Subject: In situ training in Montenegro

Place: Radovici, Krtole, Krasici, MNE

August 5-12, 2017

Procurement plan: Training, line 6

WP1

REPORT ON THE PRELIMINARY STUDY AIMED TO THE RISK ASSESSMENT OF CHIKUNGUNYA, DENGUE AND ZIKA OUTBREAK IN MONTENEGRO

2017

BACKGROUND

The fourth LOVCEN mission conducted in the period August 5th – 12th 2017 was focused to the field investigation necessary to assist the risk assessment estimation, already started in the 2015, related to the vectorial capacity of *Aedes albopictus* for the three main human pathogens threatening the Mediterranean countries: Dengue, Chikungunya and Zika viruses.

Autochthonous Dengue (DENV) cases were registered in a small village in the Peljesac peninsula in Croatia in 2010 and in Southern France in 2010, 2013, 2014 and 2015. In September 2012 the Hellenic Centre of Disease Control notified a case in an 84-year-old patient who died.

Chikungunya virus (CHIKV), a member of the Togaviridae family, is efficiently transmitted by *Ae. albopictus* in Mediterranean climate as evidenced for the first time in Northern Italy in the summer 2007, when an outbreak involved about 250 people. Other endemic infections were registered in Southern France in the 2010 and in 2014. In the summer 2017 two outbreaks happened, one in Central Italy with secondary focuses in the South Italy accounting for more than 300 cases, and the second in the Var department, southern France, with 17 cases.

Zika virus (ZIKV) is a Flavivirus isolated for the first time from a monkey in Uganda back to 1947. Before 2007 ZIKV didn't show much activity. In the 2007, ZIKV caused an epidemic in Micronesia, and in the 2013, a large epidemic was registered in the French Polynesia and New Caledonia with tenths thousands of cases. Starting in May 2015 a huge epidemic spread in the Americas with several millions of cases and a world-wide emergence declared by WHO. *Ae. aegypti* has been identified as the main vector of ZIKV with *Ae. albopictus* considered of less importance.

Control of *Aedes albopictus* developing in urban areas is extremely difficult because of the huge number of artificial breeding sites mainly dispersed in private properties. Community participation is showing to achieve only limited results allowing the population density to achieve significant epidemiological levels. Moreover, the progressive reduction of available biocides due to the EU policy is increasing the risk of resistance in the target mosquito.

For the above-depicted scenario, we consider as strategically important and timely to start a Mediterranean regional network focusing efforts on the coordinated development of genetic control methods to be implemented promptly in case of detection of new invasive *Aedes* species.

A strong increase in the interest on genetic approaches to mosquito control is ongoing mainly because of the new possibilities made available by technological advancements in the fields of mass rearing facilities, bio-genetics and informatics. Mass rearing of *Aedes* species is now relatively easy and a pilot model system has already been set up by methods developed at the Centro Agricoltura Ambiente (CAA) in Crevalcore (BO) in cooperation with the International Atomic Energy Agency (IAEA).

The IAEA has launched in August 2016 the technical cooperation project RER 5022 "Establishing Genetic Control Programs for *Aedes* Invasive Mosquitoes" with the main objective to promote efforts by member states to develop technologies and capacities for the implementations of the SIT strategy against *Aedes* mosquitoes.

The LIFE+ Environment Policy and Governance project "Development & demonstration of management plans against -the climate change enhanced- invasive mosquitoes in Southern Europe" LIFE CONOPS- LIFE12 ENV/GR/000466 (July1st, 2013-Dec 31st, 2017) supported by the EU Commission has been set to strengthen the surveillance capacity on invasive mosquito species posing risk in the Mediterranean basin.

The HORIZON 2020 - Research and Innovation Framework Programme. Research capacity for the implementation of genetic control of mosquitoes (INFRAIA-01-2016-2017) (Feb 1st, 2017 – Jan 31st, 2021) has been recently started involving a large consortium of research institutions.

