ECOMORE II

ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

ກະຊວງ ສາທາລະນະສຸກ
Ministry of Health

Dengue vector Surveillance, Insecticide Resistance and Innovative Strategies for vector control in Laos. Entomology WP, ECOMORE2

Sebastien Marcombe, P. Thammavong, S. Chonephetsarath, N. Phommavanh, K. Lakeomany, P. Luangamath, N. Xayavong, V. Vungkyly, S. Nilaxay

AFD
AGENCE FRANÇAISE DE DÉVELOPPEMENT

27-28 NOVEMBER 2019 *Settha Palace Hotel, Vientiane, Lao PDR*

ECOMORE2 project: Entomology Work Package, Vientiane, Laos

Objectives:

- Entomological Surveillance to define the Dynamics of *Aedes* vectors in dengue hotspots in Vientiane Capital

- Measure and Map insecticide resistance levels of the *Aedes* populations in Vientiane Cap.

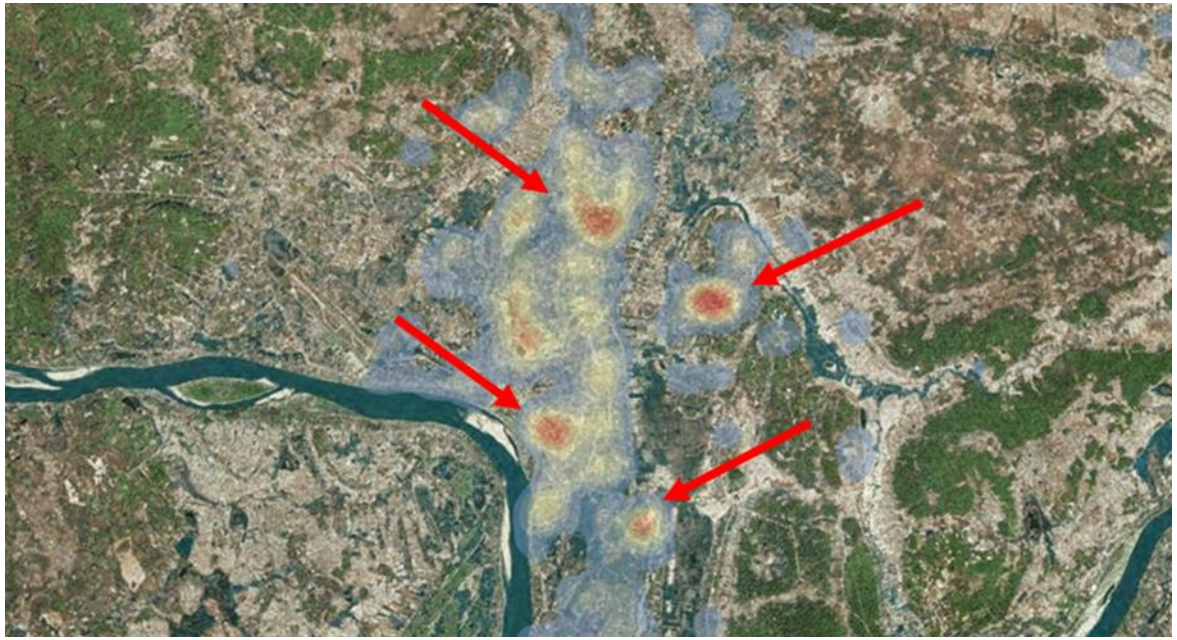
- Evaluate innovative strategies of vector control (In2Care® traps, autodissemination of pyriproxyfen)

Dynamics of vectors and surveillance in Dengue hotspots

- 4 villages selected in Vientiane capital dengue hotspots
- 4 BG sentinel traps and 2 oviposition traps / village
- ➢ Mosquito abundance (every week since may 2016)
- ➢ Arbovirus infestation rates in vectors

Ovitrap

BG trap



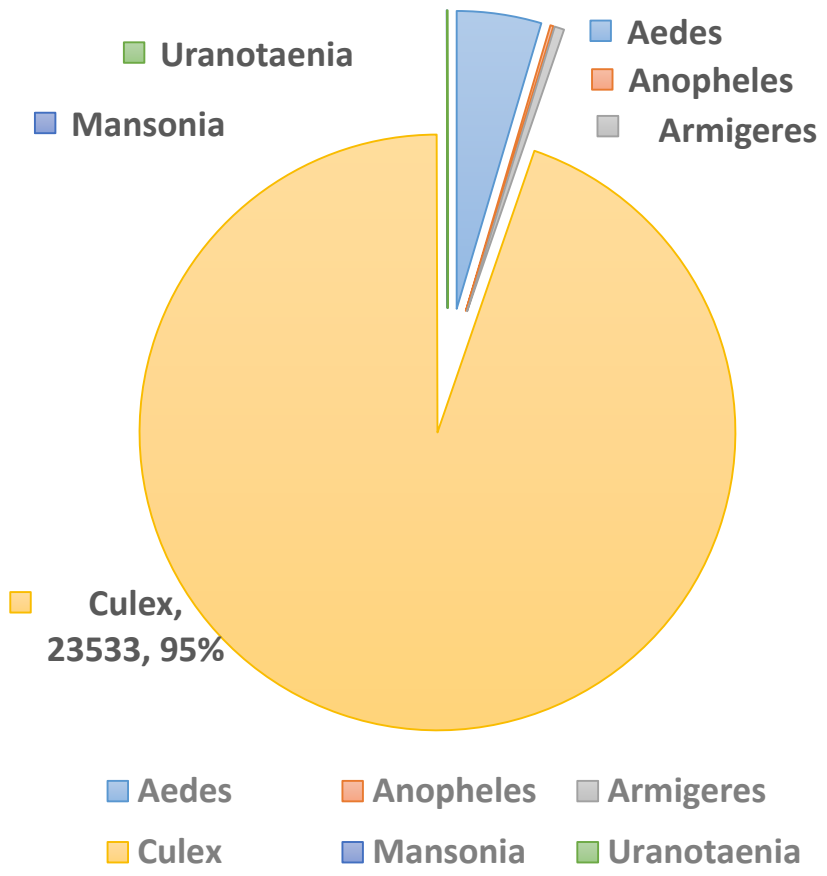
Density of dengue confirmed cases in Vientiane (2012/2016)
Virology Department IPL.

Mosquito species diversity, abundance and proportions

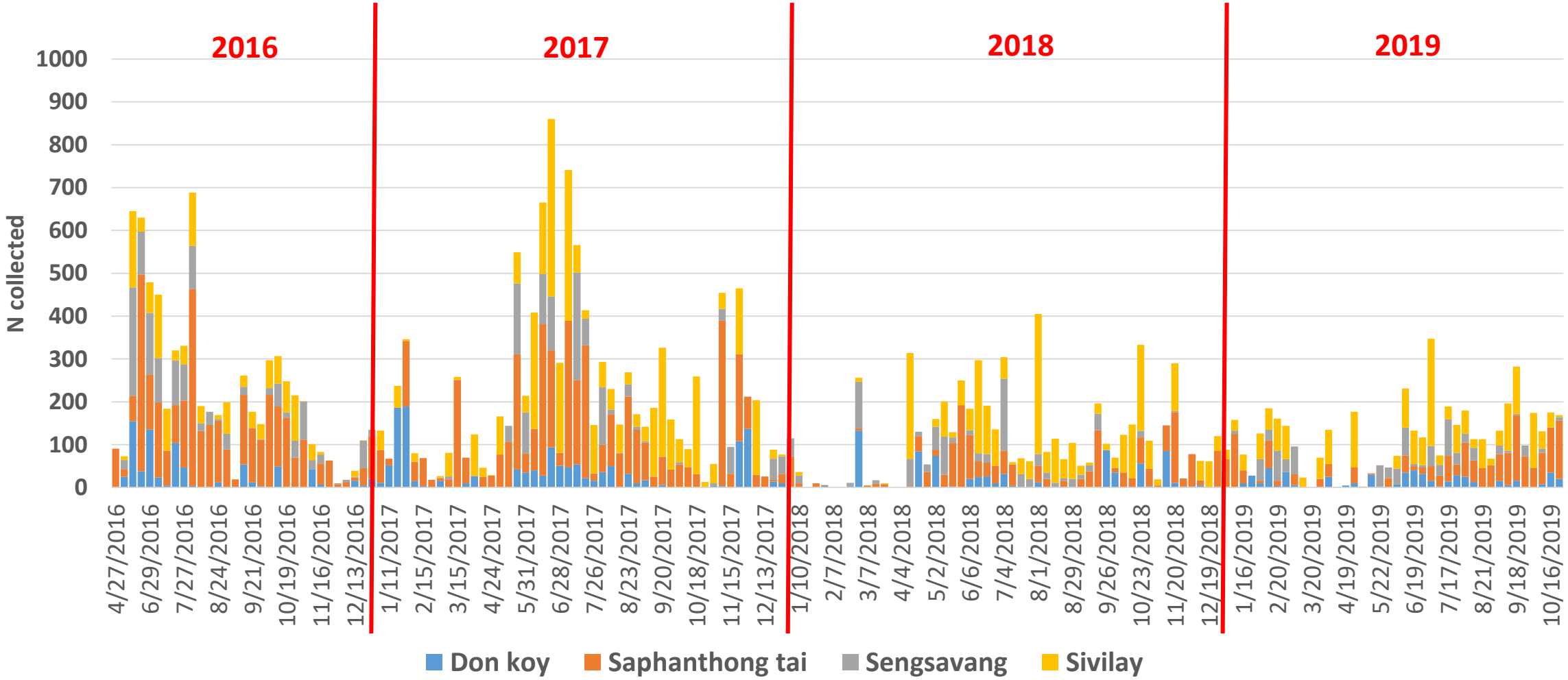
Species	n	%
Aedes	1140	5
Anopheles	42	0.2
Armigeres	130	1
Culex	23533	95
Mansonia	3	0.01
Uranotaenia	8	0.03
Total	**24856**	

Dengue vector Sp.	Total	%
Ae. aegypti	**886**	**85**
Ae. albopictus	**158**	**15**

Species
Ae.aegypti
Ae.albopictus
Ae.vexans
An.vagus
An.indefinitus
Anopheles.sp
Ar.kesseli
Ar.subalbatus
Armigeres.sp
Cx.fuscocephala
Cx.gelidus
Cx.hutchinsonia
Cx.quinquefasciatus
Cx.tritaeniorhynchus
Cx.vishnui
Culex.sp
Mansonia.sp
Uranotaenia
>20 species

