perform HLC sessions on days with good weather conditions, in the late afternoon, during the time of maximum female activity. Each technician performs four HLC sessions during the 90 min of peak *Ae. albopictus* activity to obtain 20 replications of HLC data. The mean number of females collected according to these HLC data makes it possible to estimate the expected mean number of eggs/ovitrap/two weeks using Equation (2):

$$\text{Expected No. eggs} = 111.52 * \text{No. females} - 6.33 \quad (3)$$

*valid for No. of females ≥ 1.

If the expected number of eggs is compatible with the mean number of eggs collected (within a confidence limit of 95%) the egg data are validated. If there is a significant difference, the data are considered invalid and definitely excluded from the database.

All data from SC and SP that were found valid at the city level could then be used to assess the reliability

of the monitoring system at the regional level, thus included in the calculation of the mean average of eggs per ovitrap per two weeks. Data would then be used to update the historical series of data.

3. Results and discussion

In this section, we present and discuss the results of this quality control procedure applied to the data collected during the large-scale *Ae. albopictus* monitoring operation performed in Emilia-Romagna in 2015. All data are reported as means with their standard deviations. Seasonal trend of *Ae. albopictus* egg density at the province level by the E-RMN_AA in week 23–40 during year 2015 is shown in Figure 5.

Step 1. The first validation step showed that 92.2 ± 3.1 % of the data were correctly collected, with a minimum of correctness in weeks 29–30 (85.5 ± 7.3%) and a maximum in weeks 39–40 (96.0 ± 7.0 %). The main reasons for data loss were ovitrap damage or disappearance and a lack of water in the ovitrap at the time of inspection during the driest periods (the protocol requires that ovitraps found dry at the time of inspection are not considered in the egg counting).

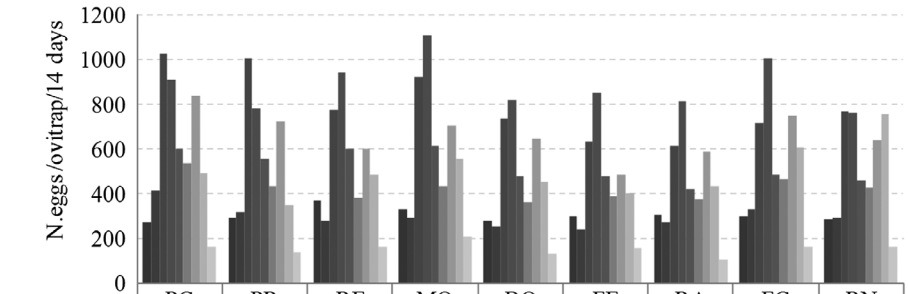
The province with the lowest percentage of correct data was Parma (80.9 ± 6.8 % SD), while the provinces with the highest correct data collections were Ravenna (96.9 ± 2.0 % SD) and Ferrara (96.6 ± 1.4 % SD).

Step 2. The second validation step was performed at the municipal/city level in the 25 SC cities. In 2015, there were five SC cities with invalid data for two consecutive fortnight collections (Table 3); therefore, these data underwent the third validation step.

Step 3. In San Giovanni in Persiceto and Comacchio, the deviation from expected data were explained by additional mosquito control activities conducted out of the regular strategy: in the former, both public and door-to-door larval control extended to three large areas were conducted; in the latter 250 h of adulticide treatment (using 450 kg of pyrethroids) were performed in the summer period. In both cases, these occurrences were considered convincing

Table 3. Number of invalid data at the second validation step (2 consecutive fortnights with AM > 0.5 and percentage of ovitraps without eggs in weeks 27–39).