OBJECTIVES OF THE FOURTH MISSION IN MONTENEGRO

The fourth mission to Montenegro in the frame of the LOVCEN project includes the following objectives:

- To evaluate the *Ae.albopictus* population density in three urban areas of MNE during the seasonal peak period (August);
- To calculate the level of correlation between ovitrap data and human landing collection data in MNE;
- To estimate the risk of outbreak in case of introduction of Chikungunya, Dengue and Zika viruses throughout viremic travelers;
- To compare the observed level of correlations between ovitrap data and human landing collection data observed in MNE and in Northern Italy;
- To compare the risk of Chikungunya, Dengue and Zika viruses outbreak estimated in MNE and in Northern Italy;
- To compare collection capacity of MNE ovitraps with CAA ovitraps.

PROTOCOL OF THE FIELD TRIALS

Three urban localities of different dimensions have been chosen as representative of the MNE environmental condition: Radovici, Krasici, Krtole.

In each locality the urban area under study has been defined and number of ovitraps fixed as follow:

- Radovici: 20 ha – 15 ovitraps;
- Krasici: 40 ha – 15 ovitraps;
- Krtole: 28 ha – 12 ovitraps.

In total 37 CAA ovitraps have been used and positioned in the same stations used in the 2016 and left working for about 9±2 days. First positioning of ovitraps was on June 29, last collection on August 29.

RADOVICI

At 5 of 10 stations in Radovici, 1 MNE ovitrap has been settled in well shaded position at a indicative distance of about 10-20 meters from the CAA ovitrap, and position of each ovitrap rotated every inspection.

At the other 5 stations one MNE ovitrap and one CAA ovitrap have been kept stationary.

KRASICI & KRTOLE

At Krasici and Krtole 5 stations each locality have been selected for their homogeneous ecological condition (well shaded area) and one MNE ovitrap has been operated at a distance of about 10-20 meters from the CAA ovitrap, and rotated position every inspection.

ADULT FEMALES COLLECTION (HLC)

In the period August 8-13, *Ae. albopictus* females have been collected in the three urban areas, by manual aspirators (Human Landing Collection sessions of 15 min) during the peak activity time (indicatively 5-7PM) by three operators. Every day, each person conducted 5 HLC sessions during the indicated two hours period, in the stations where the couple of ovitraps have been positioned (5 stations per each locality). Collected females were released back to the field immediately after the 15 min session. To avoid possible bias due to position effect the three operators (Igor, Dusan, Romeo) rotated locality every day, while to avoid possible bias due to different timing the sequence of site HLC collection were reversed every day, following the scheme:

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
RADOVICI	Operator A 38, 42, 46, 48, 51	Operator B 51, 48, 46, 42, 38	Operator C 38, 42, 46, 48, 51	Operator A 51, 48, 46, 42, 38	Operator B 38, 42, 46, 48, 51	Operator C 51, 48, 46, 42, 38
KRASICI	Operator C 18, 21, 25, 31, 34	Operator A 34, 31, 25, 21, 18	Operator B 18, 21, 25, 31, 34	Operator C 34, 31, 25, 21, 18	Operator A 18, 21, 25, 31, 34	Operator B 34, 31, 25, 21, 18
KRTOLE	Operator B 1, 5, 9, 13, 15	Operator C 15, 13, 9, 5, 1	Operator A 1, 5, 9, 13, 15	Operator B 15, 13, 9, 5, 1	Operator C 1, 5, 9, 13, 15	Operator A 15, 13, 9, 5, 1

Collected data have been processed to evaluate:

- the *Ae.albopictus* population density in the three urban localities of MNE during the seasonal peak period;
- the level of correlation between the two ovitrap type;
- the level of correlation between the ovitrap data and the HLC data;
- the risk of outbreak in case of introduction of Chikungunya, Dengue and Zika viruses throughout viremic travelers;
- the level of correlations between MNE and Northern Italy data.

RESULTS

The capacity of the three operators to collect *Ae. albopictus* mosquitoes by HLC has been compared by block ANOVA. No differences were detected between the three operators, both for females and males.

Females	SS	Degr. of Freed.	MS	F	p
Operator	9.27	2	4.63	2.17	0.23
Locality*Operat.	8.53	4	2.13		

Males	SS	Degr. of Freed.	MS	F	p
Operator	46.49	2	23.24	2.02	0.25
Locality*Operat.	45.98	4	11.49		

The *Ae. albopictus* population density in the three localities as registered by HLC has been compared by block ANOVA. Krasici resulted with significantly more females and males in comparison with Krtole and Radovici, while no differences were detected between Krtole and Radovici.