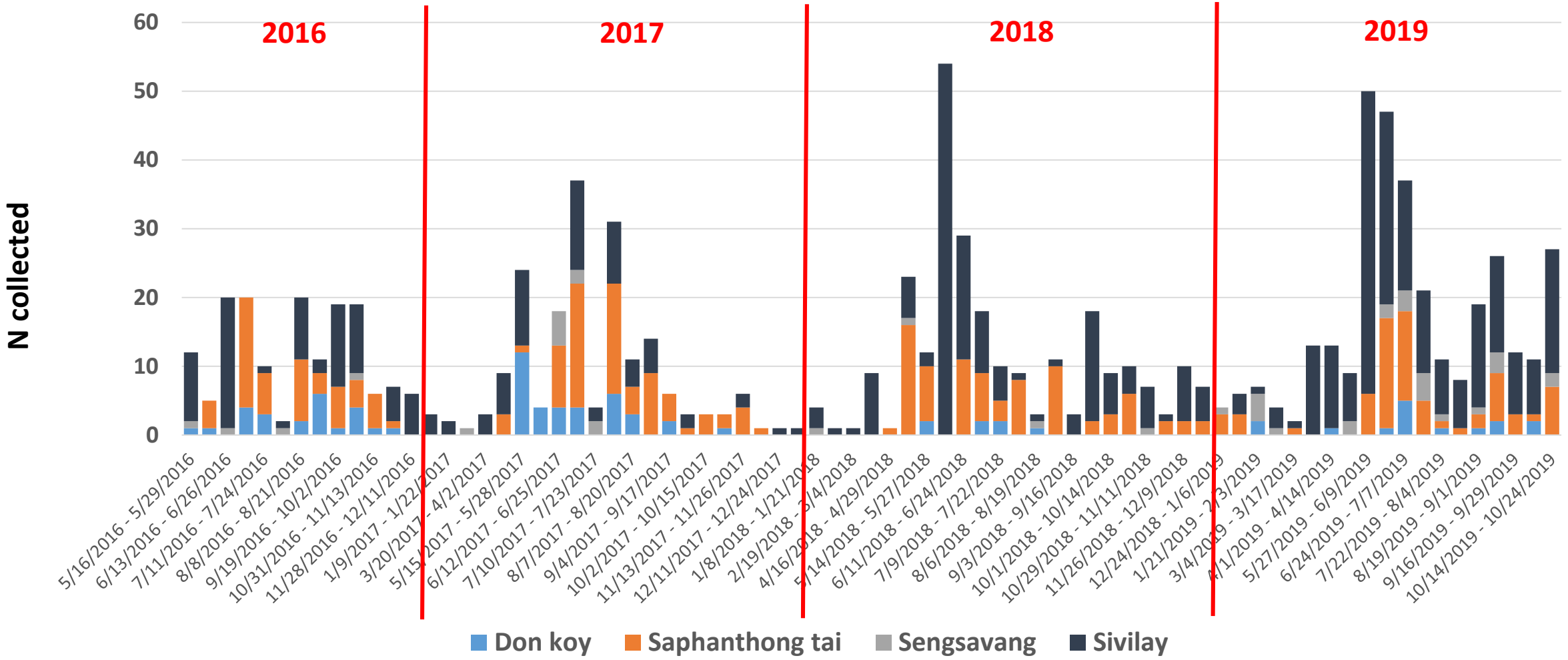


Dengue Vectors surveillance, Vientiane: Larvae



Number of Larvae *Ae. aegypti* and *Ae. albopictus* collected every week between May 2016 and October 2019 in Vientiane

Dengue Vectors surveillance, Vientiane: Adult



Number of Adult *Ae. aegypti* and *Ae. albopictus* collected every week between Jan 2017 and October 2019 in Vientiane

Insecticide resistance: Aedes mosquitoes, dengue vectors

- Levels of insecticide resistance in ECOMORE2 sites against currently used insecticides of larvae and adults *Aedes aegypti* and *Ae. albopictus* populations using WHO standard bioassays


Aedes albopictus

Temephos (Abate)
Deltamethrin
Permethrin
Malathion
DDT


Aedes aegypti

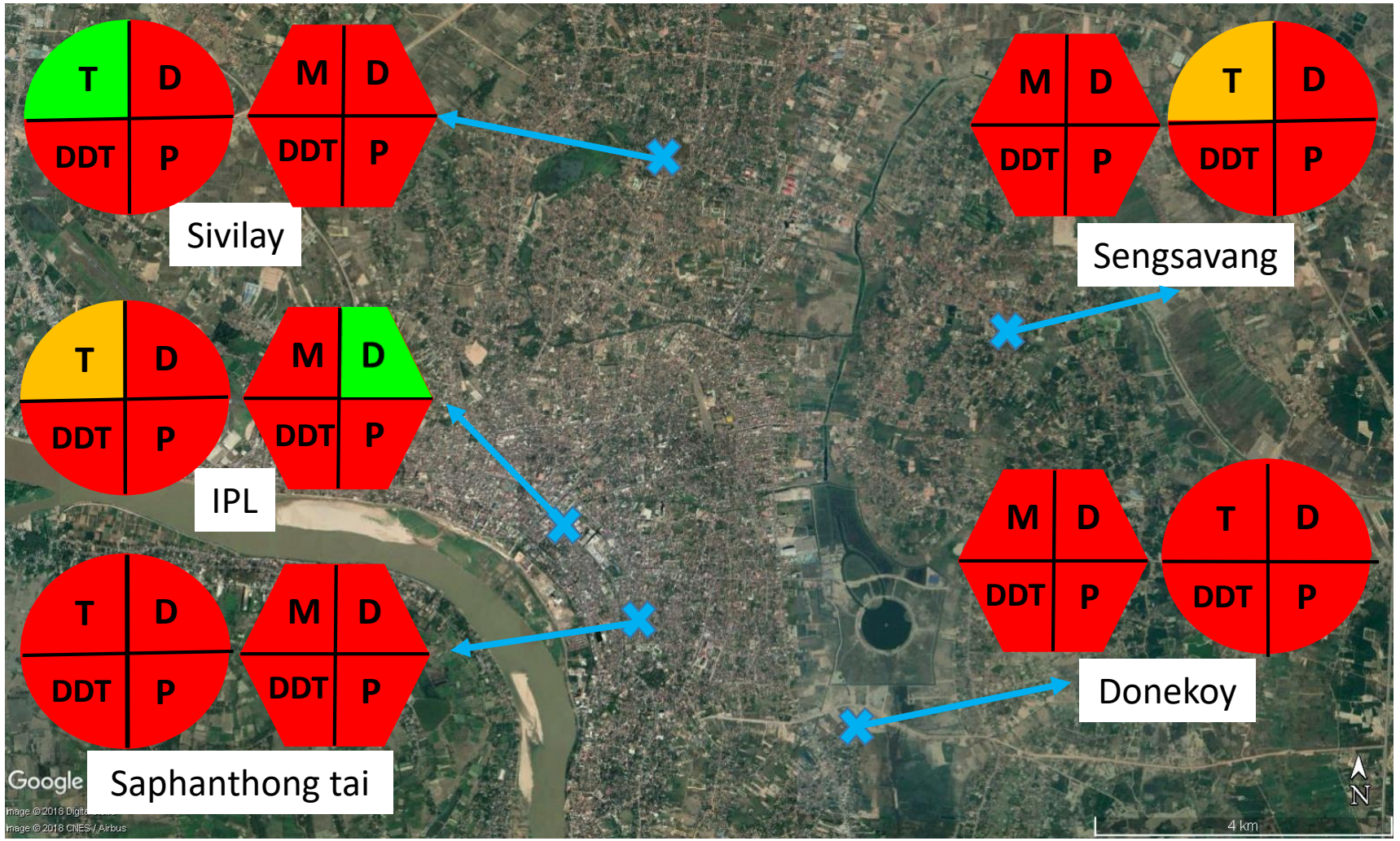
WHO criteria
- **Susceptible**

[98-100% mortality]
- **Suspected resistance**

[90-98% mortality]
- **Resistance**

[<90% mortality]

