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An Integrated Approach for Entomo-virological Surveillance in Endemic Areas for Arboviruses

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INSTITUTO OSWALDO CRUZ, BRAZIL

Introduction

Introduction

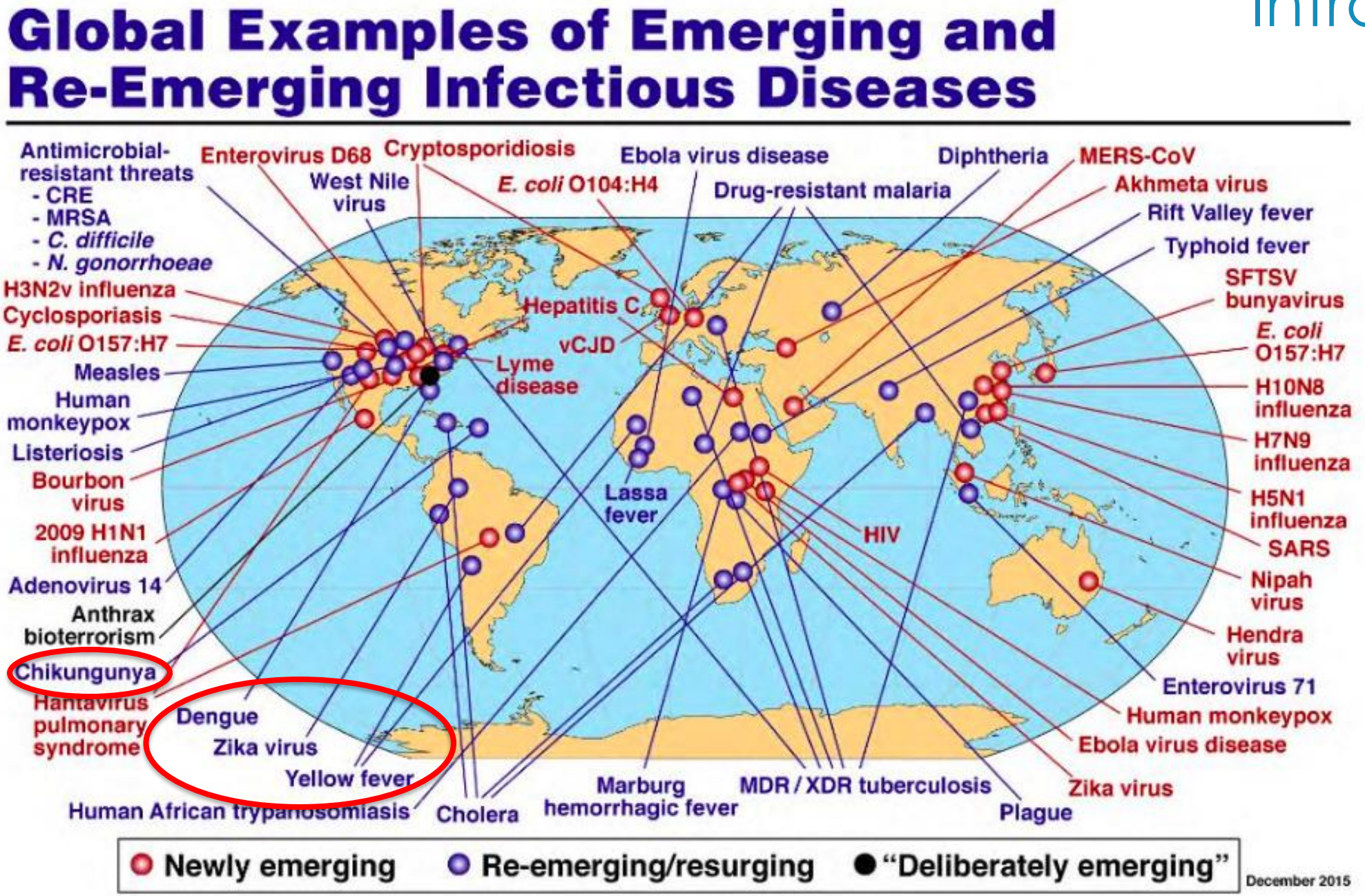
Global importance and situation in Brazil

- **Emerging infectious diseases** represent a global threat, because a disease can emerge or re-emerge anywhere in the planet and **spread quickly** to other regions through trade and travel: *"A health threat anywhere is a health threat everywhere"* (iom.int)


Global aviation network

- From all the emerging pathogens that produced outbreaks in recent years, those transmitted by **vectors** have shown increased relevance.

Introduction



Credit NIH

Introduction

Global importance and situation in Brazil

- **Mosquito-borne viruses** (DENV, CHIKV, ZIKV and YFV) major challenges in public health.

- The scenario in **Brazil** → possibility of large epidemics due to several factors:

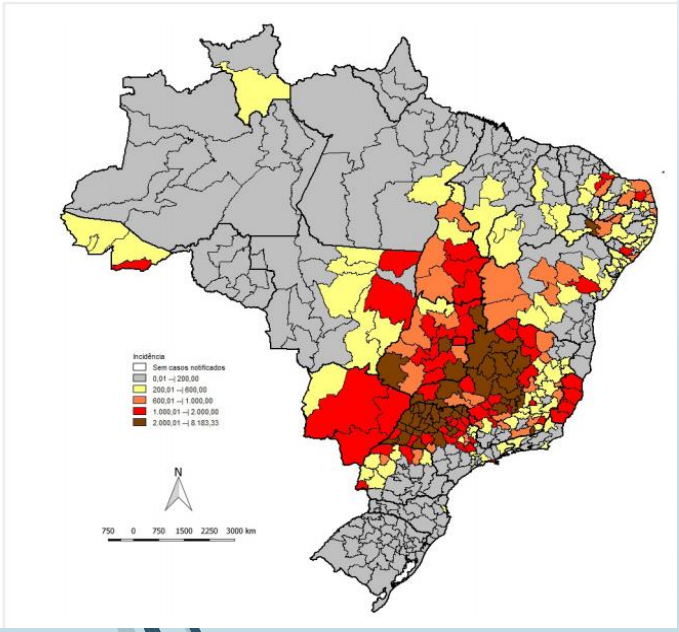
- ✓ Widespread infestation by the two main vectors, ***Ae. aegypti* and *Ae. albopictus***;
- ✓ The **simultaneous circulation** of arboviruses (DENV, CHIKV, YF, ZIKV);
- ✓ Difficulty of an accurate **diagnosis and therapeutic** approach;
- ✓ **Susceptibility** of most of the human population, favoring the rapid spread of the virus;
- ✓ Great **territorial extension** of the country, which hinders surveillance and **access to health services**, such as laboratory tests for diagnostic confirmation.

Introduction

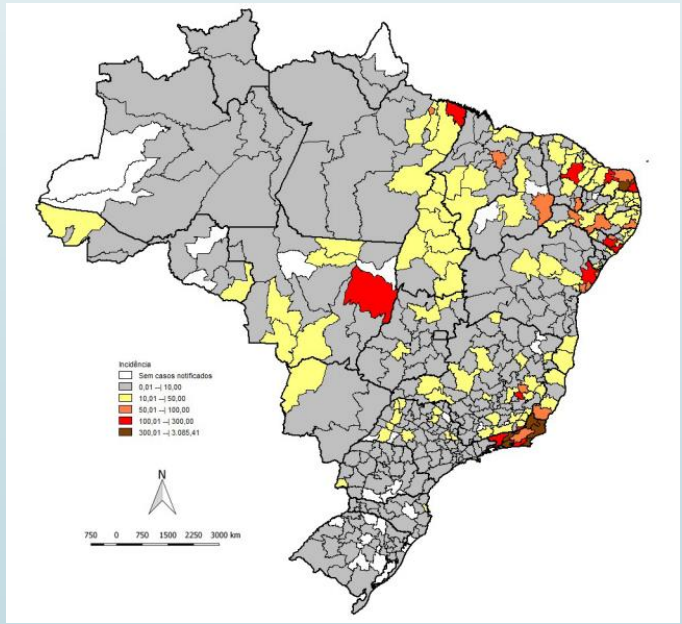
Global importance and situation in Brazil

- Simultaneous outbreaks and epidemics → simultaneous circulation of DENV-1, DENV-2, DENV-3 and DENV-4, CHIKV and ZIKV in recent years in Brazil

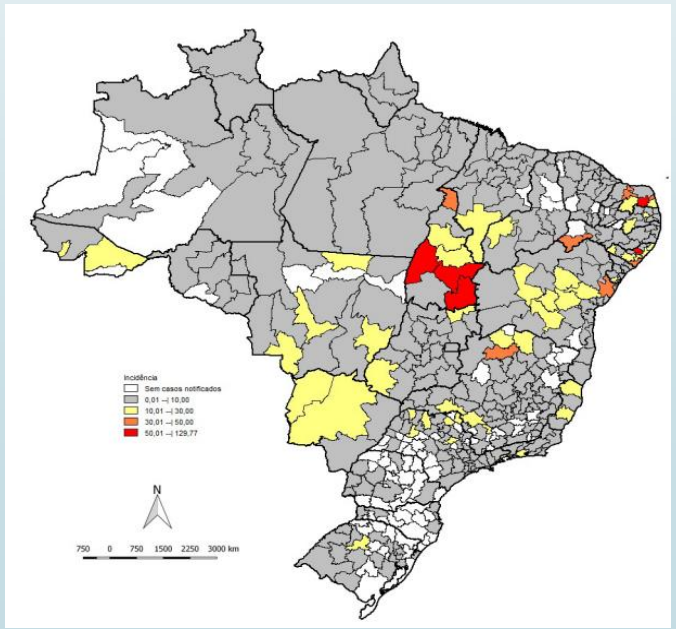
Distribuição geográfica da incidência de **dengue** por Região de Saúde, Brasil, até a SE 34 (2019). SINAN