LOCALITY	**N**	**Females**			**Males**		
		Mean	S.D.	Test N-K	Mean	S.D.	Test N-K
Krtole	**30**	1.20	1.45	a	0.37	0.61	a
Krasici	**30**	5.60	4.23	b	4.90	5.07	b
Radovici	**30**	2.30	1.99	a	1.00	1.23	a
All Grps	**90**	3.03	3.37		2.09	3.62	

	SS - Effect	**df - Effect**	**MS - Effect**	**SS - Error**	**df - Error**	**MS - Error**	**F**	**p**
Females	314.60	2	157.30	694.30	87	7.98	19.71	0.000000
Males	361.62	2	180.81	801.66	87	9.21	19.62	0.000000

The egg collection capacity of the two type of ovitraps tested (CAA14 and MNE) was compared by block ANOVA. Despite the fact that the ovitrap volume was different (CAA ovitrap was a 1.42 L black plastic container holding 800 mL water and a strip of masonite sized 15 × 2.5 cm; while MNE ovitrap was a 1.23 L black plastic container holding 750-800 mL water and a strip of masonite sized 15 × 2.5 cm) we obtained identical egg collection capacity. This may be explained by the different shape of the two ovitraps: CAA ovitrap is cylindrical with diameter 11 cm and height 15 cm; MNE ovitrap is conical with bottom diameter 10 cm, top diameter 12 cm and height 13 cm.

ovitrap	egg/day N	egg/day Mean	egg/day S.D.
CAA	54	19.93	14.57
MNE	54	23.77	13.99
All Grps	108	21.85	14.34

	SS	Degr. of Freed.	MS	F	p
ovitrap	446.73	1	446.74	2.46	0.26
sampling area*trap	363.18	2	181.59		

We did the standardization of the number of eggs collected by referring all the data to 14 days collection period (this is possible because previous studies confirmed proportionality in egg collection in the period 7-14 days of trap activation). Then we looked for correlation between the HLC data (mean per locality per year) and the number of eggs per ovitrap per 14 days. To better investigate the possible relation between females density and the egg collection, we considered three periods of egg collection: the period before the HLC data, the same period of the HLC data and the period after the HLC data.

Year	Trap	Locality	HLC	Eggs/ovitrap/14 days		
				Pre HLC period	HLC period	Post HLC period
2015	MNE	Lastva Grbaljska	4.9	761.85	453.67	321.72
	MNE	Radovici	7.4	446.02	521.20	506.80
	MNE	Tivat	5.2	600.38	635.07	573.27
2016	MNE	Krasici	6.3	480.02	396.84	508.67
	MNE	Krtole	7.9	268.59	277.31	332.76
	MNE	Radovici	10.0	336.00	285.33	378.78
2017	CAA	Krasici	5.6	304.29	255.27	213.08
	CAA_B	Krasici	5.6	284.84	293.61	193.43
	MNE	Krasici	5.6	297.50	271.29	331.33
	CAA	Krtole	1.2	459.56	583.89	440.68
	CAA_B	Krtole	1.2	335.68	469.78	292.83
	MNE	Krtole	1.2	279.05	776.84	422.57
	CAA	Radovici	2.3	312.92	281.44	333.36
	CAA_B	Radovici	2.3	260.65	313.44	364.47
	MNE	Radovici	2.3	395.50	509.19	354.38