Insecticide resistance: *Aedes aegypti*



Vientiane Capital

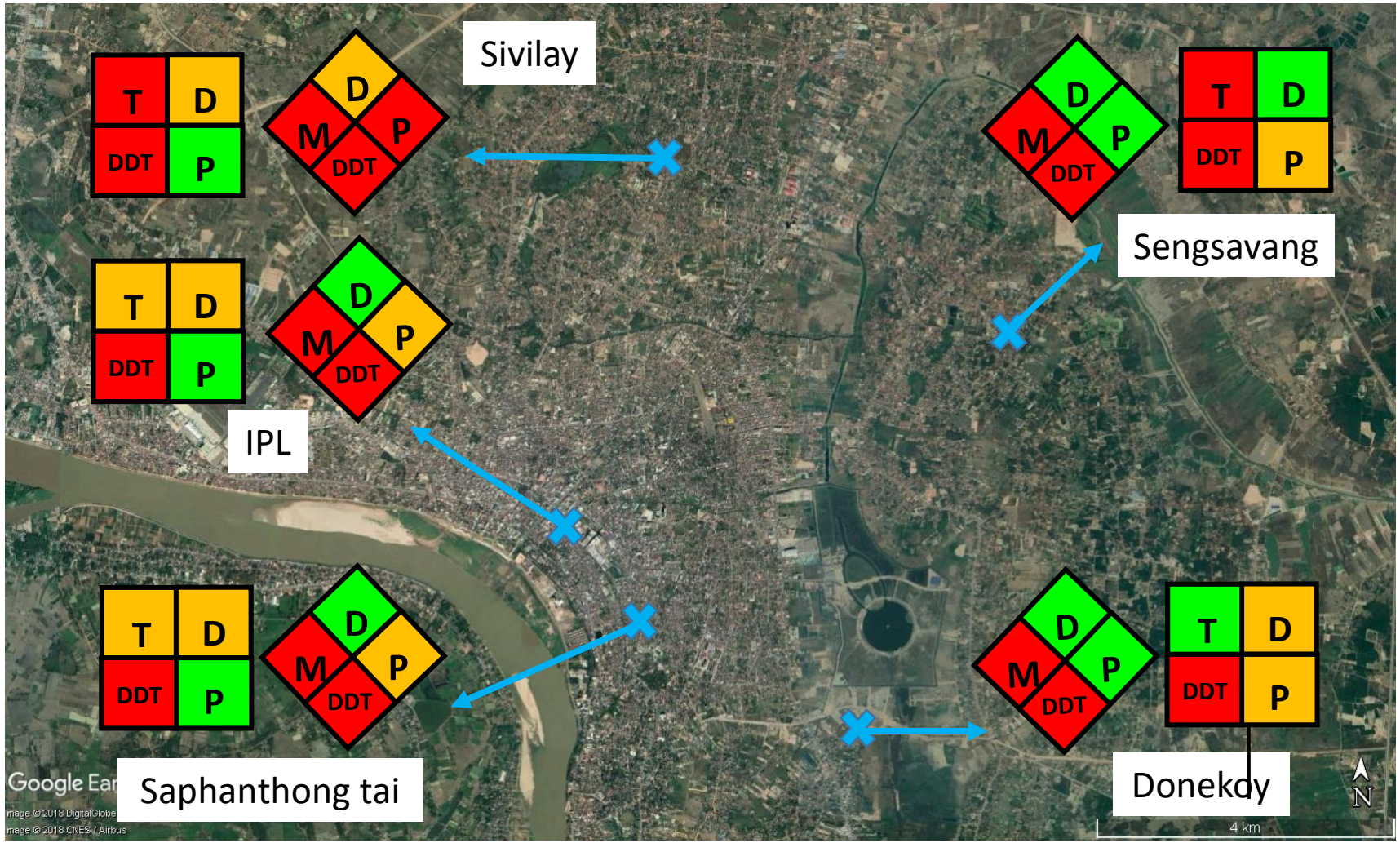
Larvae *Ae. aegypti*

Adult *Ae. aegypti*

T = Temephos
D = Deltamethrin
P = Permethrin
M = Malathion
DDT

Resistant
Suspected
Susceptible
WHO tests

Insecticide resistance: *Aedes albopictus*



Vientiane Capital

Larvae *Ae. albopictus*

Adult *Ae. albopictus*

T = Temephos

D = Deltamethrin

P = Permethrin

M = Malathion

DDT

Resistant

Suspected

Susceptible

WHO tests

Insecticide resistance monitoring, Temephos (ABATE)

LAO PEOPLE'S DEMOCRATIC REPUBLIC
PEACE INDEPENDENCE DEMOCRACY UNITY POSPERITY

Ministry of Health
Institut Pasteur du Laos
Tel: 021 285321, Fax: 021 285326

Ref: 670 /IPL
Vientiane, 26th of October 2017

New strategy for vector control in Lao PDR

Following our previous recommendations for the use of new insecticide as alternatives to Abate® for larval control in Lao PDR and by the present document we would like to introduce you a new strategy for dengue mosquito control. This strategy consists to dispose traps in a specific setting in the city of Vientiane capital with the aim to reduce mosquito abundance in the treated areas. The company In2Care® producing the traps is a private limited company registered and based in the Netherlands.

The In2Care Mosquito Trap is an outdoor contamination station against container-breeding and day-biting *Aedes* mosquitoes, with the goal to reduce the mosquito population to a level that greatly reduces nuisance and the spread of diseases such as Dengue. This auto-dissemination station lures and contaminates *Aedes* mosquitoes with a special slow-killing larvicide (pyriproxyfen) and lets them spread this to other breeding sites so that mosquito larvae are not only killed inside the trap but also in other breeding sites in its vicinity. This is combined with a biological adulticide (*Beauveria bassiana*) that kills the contaminated mosquito after a few days to prevent her from transmitting arboviruses. The product is US-EPA registered (EPA reg no. 91720-1) and allowed for professional use against *Aedes* mosquitoes. Registration processes in Lao PDR, Thailand, Cambodia and several other countries are ongoing.

The entomological team of Institut Pasteur du Laos will evaluate the In2Care Mosquito Trap persistence in the vicinity of IPL in collaboration with the In2Care correspondent, starting from December 2017. This pre-evaluation will be implemented to prepare a wider study aiming to reduce dengue incidence through mosquito reduction. This entomological study is part of the ECOMORE II project named **Development of tools for risk evaluation and control of viral mosquito borne diseases (dengue, chikungunya, zika) in urban settings** (2018 and 2019).

Furthermore, CMPE will be advised and ask to join the pre-evaluation presentation. We will share the results with CMPE.

Dr. Paul BREY
Director of Institut Pasteur du Laos

Dr. Sébastien MARCOMBE
Medical Entomologist

LAO PEOPLE'S DEMOCRATIC REPUBLIC
PEACE INDEPENDENCE DEMOCRACY UNITY POSPERITY

Ministry of Health
Institut Pasteur du Laos
Tel: 021 285321, Fax: 021 285326

Ref: 156 /IPL
Vientiane, 16th of March 2017

Recommendation for larval treatment of dengue mosquito vectors in Lao PDR

Although chemicals are widely used to treat *Ae. aegypti* larval habitats, larviciding should be considered as complementary to environmental management and – except in emergencies – should be restricted to containers that cannot otherwise be eliminated or managed.

Our recent studies showed that several mosquito populations from Vientiane C., Luang Prabang, Xayaburi, Attapue and Saravan presented moderate levels of temephos resistance. Temephos insecticide is the active ingredient of the Abate formulation, the larvicide used for dengue vector control in the Lao PDR. Those levels of insecticide resistance indicate that a selection pressure occurred in these mosquito populations. This emphasizes the need to find a substitute for the Abate formulation to avoid the development of resistance and make future public health operations useless. Furthermore, Abate is posing environmental issues and was banned in the European Union in 2009. The future use of more environmentally friendly products such as *Bti* spinosad and diflubenzon is recommended, especially knowing that no cross-resistance between these insecticides and temephos has been reported.

We recommend to start using new insecticides in rotation (i.e. a different insecticide each year) to avoid or to manage the development of insecticide resistance in the larval populations of the dengue vectors in the Lao PDR.

Bti or *Bacillus thuringiensis var israeliensis* is a bio-insecticide that has desirable properties for mosquito control because of its fast killing effect, a good toxicological profile, and the absence of cross-resistance with conventionally used pesticides. Spinosad is a bio-insecticide (naturalytes family) that is based on natural metabolites (spinosine A and D) derived from the actinomycetale *Saccharopolyspora spinosa.* Because of its unique mode of action, spinosad shows promising potential for the control of dengue vectors. Diflubenzuron is an insect growth regulator (benzoylurea family) that acts by disrupting chitin synthesis and deposition. This IGR showed a promising efficacy against several mosquito species, especially *Aedes aegypti.* All of the above insecticides are recommended by the World IIealth Organization (WHO) for use for vector control in drinking water sources and containers and may be used routinely by mosquito control services

We also recommend the development of the use of guppy fishes and copepods (small freshwater crustaceans) in large water storage containers. The usefulness of these biological methods to control dengue vectors has been proven.

Dr. Paul BREY
Director of Institut Pasteur du Laos

Dr. Sébastien MARCOMBE
Medical Entomologist

สะถาบัน ปัดสะเติ ลาว
INSTITUT PASTEUR DU LAOS

ກະຊວງ ສາທາລະນະສຸກ
Ministry of Health

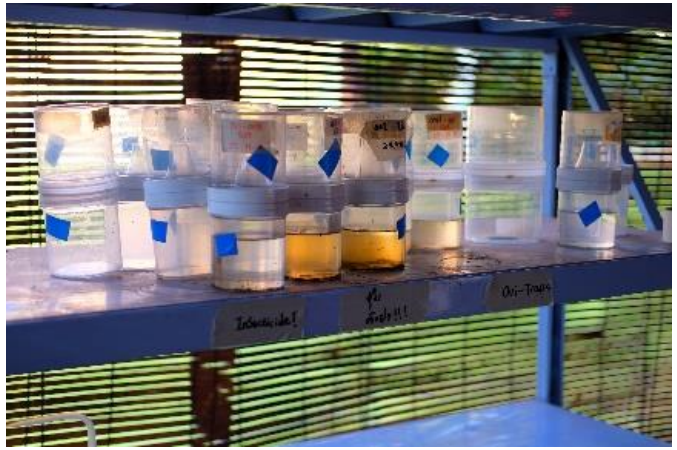
New National Strategic Plan for Dengue control in Laos (2019)

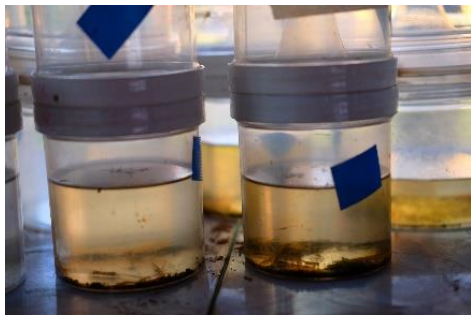
Innovative vector control strategy, In2Care traps

- Preliminary small scale study at IPL
  - Residual efficacy of the traps
  - Reduction of mosquito abundance in the area?