Distribuição de incidência de casos prováveis de **chikungunya** por Região de Saúde, até a SE 34 (2019). SINAN



Distribuição de incidência de casos prováveis de **Zika** por Região de Saúde, até a SE 33 (2019). SINAN



Introduction

- The **processes of emergence and re-emergence** of arboviruses in a territory pass through the understanding of different factors that participate in the transmission chain:

Vertebrate hosts | **Invertebrate hosts** | **Etiological agent**

- Transmission between humans in urban and periurban environments by the same species of mosquitoes:

Aedes aegypti and Ae. *albopictus*

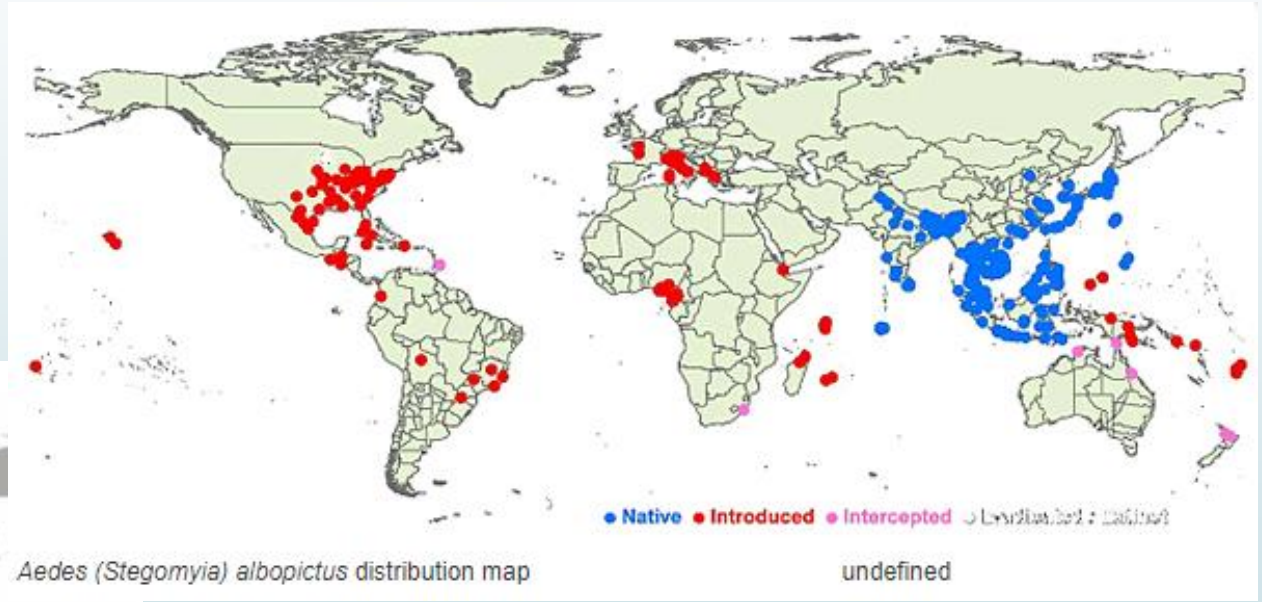
- Understanding of their biology and ecology becomes of paramount importance.

Introduction

Main vectors

- Global distribution of *Ae. aegypti* (left) and *Ae. albopictus* (right)

Khormi, 2014)

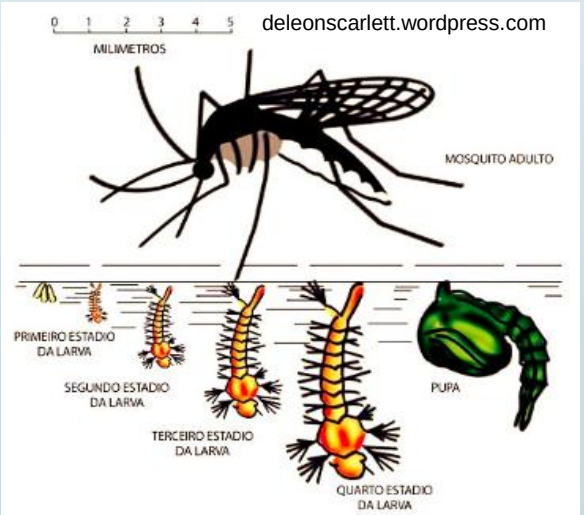


Aedes (Stegomyia) albopictus distribution map

undefined

Introduction

- Culicidae are the **most important vectors of arboviruses** to humans.
- Female is infected during **blood feeding** in a viraemic vertebrate host, or through **vertical transmission**, remaining infected throughout its life.
- **Holometabolic** biological cycle comprises 4 stages of development: egg, larva, pupa and adult.

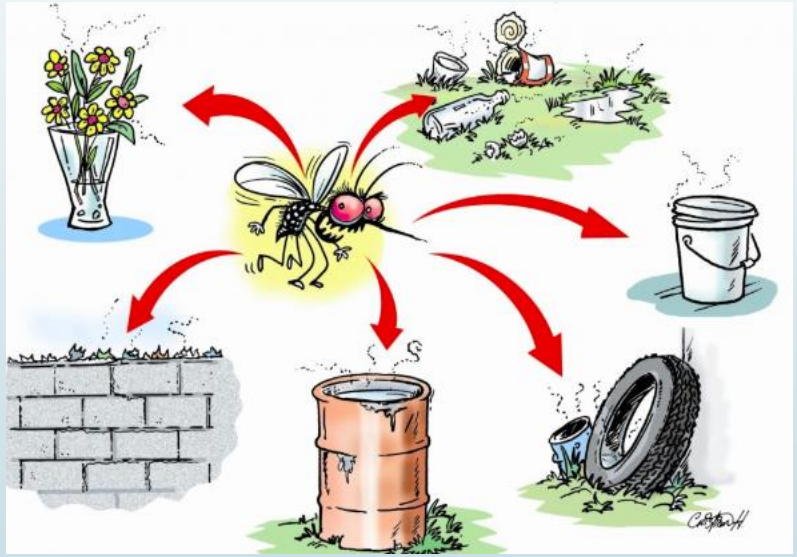


Introduction

- *Aedes* mosquitoes develop in different **natural and artificial** breeding sites:

- Animal burials
- Tree hollows
- Bamboo internodes
- Large animal footprints
- Cracks in rocks
- Fallen leaves

- Tires
- Water boxes
- Drums
- Barrels
- Pots of plants
- Disposable garbage

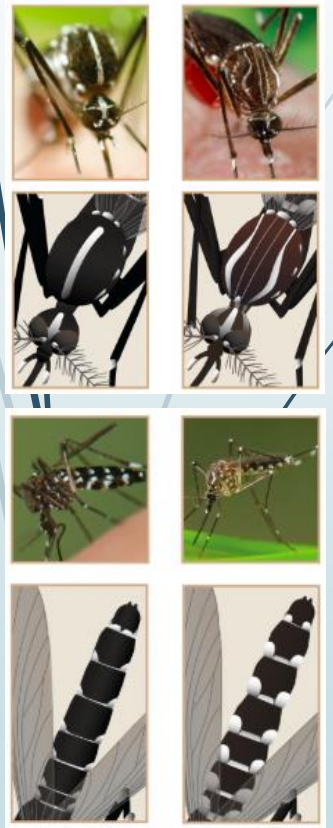


Introduction

- Only **female** blood feeding → oviposits 200 to 450 eggs, **inner walls** near the water, which remain viable for several **months**.
- Oviposition in "**jumps**", distributing small quantities of eggs in several breeding places → favors the dispersion of pathogens.

Introduction

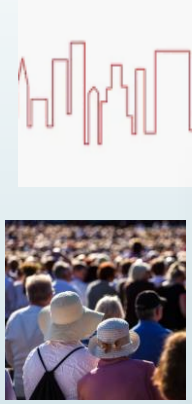
Ae. aegypti and *Ae. albopictus*



http://www.mosquitoalert.com/

Ae. aegypti

- African.
- Anthropophilic. Endophilic inside domiciles.
- High human population concentration and high concentration of residences.
- Their breeding sites are usually artificial containers



Ae. albopictus

- Southeast Asia.
- Natural vegetation.
- Low human population density. Prefer the peridomicile.
- Artificial containers, natural containers.

Introduction

Entomo-virological surveillance

- **Entomological surveillance:** activity based on indicators to detect **presence, geographical distribution and density** of vectors in time and space, to estimate the **risks of transmission** of pathogens.
- Mosquitoes are **markers for the evaluation of viral circulation**.
- **Virological surveillance** (virus detection in field-caught vectors) in endemic areas is a viable alternative for the consolidation of local surveillance and an epidemic alert system to direct control actions in critical areas.
- Many arbovirus surveillance programs in low- and middle-income countries do not have resources for pathogen screening with currently available techniques → **new alternatives**

Objectives

Main objective: perform **integrated surveillance**, as part of several prospective studies in endemic areas, to study the **transmission dynamics** of arboviruses, through entomological monitoring focused on the search for **infected culicidae**.

- ✓ *"Formation of a prospective cohort for the study of dengue in a child population"* (P. Brasil)
- ✓ *"Evaluation of transmission dynamics of dengue arbovirus (DENV), chikungunya (CHIKV) and Zika (ZIKV) in mosquitoes in an urban community in Rio de Janeiro"* (CNPq Grant Nr. 157464/2015-6) (P. Brasil and N. Honorio).
- ✓ *"Methodological proposal for stratification of risk areas for dengue, chikungunya and Zika in Brazilian endemic cities"* (ARBOALVO, N. Honorio and M. Carvalho).