Note: CAA_B are the ovitrap positoned at the HLC stations

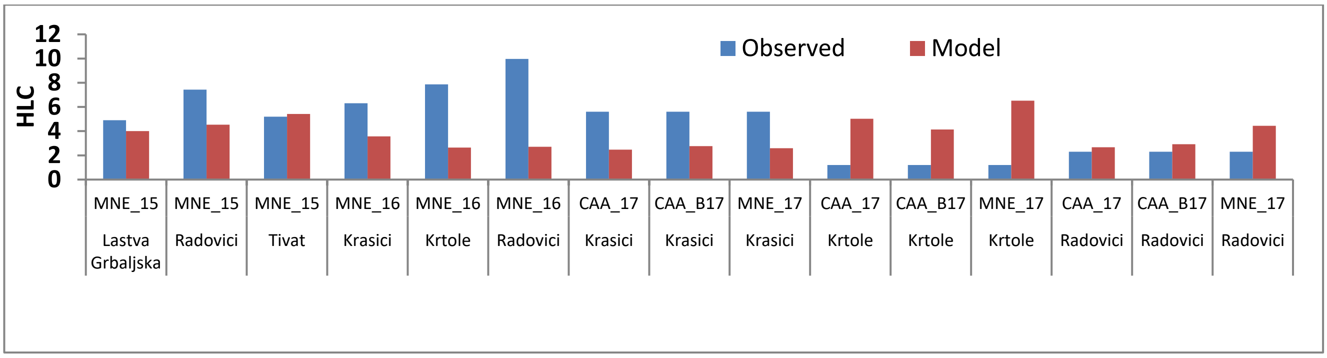
We then calculated the coefficients between the number of eggs and the number of females.

Year	Trap	Locality	Egg/female		
			Pre HLC period	HLC period	Post HLC period
2015	MNE	Lastva Grbaljska	155.48	92.59	65.66
	MNE	Radovici	60.00	70.12	68.18
	MNE	Tivat	115.46	122.13	110.25
2016	MNE	Krasici	76.19	62.99	80.74
	MNE	Krtole	34.14	35.25	42.30
	MNE	Radovici	33.71	28.63	38.00
2017	CAA	Krasici	54.34	45.58	38.05
	CAA_B	Krasici	50.86	52.43	34.54
	MNE	Krasici	53.13	48.44	59.17
	CAA	Krtole	382.97	486.57	367.23
	CAA_B	Krtole	279.73	391.48	244.03
	MNE	Krtole	232.54	647.37	352.14
	CAA	Radovici	136.05	122.37	144.94
	CAA_B	Radovici	113.33	136.28	158.46
	MNE	Radovici	171.96	221.38	154.08

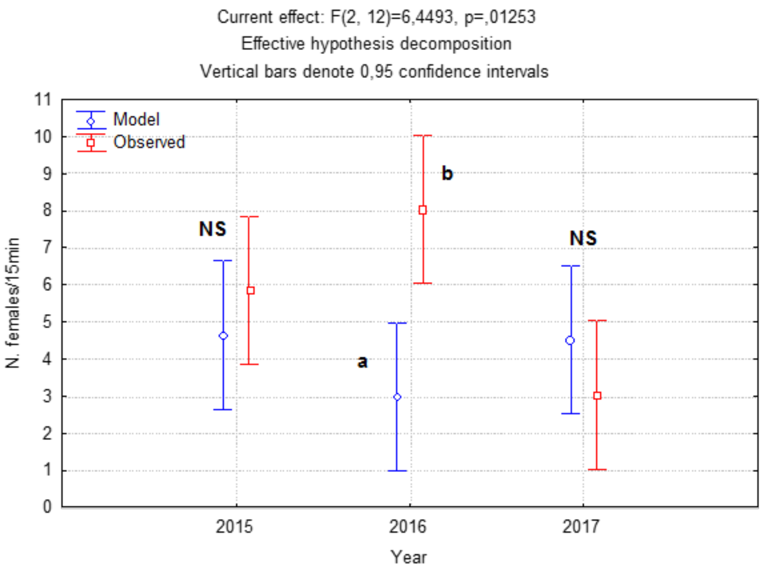
By using the number of eggs collected we then calculated the number of females expected based on the model developed in Italy.

Year	Trap	Locality	HLC	Expected N. females by model CAA		
				Pre HLC period	HLC period	Post HLC period
2015	MNE	Lastva Grbaljska	4.9	6.40	4.01	2.99
	MNE	Radovici	7.4	3.95	4.54	4.42
	MNE	Tivat	5.2	5.15	5.42	4.94
2016	MNE	Krasici	6.3	4.22	3.57	4.44
	MNE	Krtole	7.9	2.58	2.64	3.07
	MNE	Radovici	10.0	3.10	2.71	3.43
2017	CAA	Krasici	5.6	2.85	2.47	2.14
	CAA_B	Krasici	5.6	2.70	2.77	1.99
	MNE	Krasici	5.6	2.80	2.60	3.06
	CAA	Krtole	1.2	4.06	5.02	3.91
	CAA_B	Krtole	1.2	3.10	4.14	2.76
	MNE	Krtole	1.2	2.66	6.52	3.77
	CAA	Radovici	2.3	2.92	2.68	3.08
	CAA_B	Radovici	2.3	2.51	2.92	3.32
	MNE	Radovici	2.3	3.56	4.44	3.24