Innovative vector control strategy, In2Care traps

- Small scale study, IPL
  - Residual efficacy of the traps





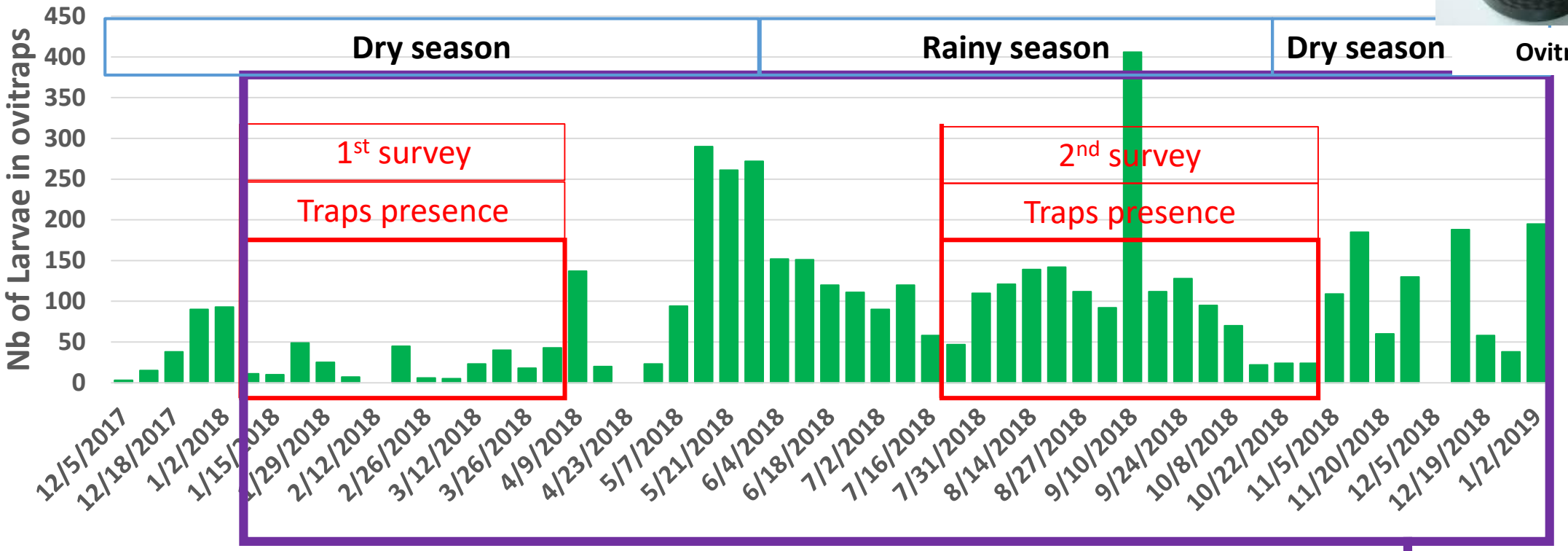
Time	Trial 1	controls	Trial 2	controls
week 4	0	100	0	92
week 6	0	100	0	100
week 8	0	100	0	96
week 10	0	80	0	80
week 12	0	88	0	88

Table. % Adult emergence in water sample collected in five traps every 2 weeks.

➢ **No emergence of mosquitoes from the traps**

IPL small scale study: Larval survey, ovitraps

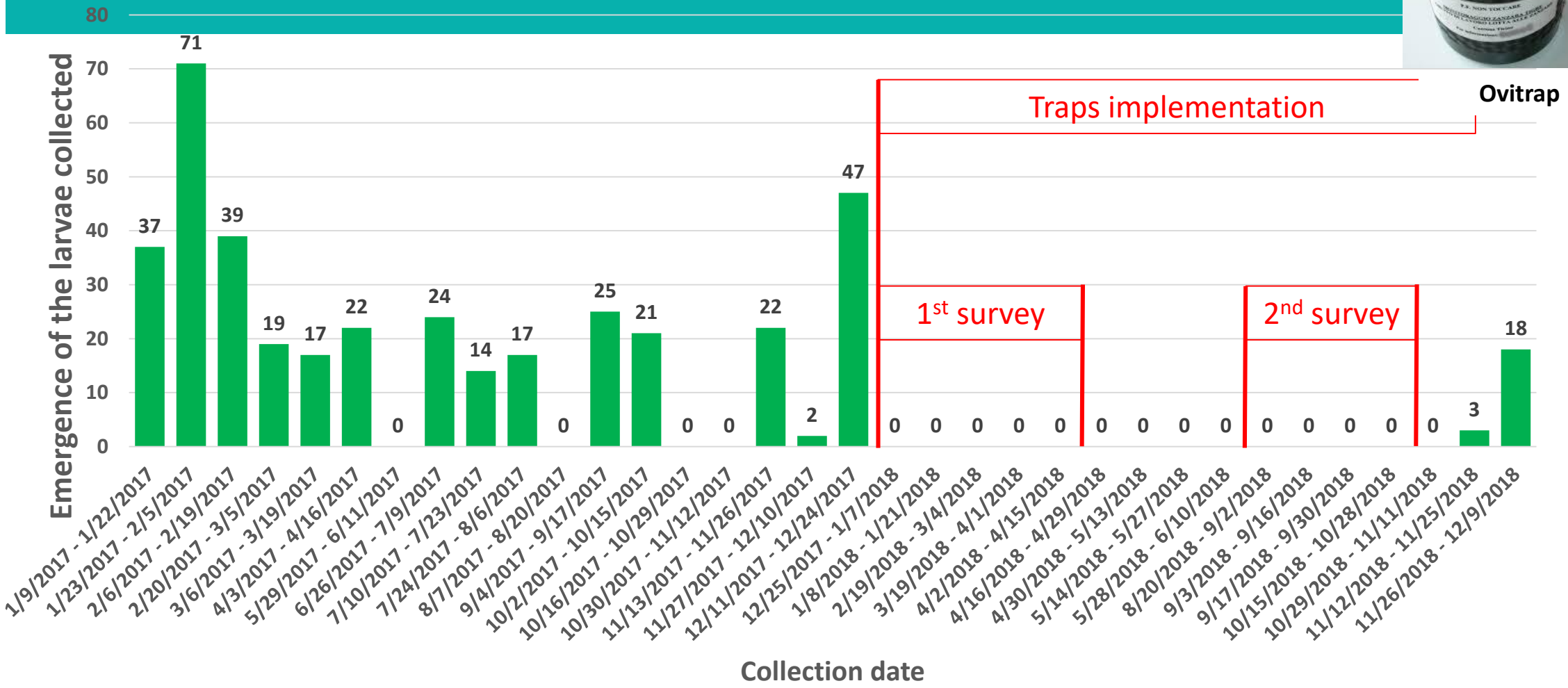
Ovitrap



Number of Larvae (*Ae. aegypti* and *Ae. albopictus*) collected in ovitraps at IPL

No emergence of adults from the larvae collected from the ovitraps during both surveys

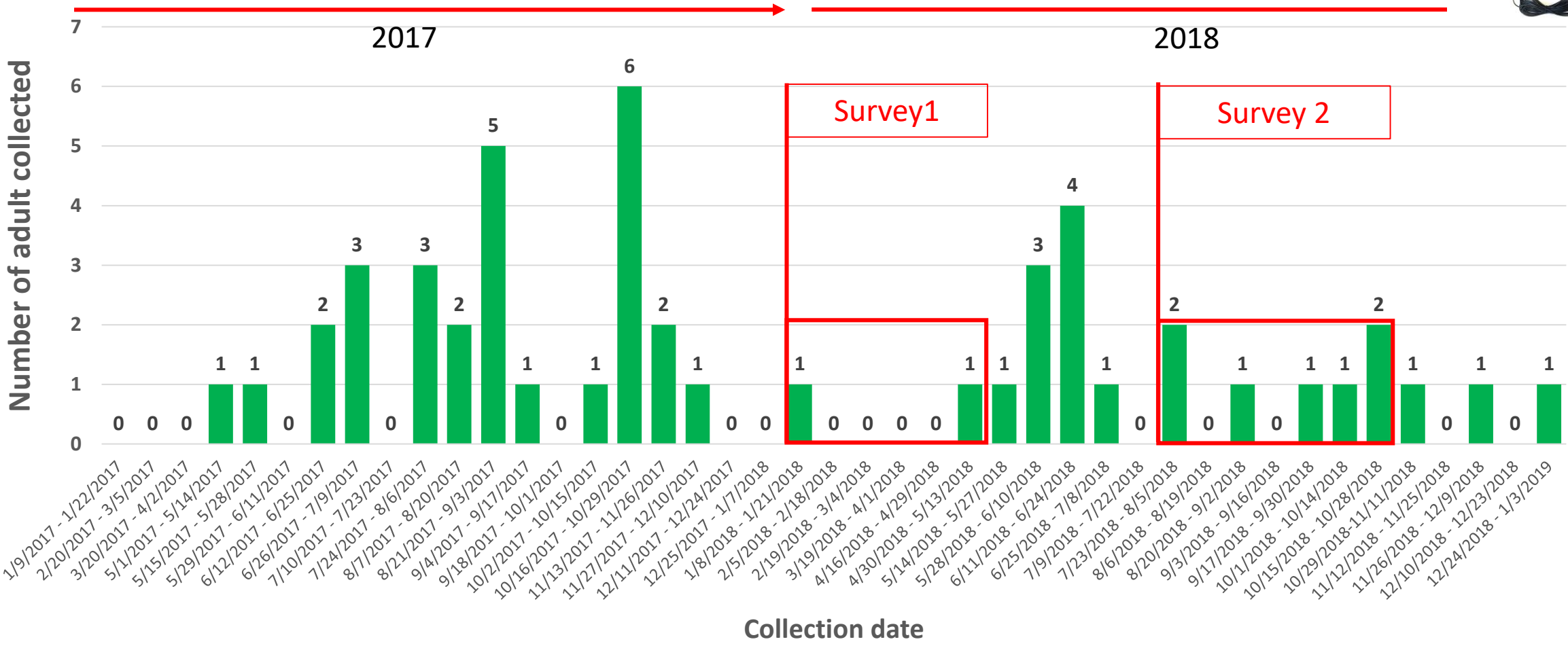
IPL small scale study: Larval survey, emergence



Number of *Ae. aegypti* and *Ae. albopictus* adult emerged from ovitraps in IPL

No emergence of adults from the larvae collected from the ovitraps

IPL small scale study: Adult survey



Number of Adult *Ae. aegypti* and *Ae. albopictus* collected with BG traps at IPL