Early Evidence for Zika Virus Circulation among *Aedes aegypti* Mosquitoes, Rio de Janeiro, Brazil

Tania Ayllón, Renata de Mendonça Campos, Patrícia Brasil, Fernanda Cristina Morone, Daniel Cardoso Portela Câmara, Guilherme Louzada Silva Meira, Egbert Tannich, Kristie Aimi Yamamoto, Marilia Sá Carvalho, Renata Saraiva Pedro, Jonas Schmidt-Chanasit, Daniel Cadar, Davis Fernandes Ferreira, Nildimar Alves Honório

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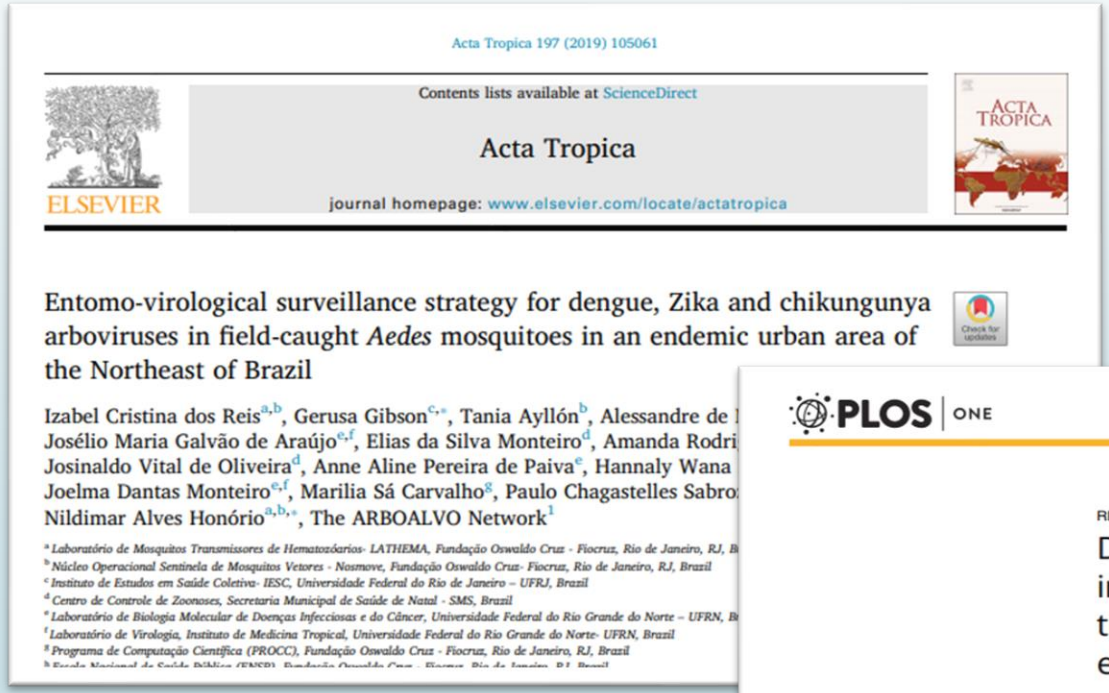
DOI: https://doi.org/10.3201/eid2308.162007



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Entomo-virological surveillance strategy for dengue, Zika and chikungunya arboviruses in field-caught *Aedes* mosquitoes in an endemic urban area of the Northeast of Brazil

Izabel Cristina dos Reis[a,b], Gerusa Gibson[c,*], Tania Ayllón[b], Alessandre de
Josélio Maria Galvão de Araújo[e,f], Elias da Silva Monteiro[d], Amanda Rodri
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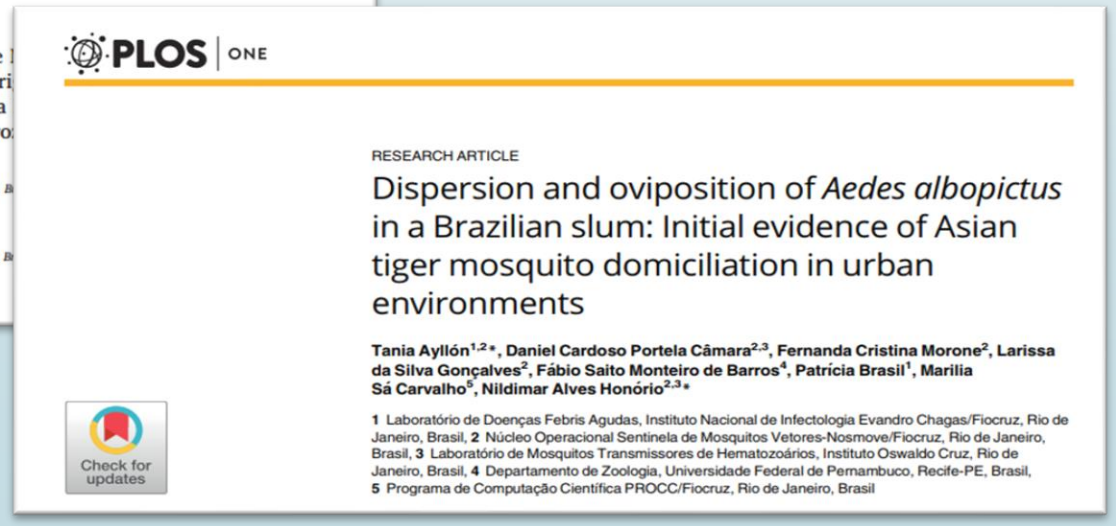

PLOS ONE

RESEARCH ARTICLE

Dispersion and oviposition of *Aedes albopictus* in a Brazilian slum: Initial evidence of Asian tiger mosquito domiciliation in urban environments

Tania Ayllón[1,2]*, Daniel Cardoso Portela Câmara[2,3], Fernanda Cristina Morone[2], Larissa da Silva Gonçalves[2], Fábio Saito Monteiro de Barros[4], Patrícia Brasil[1], Marilia Sá Carvalho[5], Nildimar Alves Honório[2,3]*

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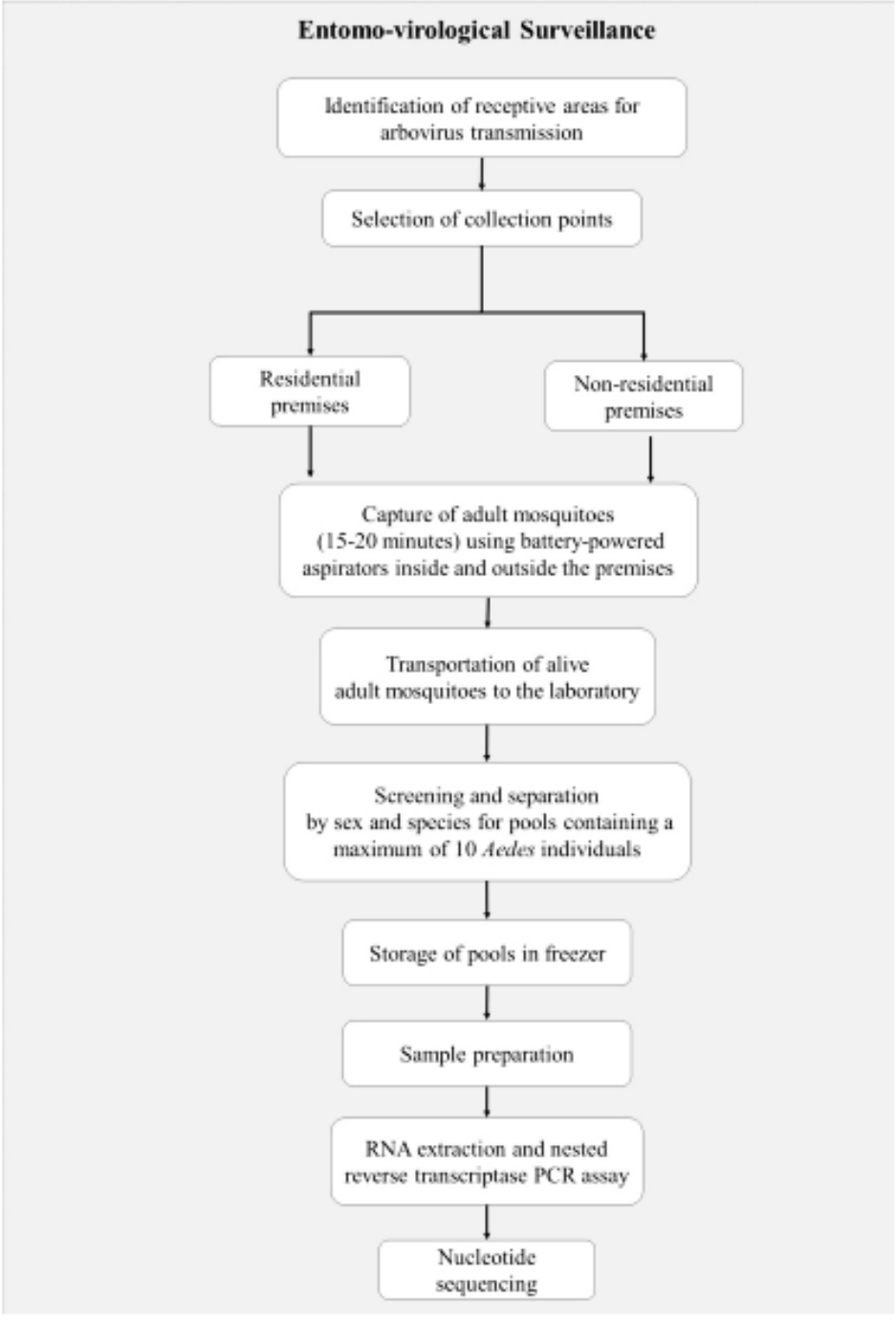
Methods