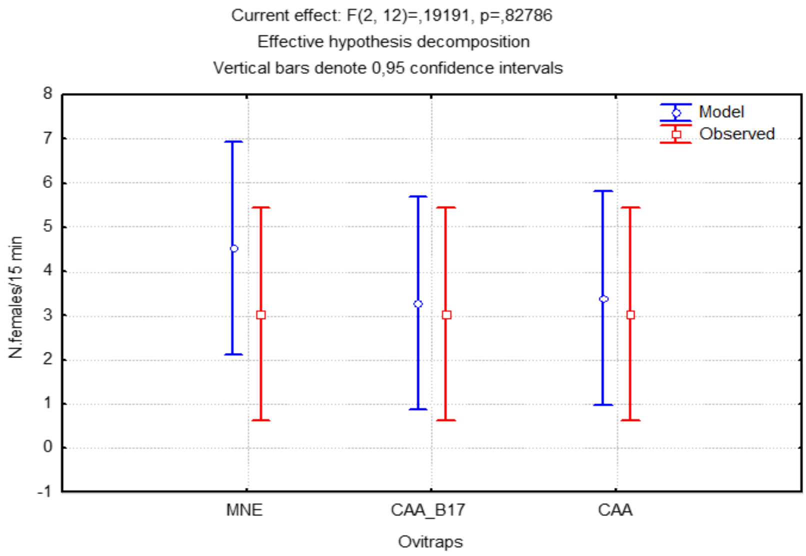
Finally, we compared the observed HLC values *VS* the HLC expected values by applying the model developed in Italy.



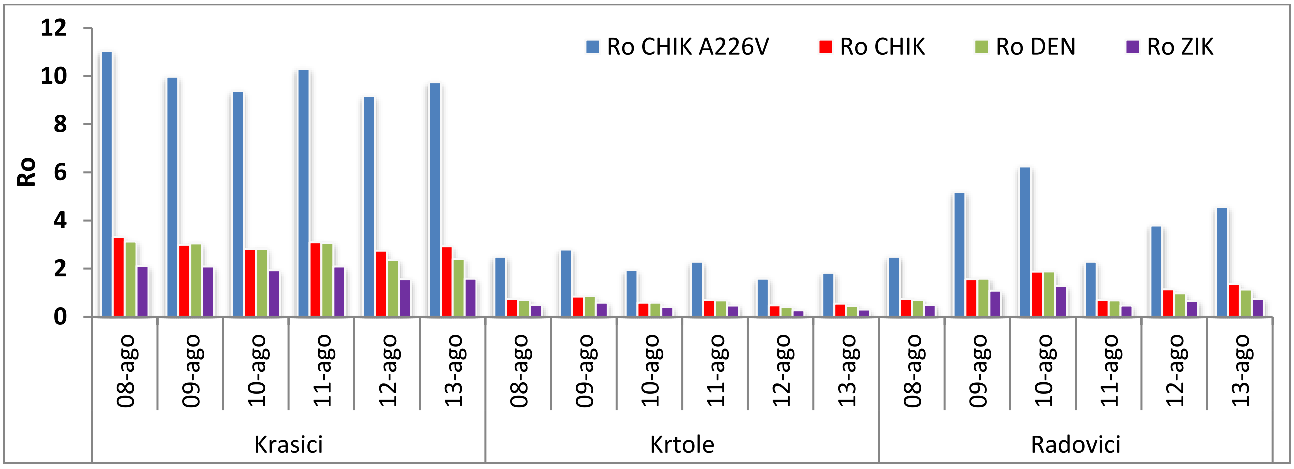
No clear correlation was observed between observed and expected HLC in the three years period. In the 2015 season the model slightly underestimated the HLC values without significant differences. In the 2016 season the model underestimated with significant difference. In the 2017 season the model overestimated without significant differences.