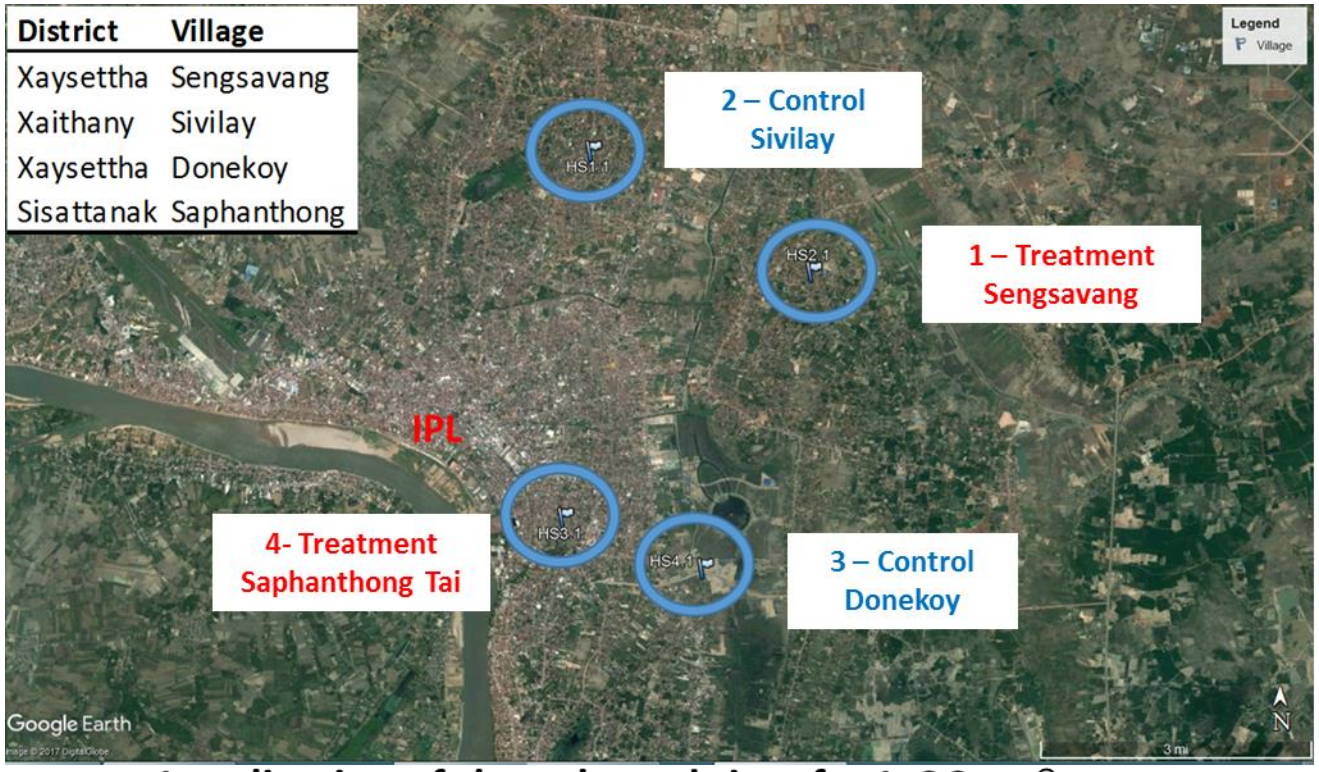
IPL small scale study: Conclusions

- No effect on the adult and larval population adundance
  - ➢Re-colonisation from outside the treated area
- Autodissemitation technique is working
  - ➢No emergence of adult in breeding sites

Innovative vector control strategy: In2Care® traps

Protocol:

- 2 Control sites vs 2 treatment sites
- August 2018 to August 2019
- Refill of the insecticides and water every 6 weeks

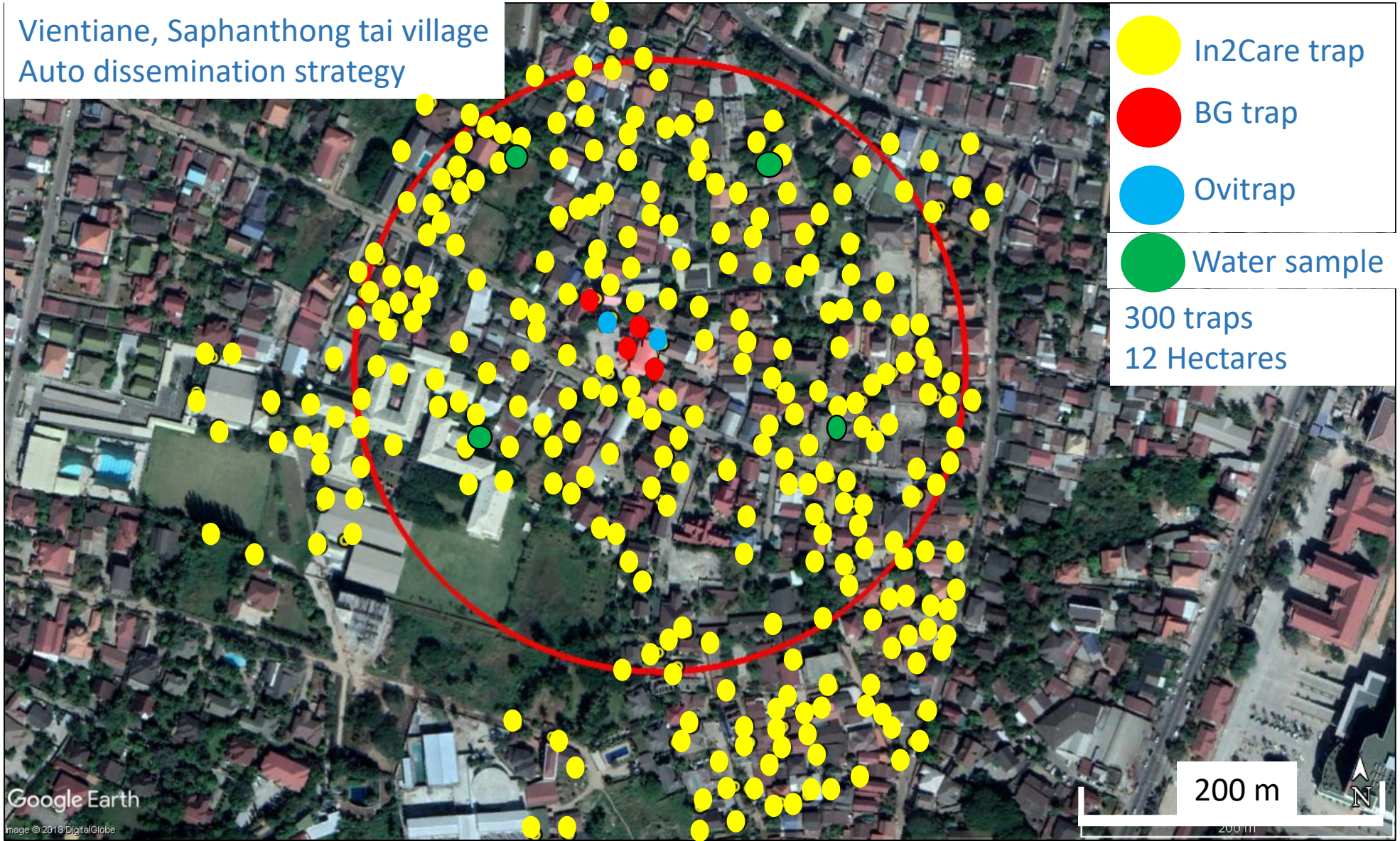


Localization of the selected sites for In2Care® traps implementation in Vientiane