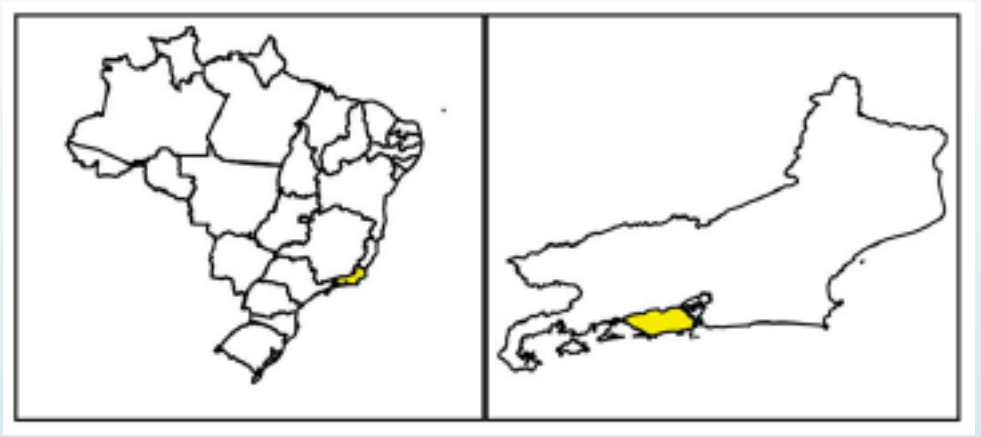
Schematic representation of the entomo-virological surveillance strategy.



Methods

Study areas

- Brazil: Rio de Janeiro
- Rio Grande do Norte

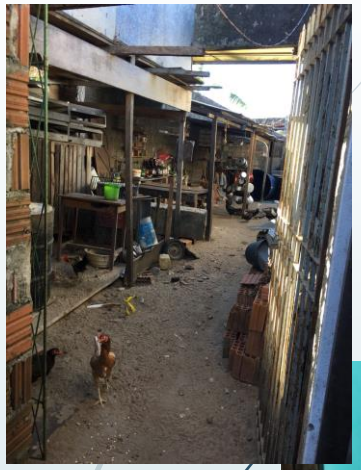


Definition of collection points

- Urban areas/periurban areas
- High-risk areas for arboviruses
- Densely urbanized

Methods

Collection points for immature and adults



- Households
- Key-sites
- Schools
- Health units

→

- Junkyards
- Thrift stores
- Factories
- Tire repair shops
- Garages
- Etc.

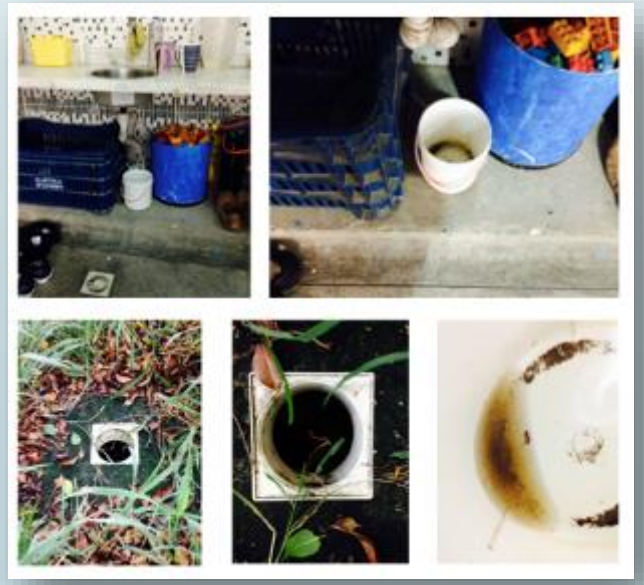
- **Households** selected according to the report of fever in children or as part of a monitoring program.
- **Schools**, **health units** and **key-sites** were selected in strategic areas. Defined as non-residential properties suitable for the maintenance of vector infestation, and characterized by higher human concentration, mobility and presence of potential *Aedes* breeding sites.

Methods

Collection of immature and adults

Immatures

- Active search for **breeding sites** and collection of *Aedes* larvae
- Maintenance of immature stages to adult →identification



Methods

Collection of immature and adults

Adults

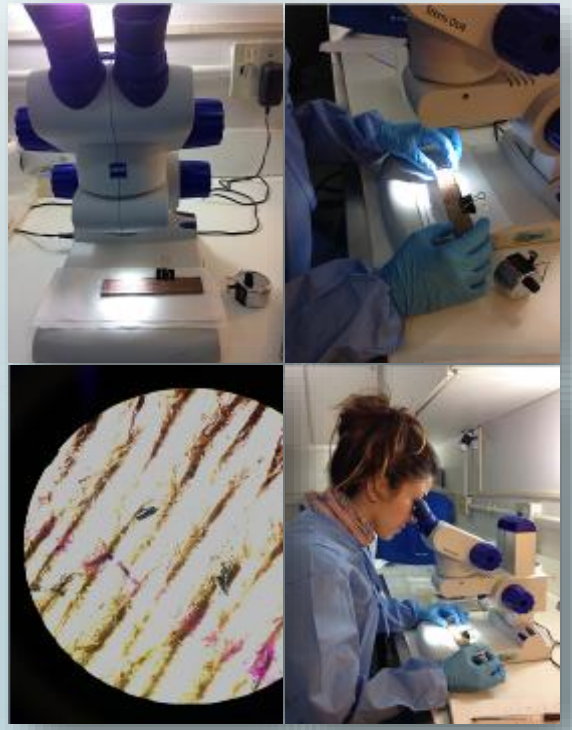
- **CDC traps** (HP Biomedica) (battery): light and CO_2, 24 hs.
- Battery-powered **aspirators** inside buildings and outdoors, 15-20 min.

Methods

Collection of immature and adults

Eggs

- **Ovitraps** remaining 1 week: black PET plastic container (Polyethylene), with a wide mouth and a palette of Eucatex containing a rough side, placed vertically inside it and attached by an aluminium clip. Filled with tap water and 30% hay infusion to attract mosquitoes.



Methods

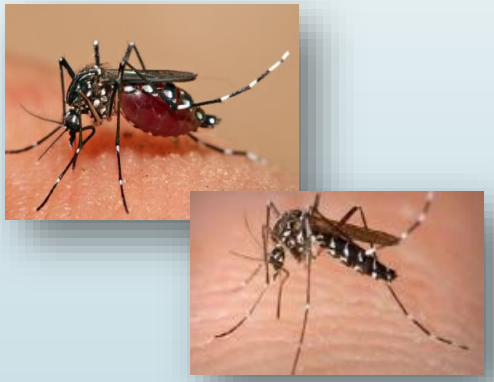
Collection of immature and adults

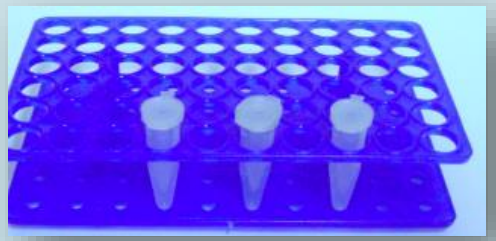
- Transport to the laboratory alive / dry ice

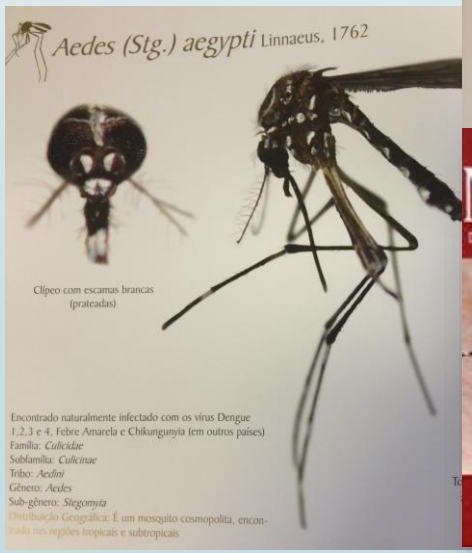
Methods

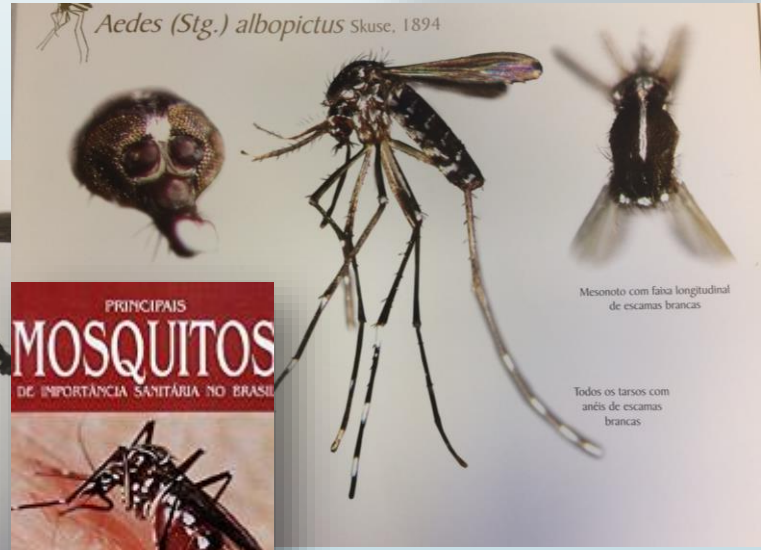
Viral detection

- Species identification
- Male and female separated
- Engorged - not engorged females separated.
- Engorged females (mostly) separated in ***pools*** (<10 mosquitoes).









Methods

Viral detection - mosquitoes

Method of choice in mosquitoes: **RT-PCR**

Other methods: hybridization probes for RNA detection

Despite its advantages, PCR is not routinely employed in arbovirus diagnostic laboratories because it is **expensive**. Improvements in automated handling of PCR as well as detection of product are being developed but are **not available to most laboratories**.