	AM > +0.5		% ovitraps without eggs		
Municipality ID_	N. fortnights	Alert	Week	%	Alert
Piacenza	2	1	0	0.00	0
Parma	0	0	0	0.00	0
Correggio	0	0	0	0.00	0
Guastalla	1	0	0	0.00	0
Reggio E.	0	0	0	0.00	0
Carpi	0	0	0	0.00	0
Formigine	0	0	0	0.00	0
Mirandola	0	0	0	0.00	0
Modena	0	0	0	0.00	0
Bologna	0	0	0	0.00	0
Casalecchio	0	0	25	7.69	0
Castel M.	0	0	0	0.00	0
Imola	1	0	25	6.38	0
S.G. in P.	4	2	0	0.00	0
Comacchio	5	1	31	6.45	1
Ferrara	2	0	25	8.11	0
Cervia	0	0	0	0.00	0
Faenza	1	0	0	0.00	0
Lugo	4	2	0	0.00	0
Ravenna	0	0	0	0.00	0
Cesenatico	0	0	0	0.00	0
Cesena	1	0	0	0.00	0
Forlì	0	0	0	0.00	0
Riccione	3	0	0	0.00	0
Rimini	6	5	25	5.26	0
All	33	12			1



PROVINCES	PC	PR	RE	MO	BO	FE	RA	FC	RN
W 23	272.3	291.6	366.7	331.7	275.7	295.8	303.3	298.5	286.3
W 25	413.0	314.4	276.5	291.1	250.3	242.1	269.8	326.2	291.8
W 27	1025.6	1003.5	772.7	924.1	734.4	633.2	614.8	719.0	764.4
W 29	909.3	782.3	939.1	1106.1	821.2	852.5	813.1	1008.5	758.0
W 31	602.2	555.2	599.8	610.9	475.8	480.1	418.7	483.8	455.4
W 33	536.6	429.3	378.4	435.0	363.7	384.1	373.6	465.1	425.9
W 35	837.4	722.7	601.7	702.1	645.0	481.3	585.9	747.8	640.8
W 37	488.6	349.7	481.7	555.4	454.2	399.2	430.5	603.6	752.3
W 39	162.0	134.4	162.5	203.9	131.9	156.2	100.9	161.9	162.9

Figure 5. Seasonal trend of the mean number of *Aedes albopictus* eggs per ovitrap per two weeks in the Emilia–Romagna provinces. Weeks 23–40, 2015 (all valid data SC+SP).

reasons for the observed decrease in egg density, therefore ovitrap data were considered valid.

Field inspections to check for correct ovitrap management (third validation step) were conducted in three cities, and incorrect ovitrap management was found in only one of these, leading to the exclusion of that city (Piacenza) from the database. Evaluation of the correctness of the egg counts was necessary for the other two cities, where the observed decrease in egg density was not justified by changes in environmental conditions or by incorrect ovitrap management. The masonite strips collected in week 33 in Lugo (38 masonite strips) and in Rimini (25 masonite strips) were re-counted. The mean relative error was 14.15 % (CI 7.26–21.04 %) in Lugo and 10.06 % (CI 5.49–14.63%) in Rimini. The data collected in Lugo were considered invalid and excluded from the database, while in Rimini the egg counts did not significantly deviate from an acceptable range of error, therefore it was necessary to proceed to the fourth validation step.

Step 3. In San Giovanni in Persiceto and Comacchio, the deviation from expected data were explained by additional mosquito control activities conducted out of the regular strategy: in the former, both public and door-to-door larval control extended to three large areas were conducted; in the latter 250 h of adulticide treatment (using 450 kg of pyrethroids) were performed in the summer period. In both cases, these occurrences were considered convincing reasons for the observed decrease in egg density, therefore ovitrap data were considered valid.

Step 4. Data collected in the town of Rimini were analyzed in detail until the fourth validation step. The process started from data presented in Figure 6, which shows egg density dynamics in Rimini. In 2014, the density of eggs/ovitrap/14 days was higher than the regional average calculated after exclusion of the Rimini data. In 2015, the egg density in Rimini was lower than the regional average, although there had been no relevant change in the mosquito control strategy between 2014 and 2015. In Rimini in 2014, AM was never invalid for two consecutive collections, while in 2015 the data were invalid at Step 2 for six monitoring rounds. Step 3, the third validation level, produced no evidence that either environmental causes could have specifically affected the *Ae. albopictus* population, or that the egg count was incorrect; therefore the data underwent the fourth validation step (i.e. a check of the correlation between the adult density estimated by HLC and that estimated by the egg density data). HLC sessions, performed on September 2, whose results are presented in Figure 7, showed that the number of females recorded was incompatible with the mean number of eggs collected. For this reason, the data collected in Rimini were considered invalid and were finally excluded from the database.