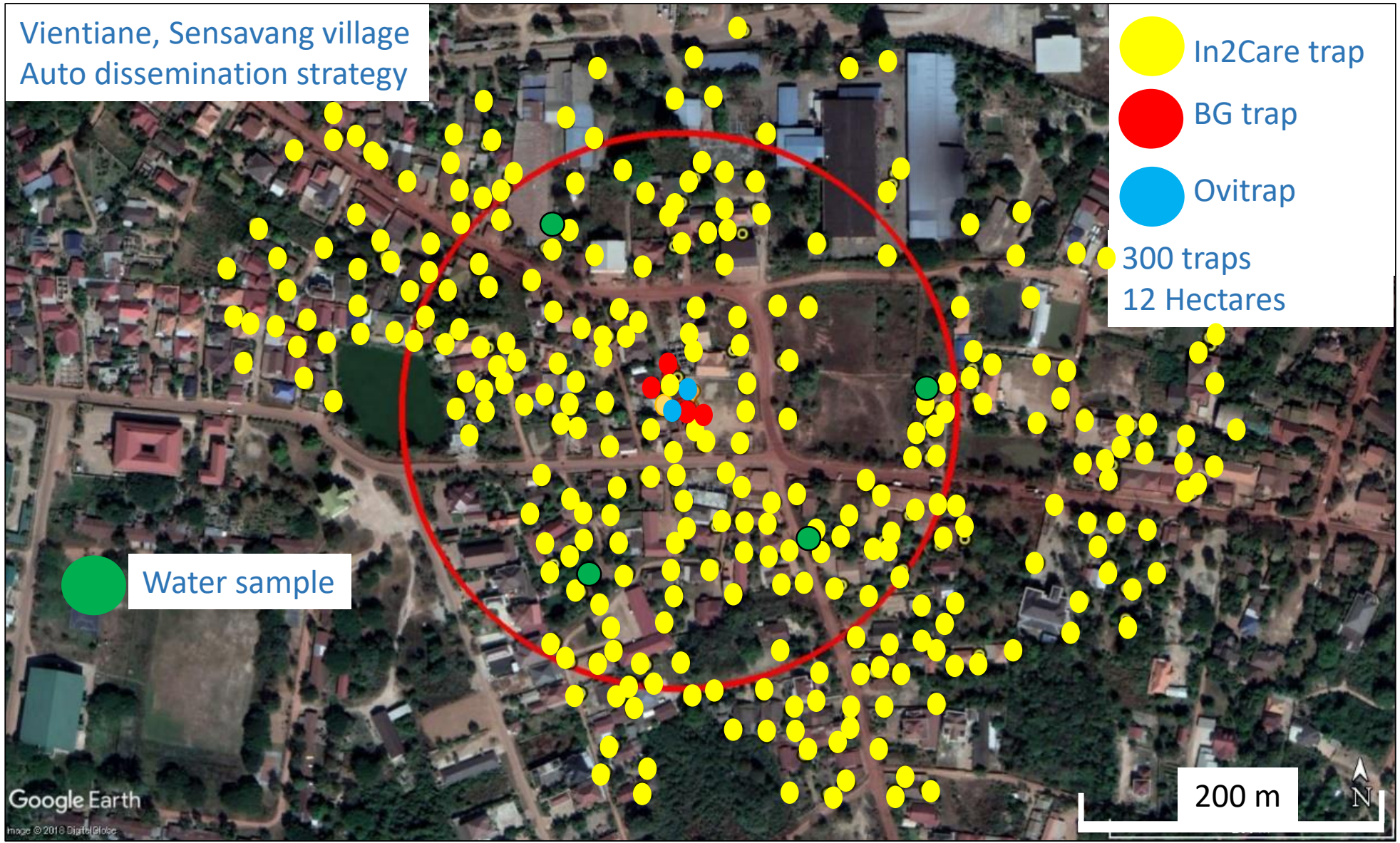
In2Care® trap

300 traps / village
12 Hectares treated / village
1 trap for 400m2

Traps location

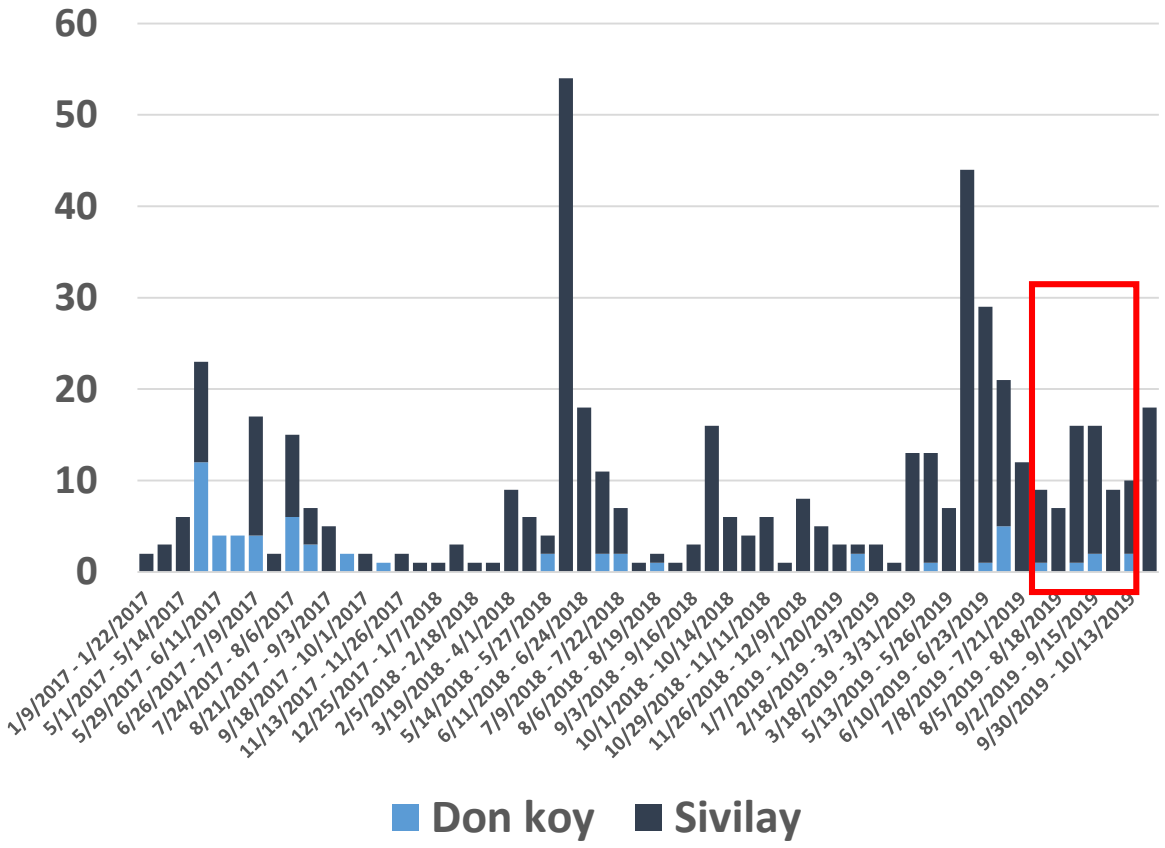


Traps location

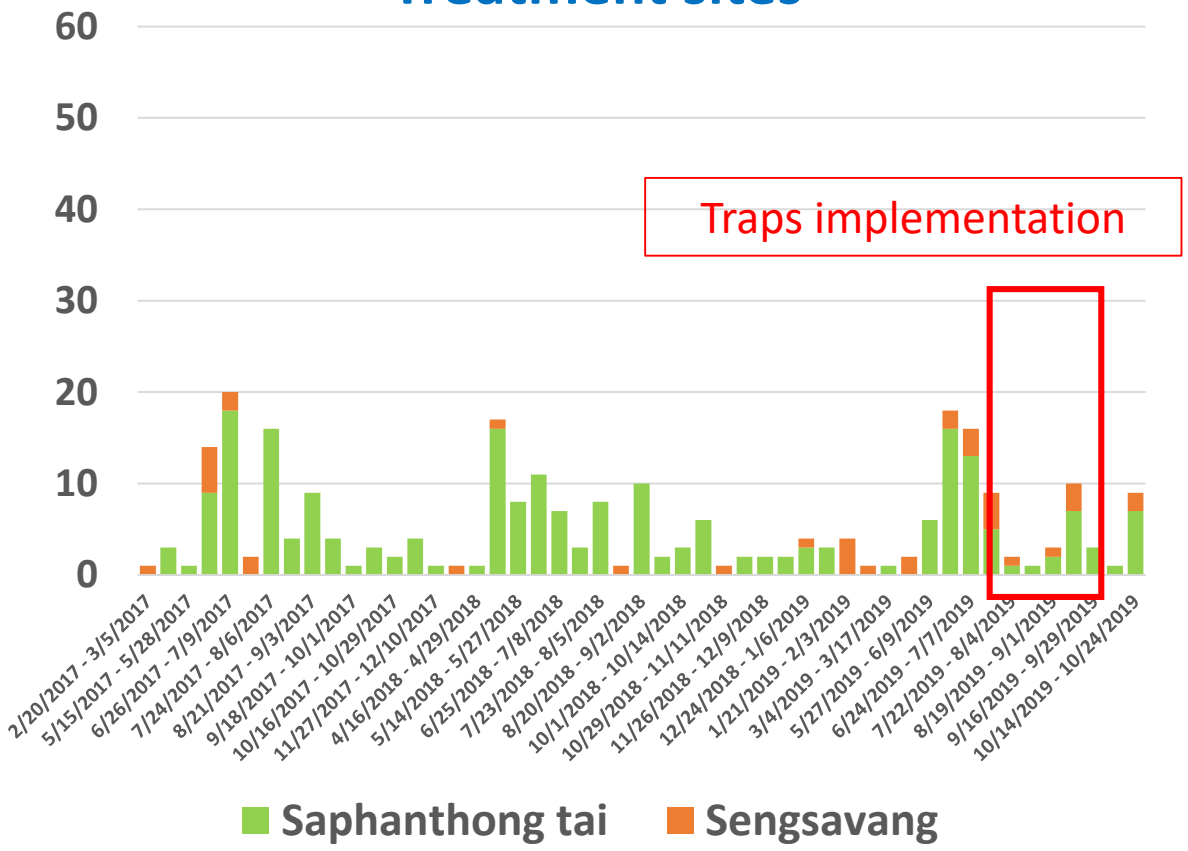


New vector control strategy: Adult collection

Control sites

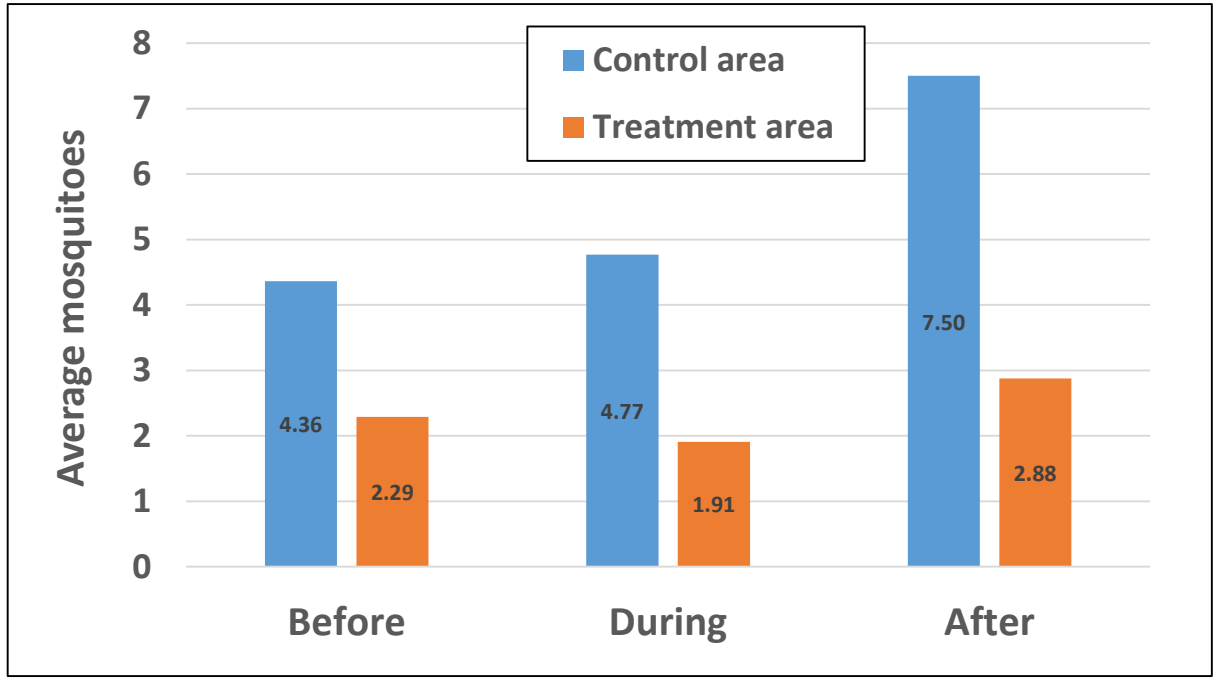


Treatment sites



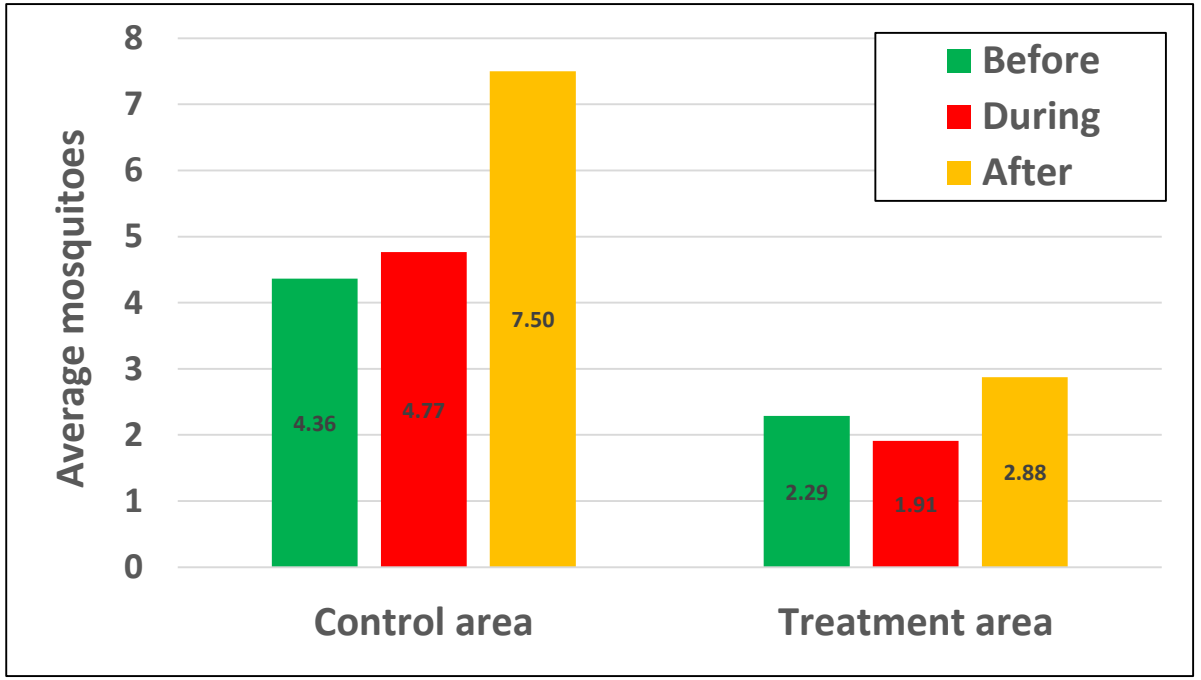
Number of Adult *Ae. aegypti* and *Ae. albopictus* collected between January 2017 and October 2019 in Vientiane. Treatment with In2Care traps between August 2018 and August 2019

New vector control strategy: Statistical analysis, Adult