Methods

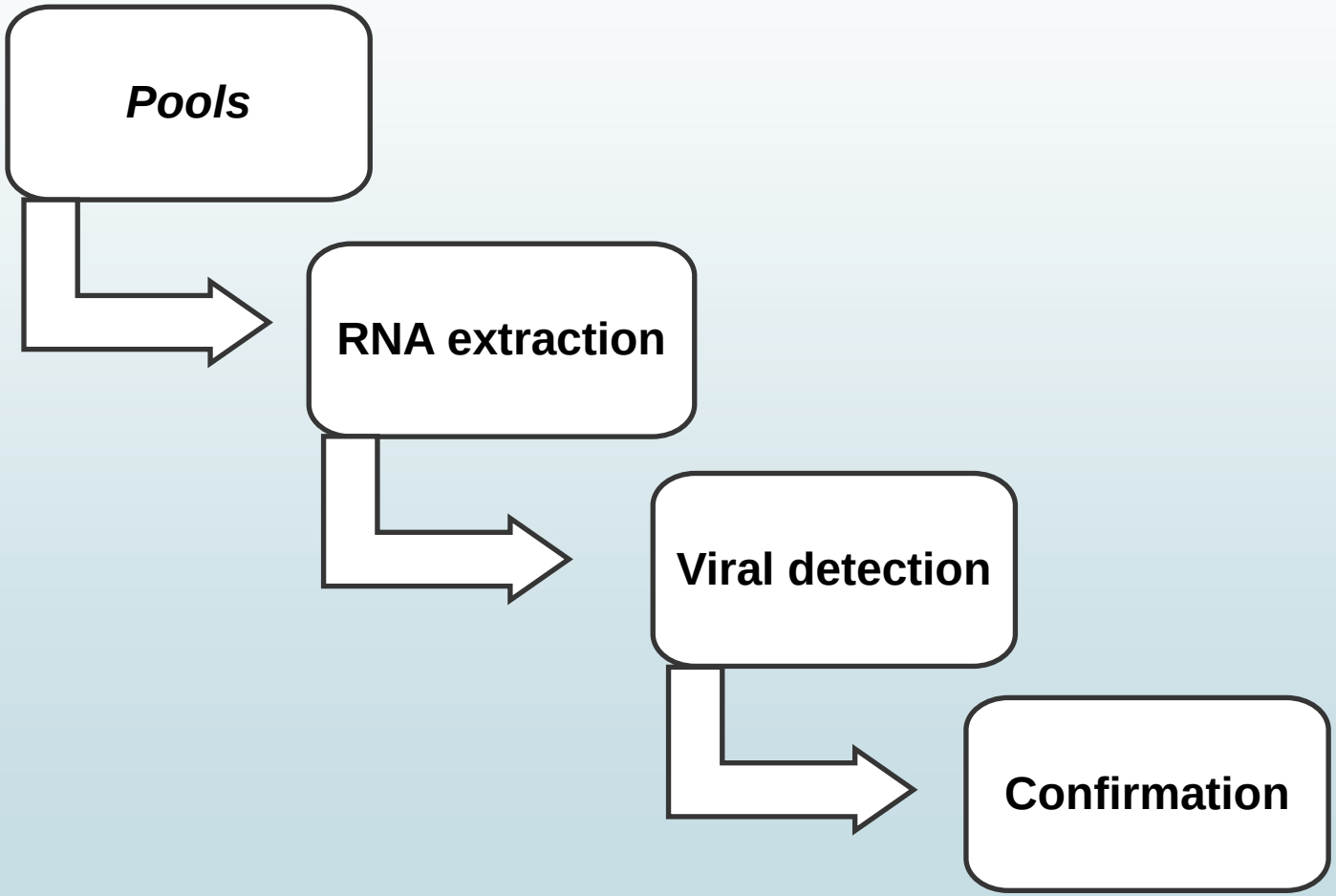
Molecular detection

- The Polymerase Chain Reaction (PCR) widely used for detection and diagnosis of infectious diseases **and virus detection in mosquitoes → fundamental method of surveillance** in arboviruses prevention programs.

- Circulating arboviruses can be evaluated to **predict future epidemic outbreaks**.

Methods

Molecular detection - steps



Methods

Pools

- *Aedes aegypti and Ae. albopictus*
- Species, date, collection place;
- Up to 10 mosquitoes / *pool*;
- Macerate;

RNA extraction

- QiaAmp Viral RNA Mini Kit (Qiagen);

Viral detection

- **RT-qPCR;**
- Kit QuantiTect Probe RT-PCR **(Qiagen);**
- **DENV, CHIKV, ZIKV, FASV;**
- **Cq (cut-off point: 28 cycles)**

Confirmation

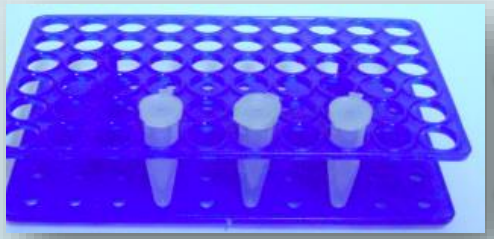
- **Conventional PCR;**
- **Sequencing and phylogenetic analysis** to compare with other strains from other countries;
- **Cell culture** C6/36 for viral isolation

Colaboratio with "Departamento de Virología- UFRJ", "Laboratório de Entomologia do Centro de Controle de Zoonoses de Natal" and UFRN.

Methods

Pools

- *Aedes aegypti and Ae. albopictus,* females;
- Up to 10 mosquitoes / *pool*;
- Species, date, collection place;
- Macerate (pearls, plastic pristiles);



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Methods

RNA extraction

- QiaAmp Viral RNA Mini Kit (Qiagen);
- “Kitchen recipe”;
- RNA separated and ready for molecular detection

Methods

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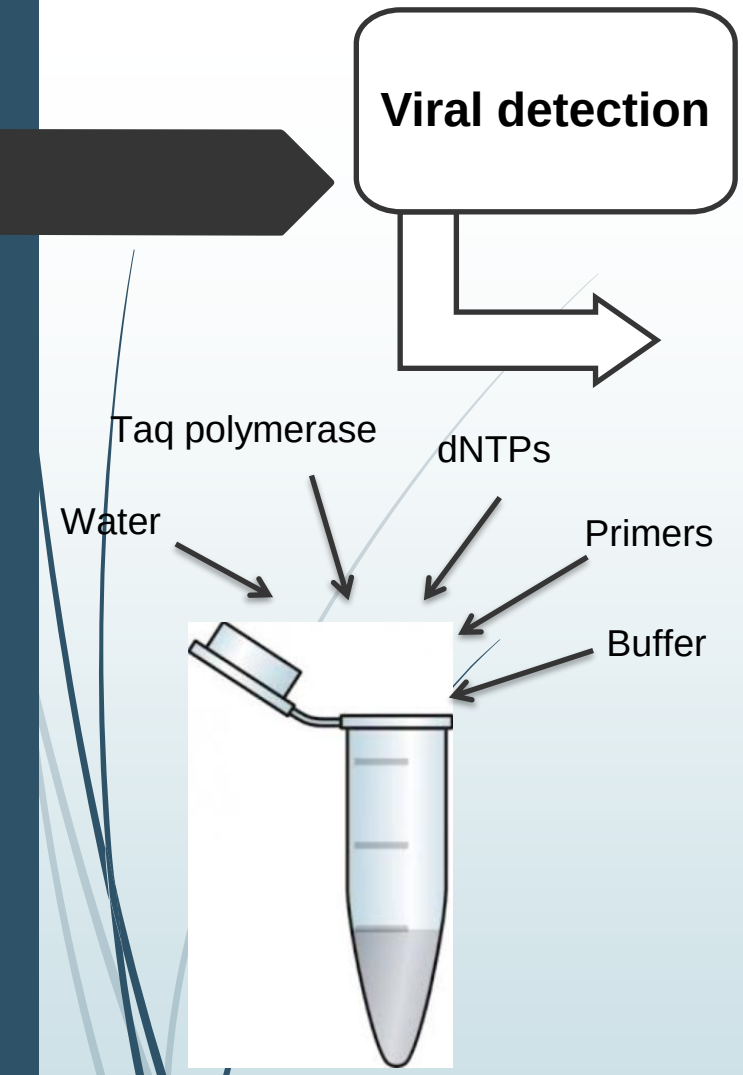
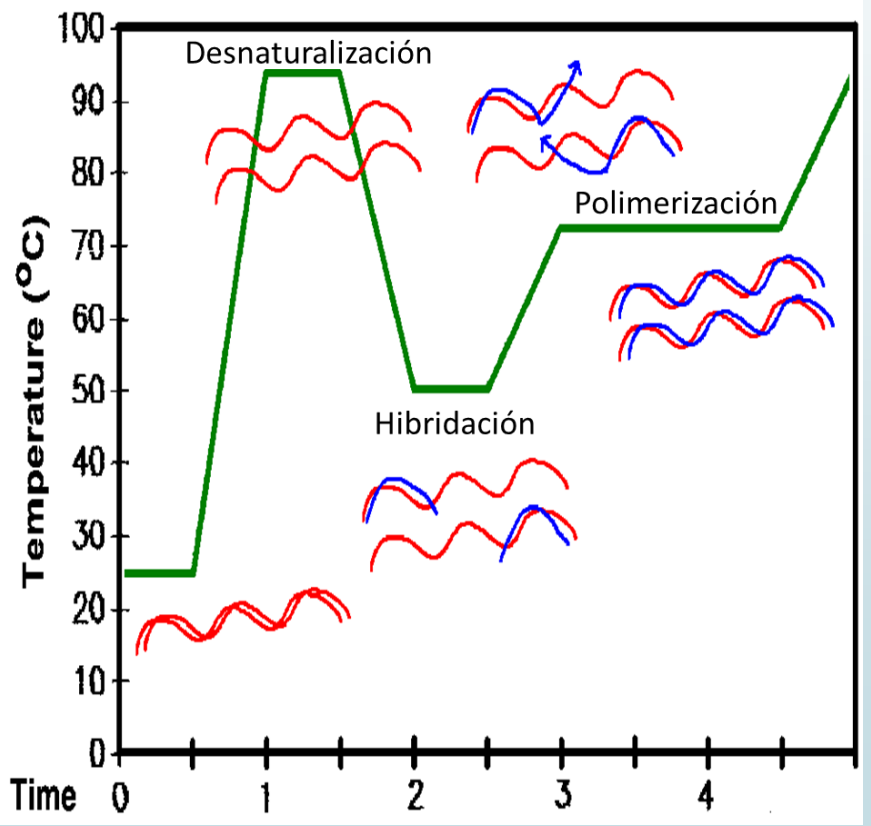
Methods