By analysing the 2017 data we observed that the HLC values estimated by the model were not different from the observed values.



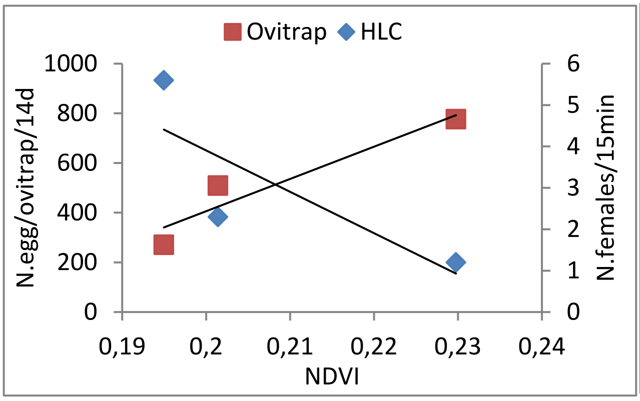
The epidemiological risk for the viruses vectored by *Ae. albopictus* was assessed by using the HLC observed values in August 2017 in the three study locality. The risk was high for the CHIK A226V strain in Krasici, while much less in the other locality. Much less risk was calculated for CHIK, DEN and ZIKA viruses.

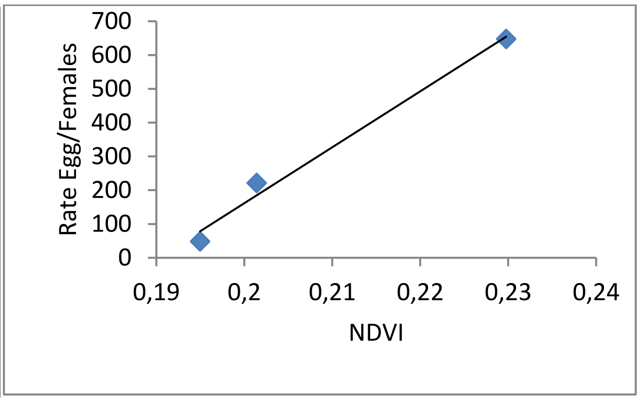


Discussion

We were not able to find a significant consistent correlation between ovitrap and HLC data collected in several localities of Montenegro during the three-year period.

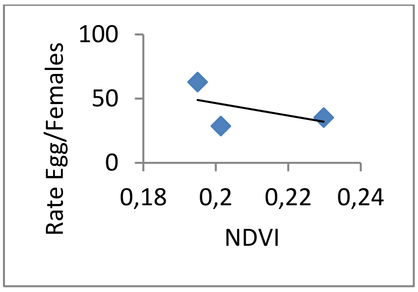
We analyzed possible influence of the vegetation covering in each locality as expressed by the NDVI on the HLC and ovitrap data. In the 2017 we observed a negative correlation between NDVI and HLC, while positive correlation was observed between NDVI and ovitrap data. A correlation therefore exist between NDVI and the ratio ovitrap data/HLC data in 2017.

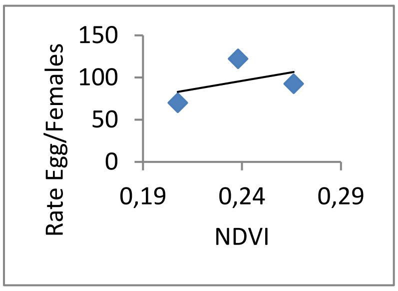


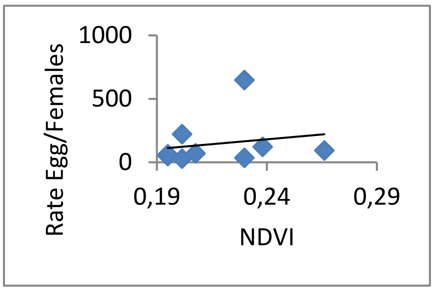


But when we analysed for the NDVI and the ratio ovitrap data/HLC correlation in the other set of data we cannot find any similar correlation. Also by pooling together the whole three year period collected data no consistent correlation was found.

2016 **2015** **2015-17**







Because climatic condition were similar in the three years period, possible explaining hypothesis to be investigated remains the short period we were able to collect HLC data together with small sampling data.

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