Treatment effect before, during and after treatment in treated and control areas, adult abundance

Anova	Df	Sum sq	Mean sq	F value	Pr (>F)	
Treatment	2	52	26.2	0.649	5.23E-01	NS
Location	1	486	486.3	12.048	0.000592	***
Treatement x Location	**1**	**923**	**322.9**	**26.456**	**4.77E-07**	**NS**
Residuals	312	10884	34.9			



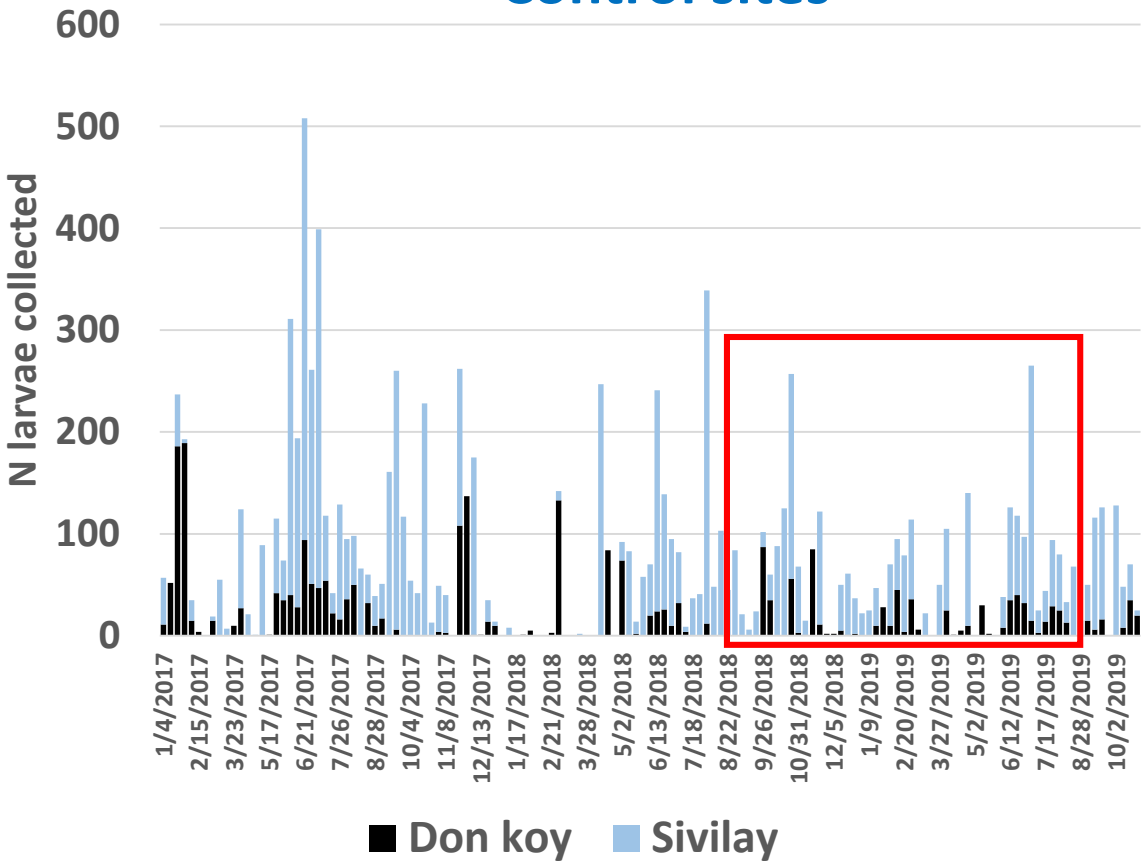
Comparison of control vs treated areas before and after treament, adult abundance

- **Statistically no effect of the treatments observed on adult abundance between treated and control areas**
- **Slight decrease of adult abundance during the treatment but not statistically validated**

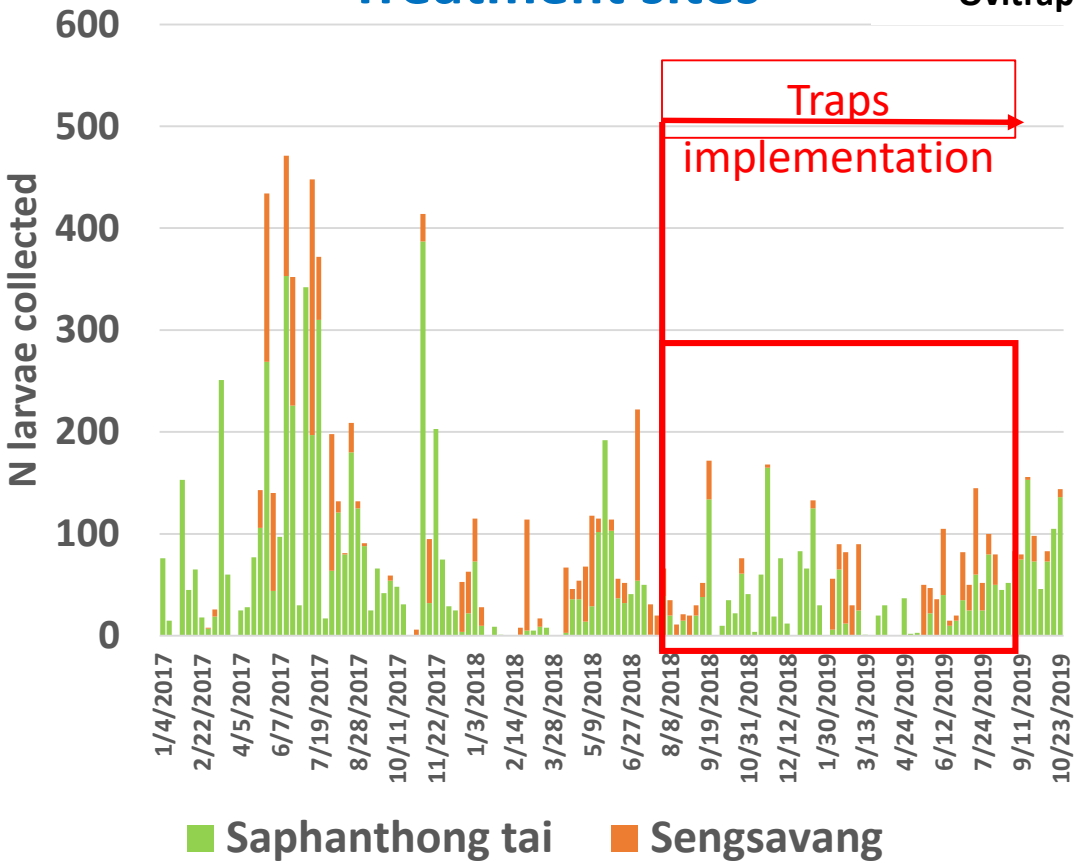
New vector control strategy: Larval collection