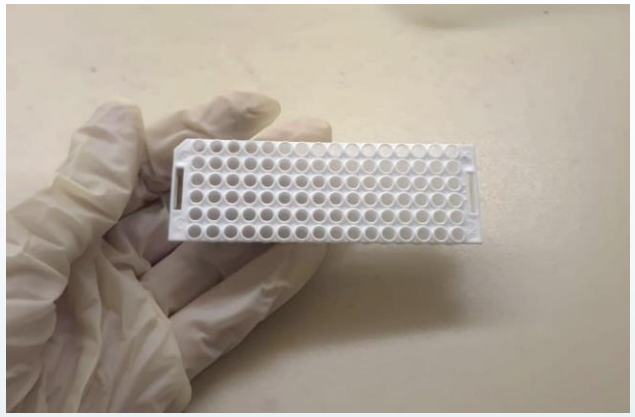
Amplified region	Primers	References
ENV-enconding gene (qPCR)	ZIKAF-5´-CCGCTGCCCAACACAAG-3´ ZIKAR-5´-CCACTAACGTTCTTTTGCAGACAT-3´	Lanciotti et al., 2008
NS3-encoding gene	ZikaDiagF-5'-CAGAGACTGATGAAGACCAT-3´ ZikaDiagR-5´-CCAGCCAAACAGGAAGAT-3´	Waehre et al., 2014
NS3-encoding gene	ZIKANS3-F-5´-AGAGAGCCTGGAGCTCAGGCT-3´ ZIKANS3-R-5´-CTTCCATTATGGTGTTGTTG-3´	Waehre et al., 2014
NS5-encoding gene	ZikaD_NS5F-5´-CCAATYGATGATAGGTTTGC-3´ ZikaD_NS5R-5´-TCARTTCATCTTGGTGGCG-3´	Cadar et al., 2015
Envelope protein	ZIKVENVF-5´-GCTGGDGCRGACACHGGRACT-3´ ZIKVENVR-5´-RTCYACYGCCATYTGGRCTG-3´	Faye et al., 2008
prM	ZIK835-TTGGTCATGATACTGCTGATTGC ZIK911c-CCTTCCACAAAGTCCCTATTGC	Lanciotti et al., 2008
nsp1 gene	ChikS- 5´-TGATCCCGACTCAACCATCCT-3´	Panning et al., 2008

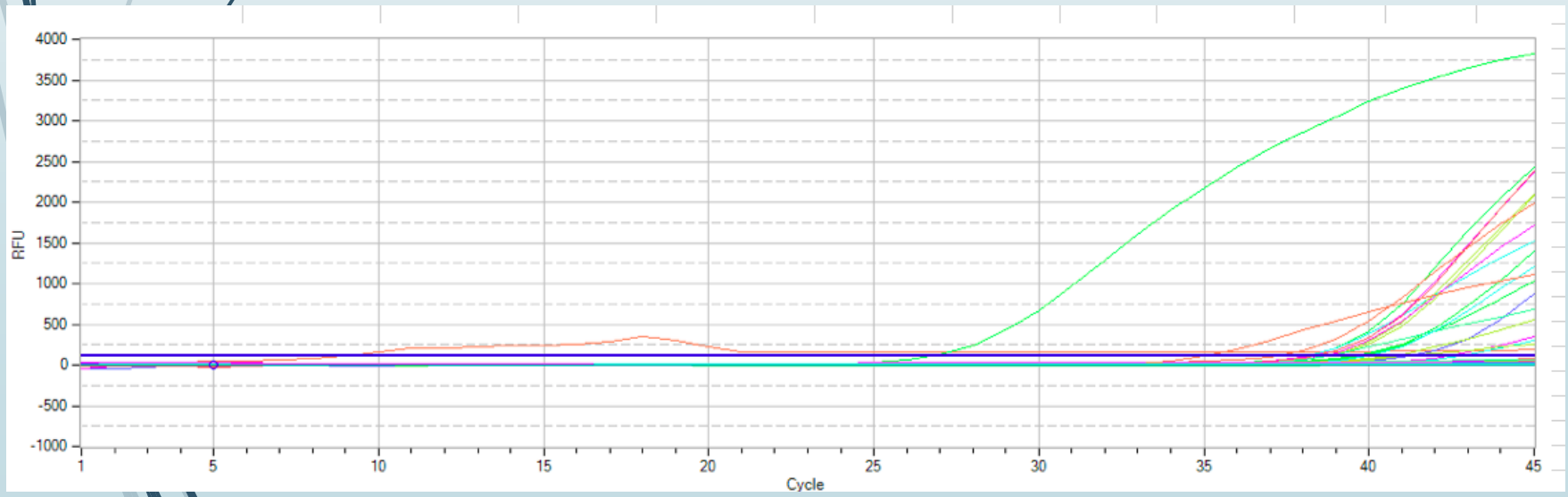


Methods

Viral detection

- **RT-qPCR → quantitative PCR;**
- Kit QuantiTect Probe RT-PCR **(Qiagen);**
- **DENV, CHIKV, ZIKV, YFV;**
- **Cq (cut-off point: 28 cycles)**





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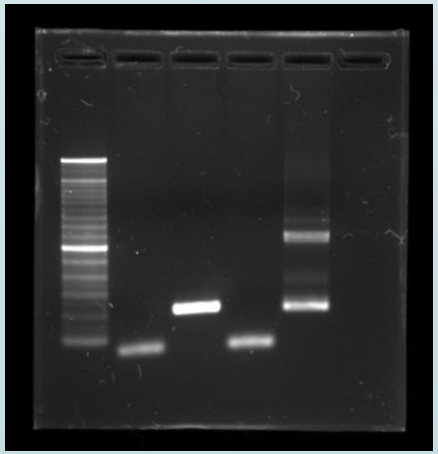
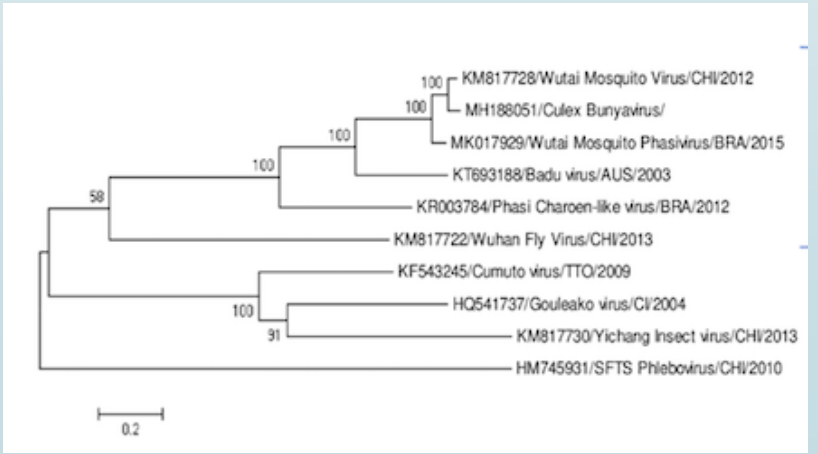
Methods

Confirmation

- **Conventional PCR;**
- **Cell culture** C6/36 for viral isolation
- **DNA Purification;**
- **Sequencing and phylogenetic analysis** to compare with other strains from other countries;
- Phylogenetic **trees /** common ancestors and time-scale phylogeny



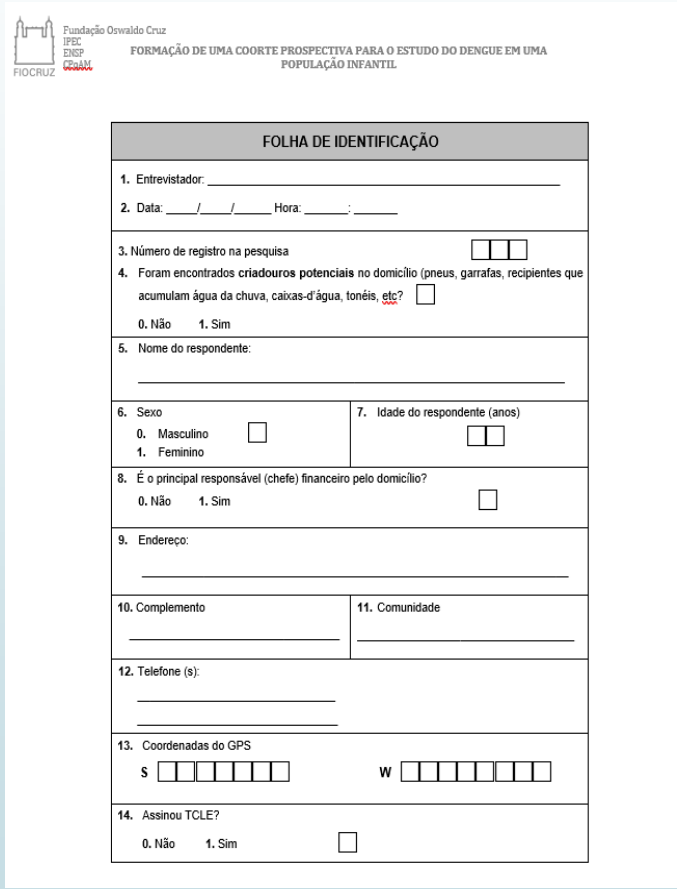


Methods

Socio-environmental and climate data

- Socio-environmental data → **questionnaires** to obtain socioeconomic data and the conditions of the domicile.

