

## SHORT COMMUNICATIONS

## An experimental quality control related to the regional monitoring plan against *Aedes albopictus* (tiger-mosquito)

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**Allestimento di un controllo di qualità interno sperimentale in relazione al piano di monitoraggio regionale per la sorveglianza di *Aedes Albopictus* (zanzara tigre)**

### SUMMARY

Following the epidemic disease caused by the Chikungunya virus detected in the Provinces of Romagna during 2007, a specific monitoring-plan against the bug-vector *Aedes albopictus* was set up by the Agenzia Regionale Prevenzione e Ambiente dell'Emilia Romagna (ARPA) in the Emilia-Romagna region (Italy). The analytical method consisted in the simple enumeration of the mosquitoes eggs spawned on a appropriate substratum, using an optic microscope. The aim of this study was to guarantee data comparability among the several laboratories involved in the project. Using the Analysis of Variance (ANOVA), homogeneous and comparable analytical data were emphasised.

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### INTRODUCTION

Since 2004, several million indigenous cases of Chikungunya virus disease occurred in Africa, the Indian Ocean, India, Asia and recently Europe. The virus, usually transmitted by *Aedes aegypti* mosquitoes, has now repeatedly been associated with a new vector: *A. albopictus*. (3) During summer 2007, an outbreak due to the local transmission of Chikungunya virus by *Aedes albopictus* mosquitoes occurred moreover in Italy, Emilia-Romagna Region, in the areas of Ravenna, Forlì-Cesena, Rimini and Bologna cities. After case recorded on August 9th the epidemic outbreak then spread out, thus giving rise to smaller secondary outbreaks and further sporadic cases in the same area, for a total of 337 suspected cases, 217 of which confirmed by blood analysis. Chikungunya virus has been isolated and characterized on both blood and mosquito samples (1). Following this epidemic disease caused by the Chikungunya virus detected in the Provinces of Romagna during 2007, a specific monitoring-plan against the bug-vector *Aedes albopictus* was set up by Emilia-Romagna Region.

### MATERIAL AND METHODS

The monitoring-plan consisted of standard traps constituted by black containers filled with approximately 0.5 litres of water. Masonite, a specific substrate for deposition of the mosquitoes eggs was fixed inside the containers. (2)

The Agenzia Regionale Prevenzione e Ambiente dell'Emilia-Romagna (ARPA) the environmental protection agency of Emilia Romagna region (Italy), was recognised as professionally qualified for the reading of the substrata, in consideration of the experience acquired in this activity during the past season.

The analytical method consisted in the simple enumeration of the eggs spawned on the substratum, using an optic microscope. Since the eggs' mean-size is approximately of 0.610mm X 0.193mm, is necessary a 100X enlargement to distinctly observe them (2).

Even if the method was not particularly sophisticated, in order to guarantee data comparability, an interlaboratory comparisons among the laboratories involved in the project was organized (5). Five ARPA Emilia-Romagna centres (Modena,

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Bologna, Reggio Emilia, Forlì-Cesena and Rimini) and two university Laboratories (Ferrara and Parma) were involved, for a total of 14 operators so parted:

1. Arpa-Modena: 2 operators
2. Arpa-Bologna: 2 operators
3. Arpa-Reggio-Emilia:3 operators
4. Arpa-Forli-Cesena: 2 operators
5. Arpa-Rimini: 2 operators
6. Ferrara University: 2 operators
7. Parma University: 1 operator

The goal in the interlaboratory comparisons was to reproduce, as more as possible, natural environmental conditions for *Aedes albopictus*' eggs: whole, opened, dehydrate. So, 9 substrates, coming from traps belonging to a controlled breeding of *A. albopictus*, were analyzed by each technician.

The same microscope, making a single reading for every substratum, was used by all the analysts and analytical data were statistically elaborated with "XLSTAT" program (Microsoft Office).

In Table 1 collected data-set is reported. The number of eggs read by each operator is reported for every trap.

**RESULTS**

In order to check at first, if the performances provided by the different laboratories, and after, by each operators, were significantly different, analysis of variance (ANOVA) was performed. The statistical elaboration was made considering a 95% confidence level. In general, the purpose of an ANOVA approach is to test for significant differences between means, and, in an ideal case, all variability in measurements will be due to the part-to-part variation, and only a negligible proportion of the variability will be due to operator reproducibility and trial-to-trial repeatability.