Ovitrap

Control sites

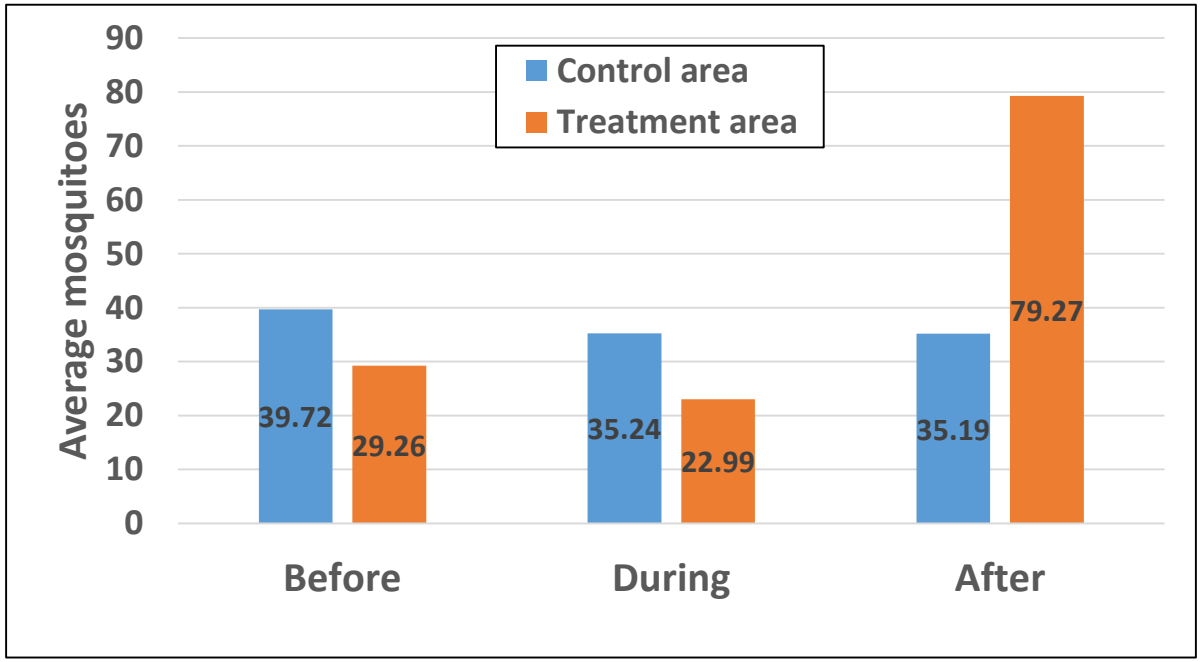


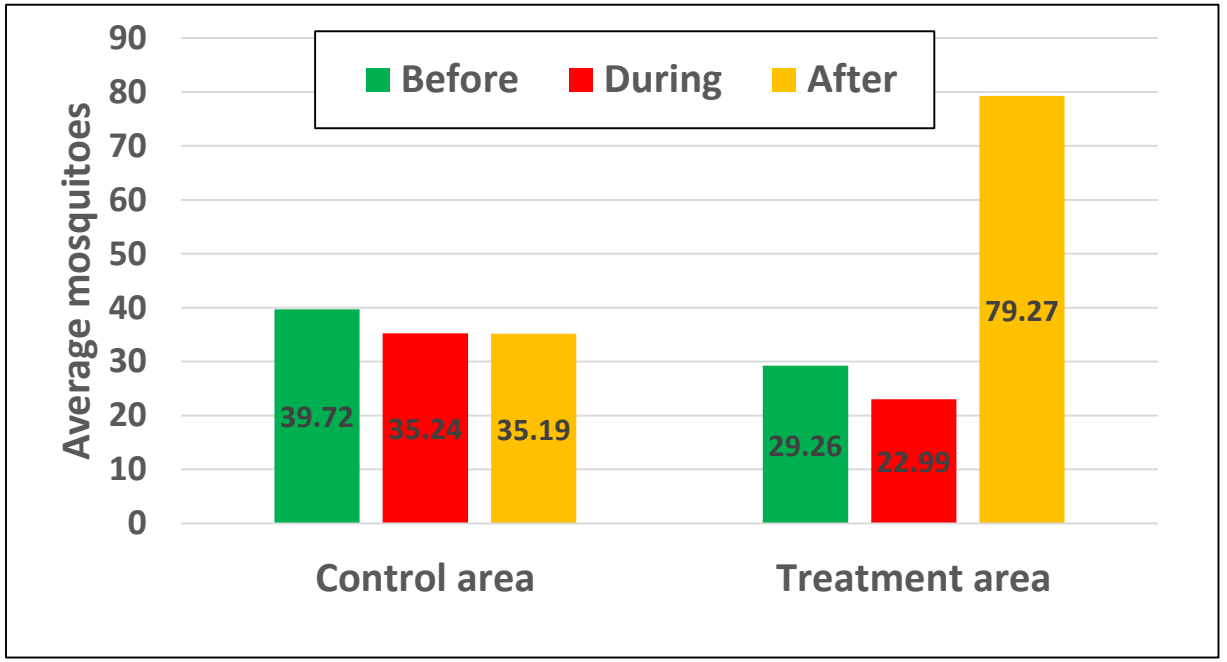
Treatment sites



Number of Larvae *Ae. aegypti* and *Ae. albopictus* collected weekly between January 2017 and October 2019 in Vientiane. Treatment with In2Care traps between August 2018 and August 2019

New vector control strategy: Statistical analysis, Larvae





Treatment effect on larval abundance before, during and after treatment

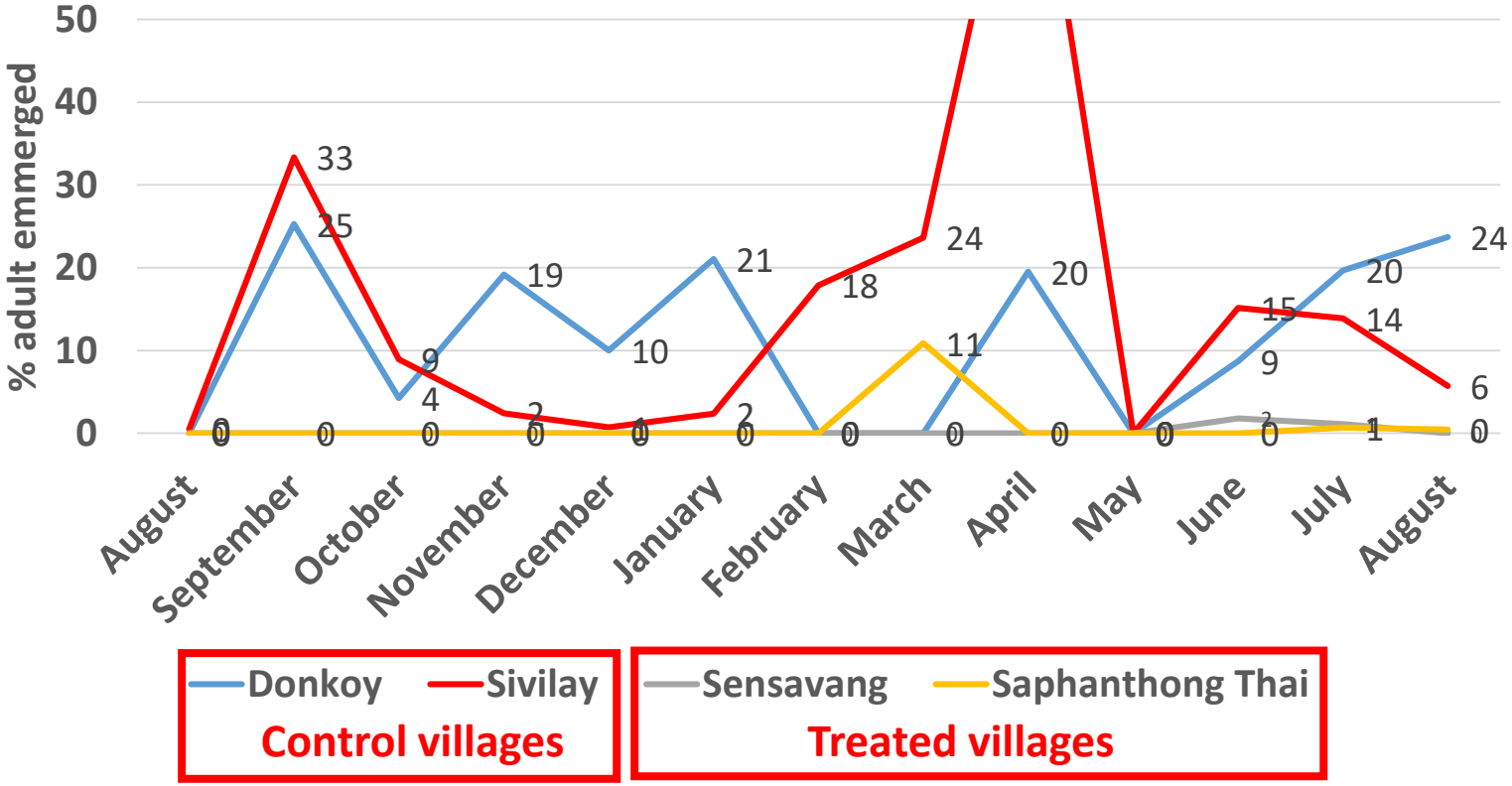
Anova	Df	Sum sq	Mean sq	F value	Pr (>F)	
Treatment	2	190282	95141	24.154	7.49E-11	***
Location	1	4212	4212	1.069	0.30147	NS
Treatement x Location	**2**	**38888**	**19444**	**4.936**	**7.45E-03**	**
Residuals	665	2742794	4125			

Comparison of larval abundance between control vs treated aeas before and after treament

- No statistical treatment effect before and during the treatment between treated and control areas
- Significant effect (increase) between before/during and after treatment

New vector control strategy: Emergence from ovitraps

Ovitrap



Emergence rate of *Ae. aegypti* and *Ae. albopictus* adult from ovitraps in control and treated areas

- **No emergence of adults from the larvae collected from the ovitraps during the treatment period**

Field implementation remarks: Technical organization

Field implementation remarks: Technical organization

Field implementation remarks: Laos

- Importation of the traps
- Number of people available
- Houses access: more than 200 houses visited per village
- Where to put the traps and time to install them
- Weather conditions
- Refusal:
  - No mosquito so no need of the traps
  - More mosquitoes after implementation
  - Not understanding the use/principle of the traps
  - Danger for kids and animals?
  - Larvae always present in the traps: IGR






New vector control strategy: Other results

Currently under analysis:

- Indexes (Breteau, house and container index) in the 4 villages before, during and after treatment
- Efficacy of autodissemination: Water samples randomly collected in the treated areas: larval emergence vs container volume and, number of breeding sites
- Fungus efficacy: mortality of the mosquitoes exposed to gauzes from the field
- Dengue incidence in treated areas vs control areas and screening of the virus in mosquitoes collected

Conclusions on the new strategy:

- No statistical effect on the adult and larval population adundance
  - ➢ Re-colonisation from outside the treated area
- Autodissemitation technique is working
  - ➢ No emergence of adults in control oviposition containers
  - ➢ Limitations: number of breeding sites and volume
- Autodissemination technique should be applied in combination with other conventional and new strategies

Acknowledgements

- IPL technicians, drivers and scientists
- Military staff
- Village volunteers
- Mayors
- District health officers

ກະຊວງ ສາທາລະນະສຸກ
Ministry of Health

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AGENCE FRANÇAISE
DE DÉVELOPPEMENT

ສະຖາບັນ ປັດສະເຕີ ລາວ
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