- **Climate** data → data repository of the International Research Institute for Climate and Society (IRI) - Columbia University, USA:
  - **Accumulated precipitation** data (CMORPH and CMAP - NOAA, TRMM-3B42 and GPCP - NASA).
  - Minimum, medium and maximum atmospheric and soil **temperatures** (LST MODIS - USGS).
  - Relative **humidity**.



Fundação Oswaldo Cruz
IPEC
ENSP
CPqAM
FIOCRUZ

FORMAÇÃO DE UMA COORTE PROSPECTIVA PARA O ESTUDO DO DENGUE EM UMA POPULAÇÃO INFANTIL

FOLHA DE IDENTIFICAÇÃO

1. Entrevistador: _____
2. Data: ____/____/_____ Hora: _____: _____
3. Número de registro na pesquisa ☐☐☐
4. Foram encontrados **criadouros potenciais** no domicílio (pneus, garrafas, recipientes que acumulam água da chuva, caixas-d'água, tonéis, etc? ☐
   0. Não 1. Sim
5. Nome do respondente: _____
6. Sexo
   0. Masculino ☐
   1. Feminino
7. Idade do respondente (anos) ☐☐
8. É o principal responsável (chefe) financeiro pelo domicílio?
   0. Não 1. Sim ☐
9. Endereço: _____
10. Complemento _____
11. Comunidade _____
12. Telefone (s): _____ _____
13. Coordenadas do GPS
    S ☐☐☐☐☐☐ W ☐☐☐☐☐☐☐
14. Assinou TCLE?
    0. Não 1. Sim ☐

Results

Results

Mosquito collections

- **Direct aspiration**:
  - >10,000 mosquitoes captured
  - ***Ae. aegypti*** predominated over *Ae. albopictus*
  - Other species detected in Rio de Janeiro: *Ae. scapularis*, *Ae. fluviatilis* and *Culex quinquefasciatus*

- **Ovitraps**: >45,000 *Aedes* spp. eggs counted → *Ae. aegypti* predominates over *Ae. albopictus.*

- **CDC** traps: >1,000 Culicidae collected, both from strategic points and schools.

Results

Mosquito collections - species

- *Ae. aegypti* and *Ae. albopictus* **collected indoors** in an urban endemic area.

Results

Mosquito collections → *Ae. albopictus*

- ***Aedes albopictus***, originally considered as a **secondary vector** for arbovirus transmission, especially in areas where this species co-exist with *Ae. aegypti*.

- Presence of *Ae. albopictus* within a **highly urbanized and densely populated** area (not commonly described in urban areas).

- As *Ae. albopictus* can easily move between **sylvatic** and urban environment, the entomological monitoring of *Ae. albopictus* should be an integral part of mosquito surveillance and control.

Fig 1. Spatial distribution of collections of *Aedes albopictus* adults in Manguinhos, Rio de Janeiro. Yellow triangles, circles, and stars represent the households, key-sites and schools, respectively, where *Ae. albopictus* adults were collected.

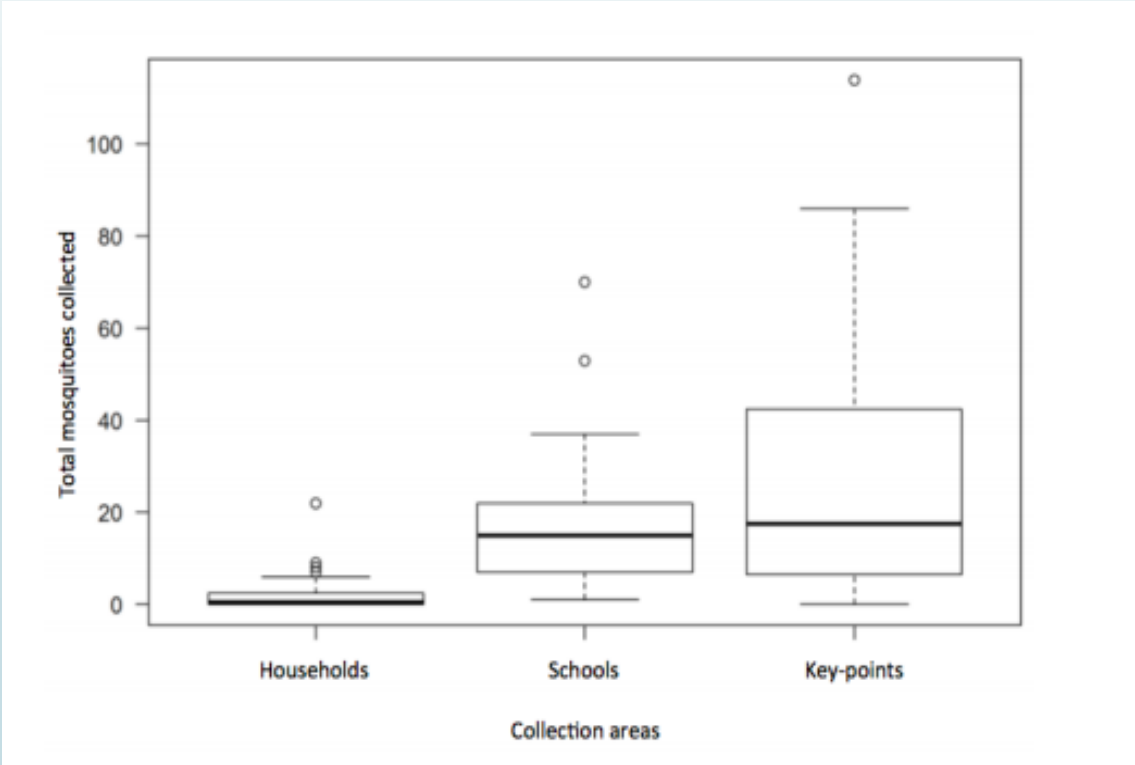
https://doi.org/10.1371/journal.pone.0195014.g001

Results

Mosquito collections - sites

- More specimens collected in **key-sites, health units and schools**. Key-sites highly favorable to mosquito infestation.
- The high infestation of mosquitoes observed in **schools** shows a high risk of arbovirus infection in children, an age group particularly vulnerable to these infections.

Key-sites, characterized by high human influx and presence of potential *Aedes* breeding sites, **should be included in entomological monitoring**.



Results

Socio-environmental and climate data

- Both species collected during **all seasons.**

- Generalized linear model: we can identify the **area of collection, precipitation and average temperature** in the previous week as important predictors of the number of *Aedes* sp. collected.