In this Control Circuit the choice of the ANOVA approach was due to:

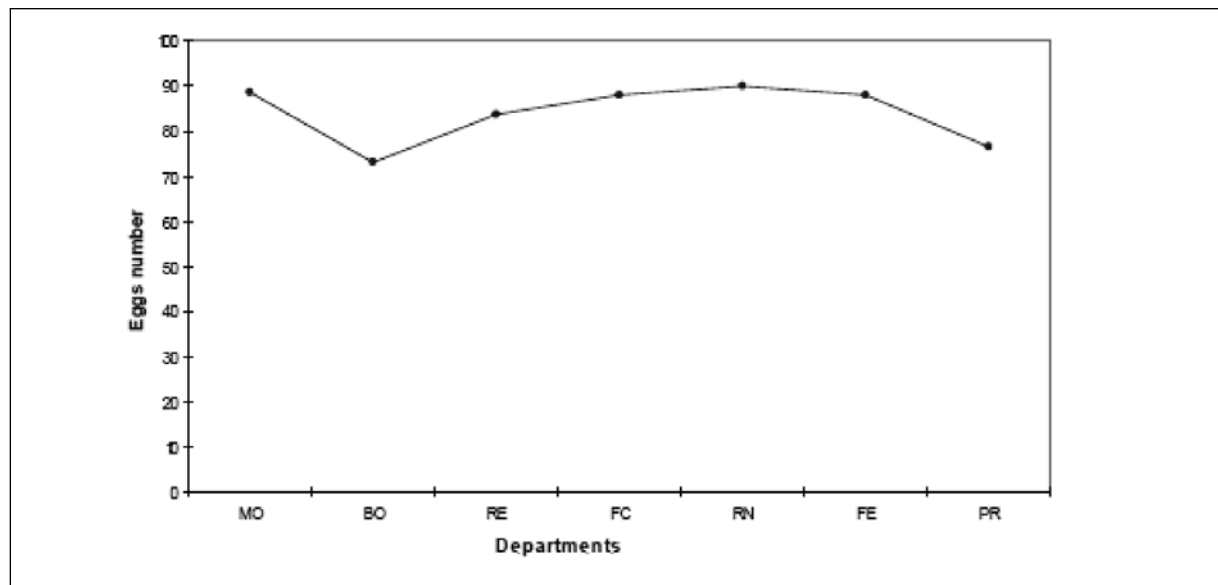
1. the lack of experimental data to justify the use of a z-score test; (4)
2. the lack of a true-value (mean of "population") to compare the readings with.

The first level of data analysis is reported in Figure I. Graphic shows the average number of the eggs numerated in every departments.

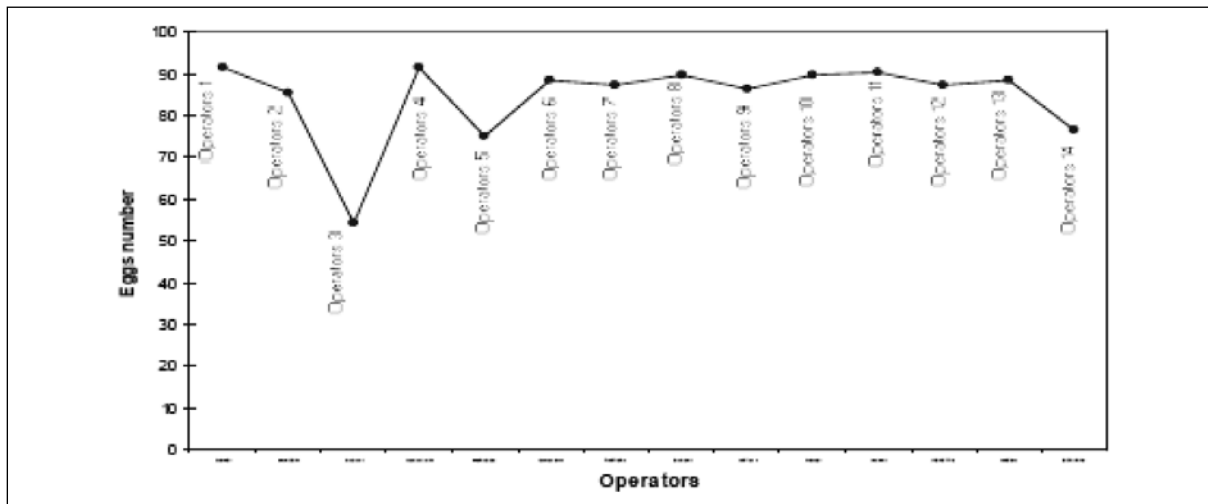
In a second level analysis, the goal was to control possible variance among each operator traps reading. Using the same chart, the mean eggs' number totally read by each operators, was reported in the