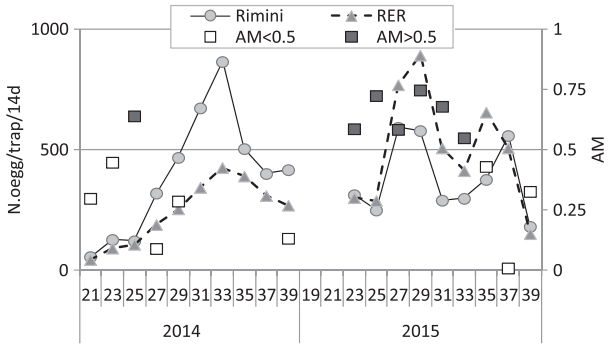


Figure 6. Seasonal trend of No. of eggs/ovitrap/two weeks collected in Rimini and AM coefficient of quality validation (Step 2).

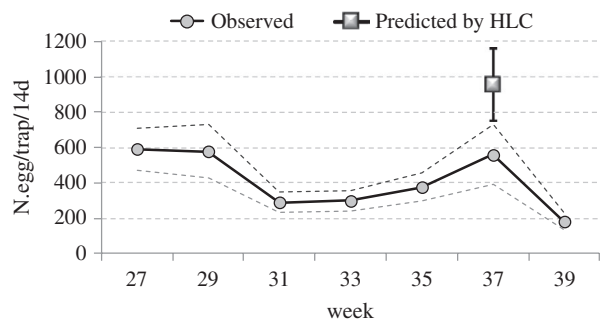


Figure 7. Step 4 of quality validation by HLC. No. of eggs/ovitrap/two weeks observed and predicted based on the number of females recorded by HLC.

4. Conclusions

Large-scale vector monitoring systems provide useful data for risk assessment of vector-borne diseases such as dengue, chikungunya, and Zika, which are strongly influenced by vector density [18]. Quantitative vector monitoring may also show the impact of vector control campaigns on the target species population density. Routine field management of these monitoring systems may require the involvement of a number of operators belonging to different organizations, so that regular operations require efficient coordination. It therefore becomes vital to develop a quality control protocol to enable analysis and validation of field collected data.

For several years we have been working to implement and optimize E-RMN_AA and to develop tools useful to verify the reliability of the E-RMN_AA data. The main aim was to develop a suitable procedure able to highlight possible cases of incorrect ovitrap management that may make the collected data untrustworthy. Accurate management of the monitoring system ensures that vector density is correctly calculated to assess the risk of related vector-borne disease. To achieve this objective,

we have pre-defined the precision level of the data collected, described the criteria for the choice of ovitrap stations, and developed and implemented a procedure for data validation.

In this paper, we presented the quality control procedure developed from 2012 to 2014 and tested in 2015. The quality control procedure consists of successive steps; the first step is performed automatically by the system, which highlights outlier data, and further control steps are performed only when an anomaly is detected.

If data are invalid after following the procedure up to the fourth level of control, they are excluded from the database, and not considered in epidemiological risk assessment or used for any further analysis.

A number of incorrect management practices were observed during the study period, allowing improved evidence-based communication between the actors and stakeholders of the E-RMN_AA. This quality control approach may improve the efficiency of operators involved in the monitoring activities, as it defines specific responsibilities of operators and their organizations, and we hope that this will prevent reductions in quality due to careless behavior and to conflicting interests.

Application of the quality control procedure year after year will build-up a historic database of reliable *Ae. albopictus* egg density data, thereby allowing analyses to be performed over time at the regional scale.

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