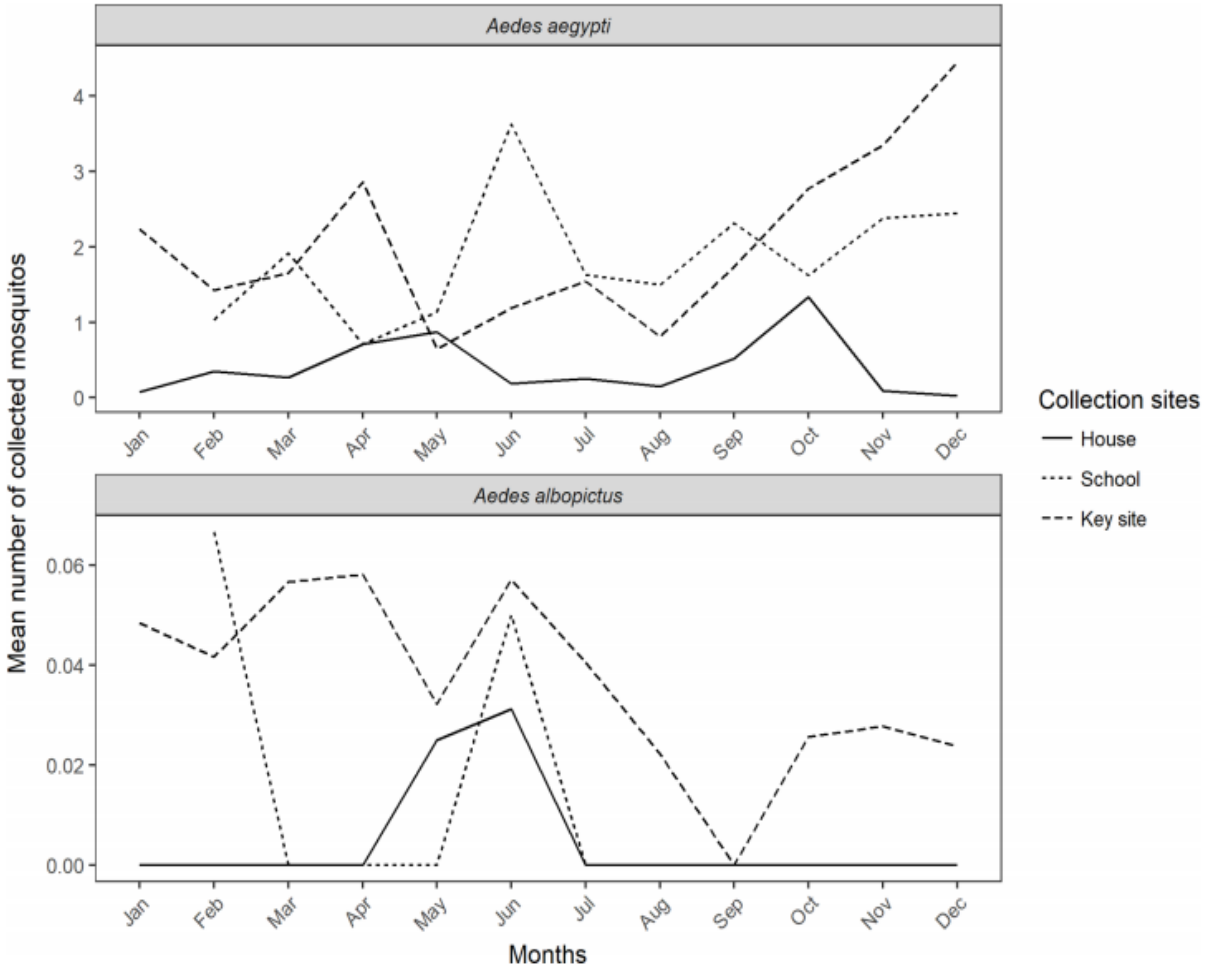


Fig 2. Monthly mean abundance of *Ae. aegypti* and *Ae. albopictus* during the three-year study period in Manguinhos, Rio de Janeiro. The figure shows the mean number of *Ae. aegypti* and *Ae. albopictus* mosquitoes collected per month during the study.

https://doi.org/10.1371/journal.pone.0195014.g002

Results

Socio-environmental and climate data

Questionnaires applied in the households:

Presence of **slab-concrete** in the roof and the **ceramic** in the floor had a negative influence on the presence of *Ae. aegypti* in households ($p = 0.02583$ and $p = 0.03809$, respectively).

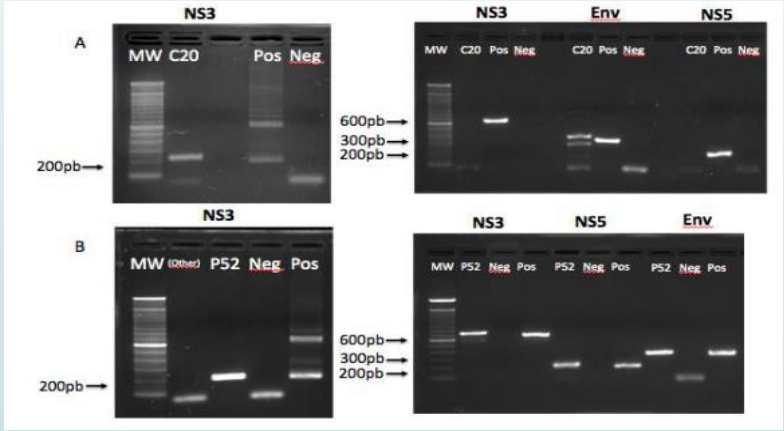
Importance of collecting data about environment and social conditions **to define risk factors for vector infestation**

Results

Molecular results

- Mosquitoes PCR negative for YFV and CHIKV.
- ZIKV positive mosquitoes (GenBank nr. KY354186 and KY354187) **weeks before** reporting autochthonous cases; DENV-3 positive mosquitoes **in low infestation period**.

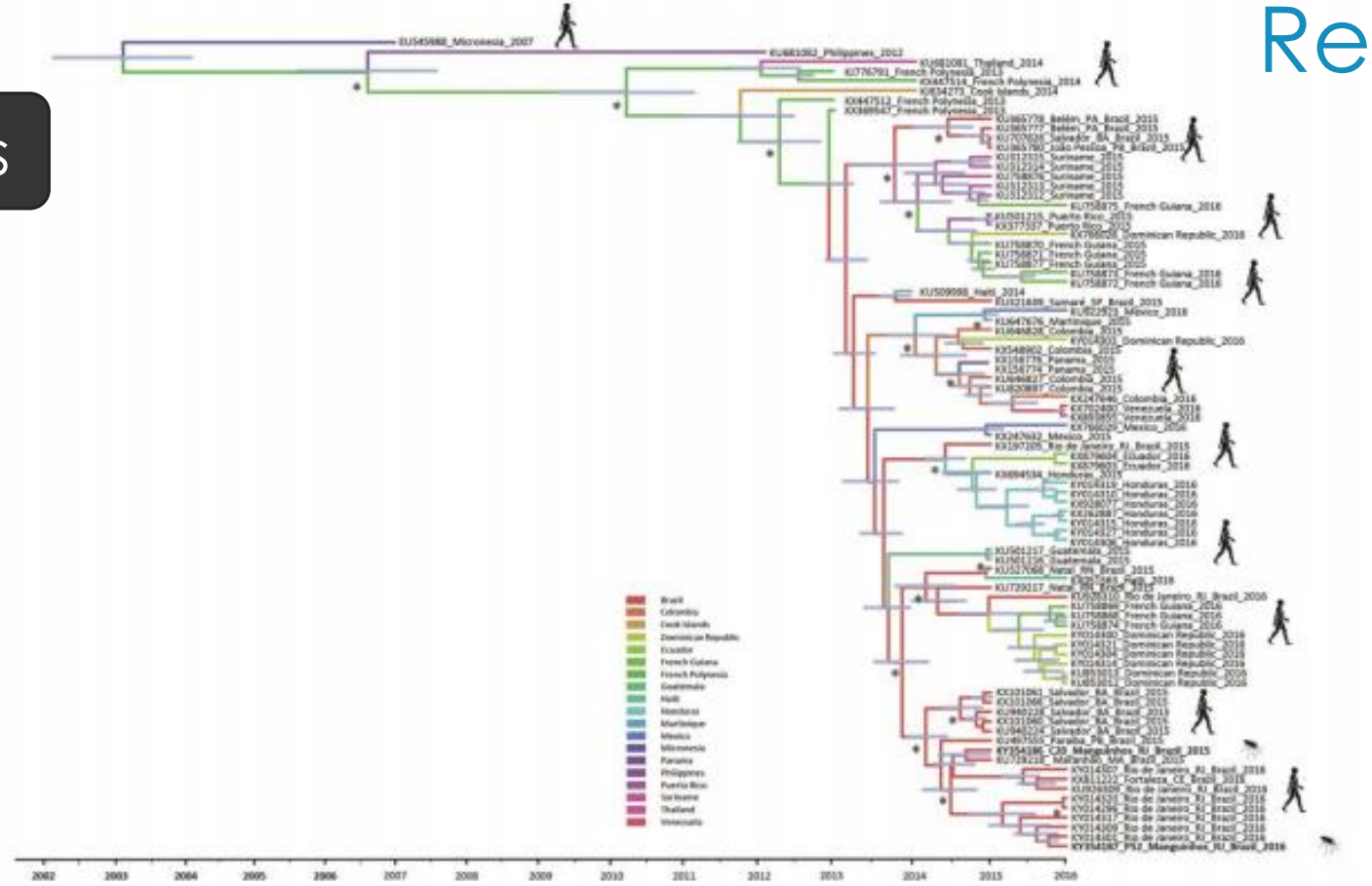
Tool for **early detection** of arbovirus circulation, including "low-season"



- Detection of arboviruses in **males** → transovarian/venereal transmission → males could play a role in the maintenance of arboviruses in nature → test both sexes!

Results

Molecular results



Technical Appendix Figure. Bayesian maximum clade credibility (MCC) tree representing the time-scale phylogeny of the Zika virus outbreaks in the Americas. The time-scaled phylogeny was performed by using the Bayesian Markov chain Monte Carlo tree-sampling method with BEAST version 1.8.3 (http://beast.bio.ed.ac.uk/) and in parallel the maximum-likelihood method (not shown) using PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/versions.php) with 1,000 pseudoreplicates based on near-complete envelope coding region sequences. The Akaike information criterion was chosen as the model selection

Conclusions

Conclusions

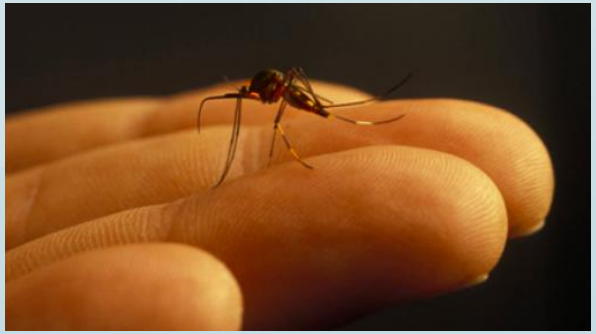
- **Integration between entomological and virological surveillance:** detection of natural infection in mosquitoes captured in the field is essential for **detecting the prevalence and circulation of new serotypes or viruses in the community**, as well as serving as a **surveillance tool for detection and anticipation of epidemics**.

- **Continuous** virological surveillance for *Aedes* mosquitoes in municipal government routines as a tool for monitoring arbovirus circulation in receptive areas, to **point out high risk areas** for virus dissemination and as an epidemic alert system to direct control actions in critical areas.

- Strategies for virological surveillance of *Aedes* mosquitoes are commonly focused on **females** (hematophagous). Vertical and venereal transmission suggested as a mechanism to maintain arbovirus circulation in vectors →**Include MALES**.

- Pursue more access to viral detection tools and foster collaborations with other research institutions (integration **academia and health services**).

- **Other systems being developed**, such as modifications to common mosquito traps that will allow the collection of mosquito excreta, or liquid waste, from which signs of viral infection can be detected.



Thank you for your attention

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