**Table 1.** Raw data collected after operators' readings. In every column are reported the performance of each technician

	ARPA Modena	ARPA Modena	ARPA Bologna	ARPA Bologna	ARPA Reggio Emilia	ARPA Reggio Emilia	ARPA Reggio Emilia	ARPA Forli Cesena	ARPA Forli Cesena	ARPA Rimini	ARPA Rimini	University Ferrara	University Ferrara	University Parma
Trap oode	Operator 1	Operator 2	Operator 3	Operator 4	Operator 5	Operator 6	Operator 7	Operator 8	Operator 9	Operator 10	Operator 11	Operator 12	Operator 13	Operator 14
1	57	57	52	58	67	68	51	70	55	74	69	68	73	55
2	91	73	27	102	85	91	74	80	71	78	92	90	90	74
3	52	48	26	43	43	48	44	50	48	51	47	31	49	47
4	95	75	32	62	74	72	65	85	81	68	73	64	69	59
5	59	38	42	51	58	64	63	60	64	67	69	68	54	68
6	48	48	37	53	49	45	60	48	44	50	49	75	64	45
7	182	182	105	172	171	196	190	186	177	190	202	174	171	148
8	142	151	107	145	85	136	147	148	147	148	152	134	143	137
9	85	76	61	99	41	77	82	81	79	88	74	80	84	55



**Figure I.** In This graphic are reported, in the x-axis the departments involved in the project (Modena, Bologna, Reggio Emilia, Forli-Cesena, Rimini, Ferrara and Parma) and in the y-axis the mean eggs' number totally read in each laboratories



**Figure II.** Graphic reports in the x-axis every operators involved in the project, and in the y-axis the mean eggs' number totally read by every single technician

Figure II. Even if in Figure II a peak underlining tendency to underestimates of operator 3 is presents, the ANOVA analysis points out a sufficient alignment among technicians' readings, as well as a sufficient alignment among all laboratories.

The concept is stressed by a further clustering made by statistical software, either of the "laboratory" variable, or of the "operators" variable, as represented in Table 2 and 3. In fact, in each tables, the two variables ("laboratory" and "operator") were put in the same cluster "A" by the statistical software, underlining the concept that there wasn't significant reading variance among reading performances.

**CONCLUSIONS**

At the end of the present article, according to the observations previously exposed, we can assert that the enumeration of *Aedes albopictus*' eggs in the different laboratories involved in the Internal Quality Control was not statistically different (at 95% of confidence level); in addition, we can also say that either laboratories or each operator involved in the regional project of "*Aedes albopictus*"'s surveillance, provided aligned, homogeneous and comparable analytical data.

**REFERENCES**

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**Table 2.** Variables "laboratory" puts in the same group "A". The "estimate mean-column" represents the mean eggs' number totally enumerated in each laboratory. The "groups-column" indicates an absence of significant difference performance among laboratories

Caboratories	Estimate Mean	Groups
RN	90	A
MO	88	A
FC	88	A
FE	88	A
RE	84	A
PR	77	A
BO	88	A

**Table 3.** Variables "operator" puts in the same group "A". The "estimate mean-column" represents the mean eggs' number totally enumerated by each operator. The "groups-column" indicates an absence of a significant difference performance among operators

	Estimate Mean	Groups
Operators 1	92	A
Operators 2	92	A
Operators 3	90	A
Operators 4	90	A
Operators 5	90	A
Operators 6	89	A
Operators 7	89	A
Operators 8	87	A
Operators 9	87	A
Operators 10	86	A
Operators 11	85	A
Operators 12	77	A
Operators 13	75	A
Operators 14	54	A