Symposium “Research and Innovation for the control of vectors of emerging arbovirus”

# Use of Densoviruses as potential biological control agents


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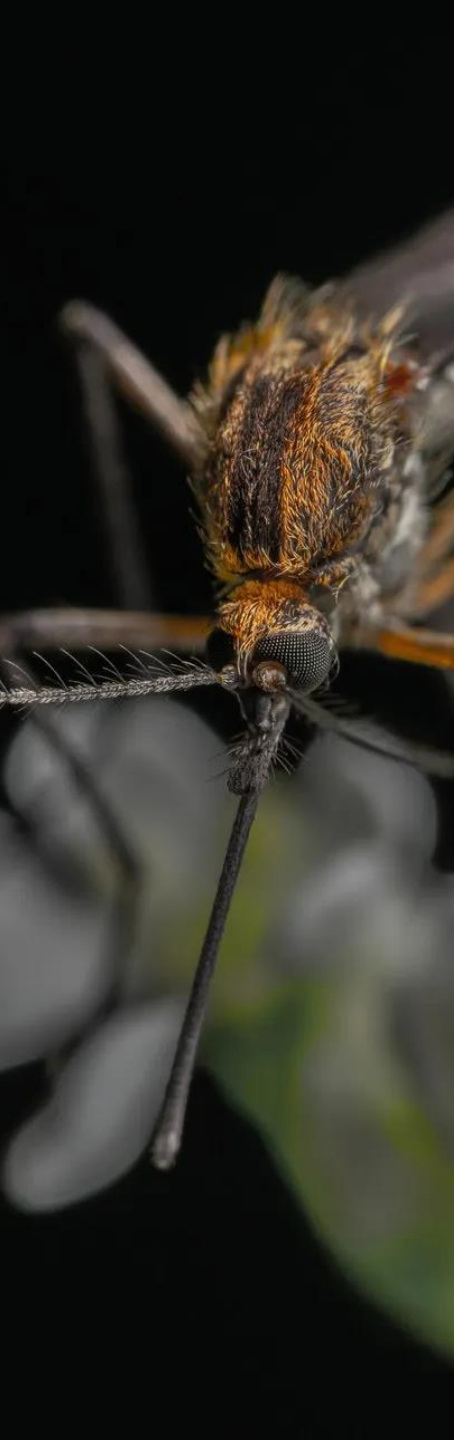
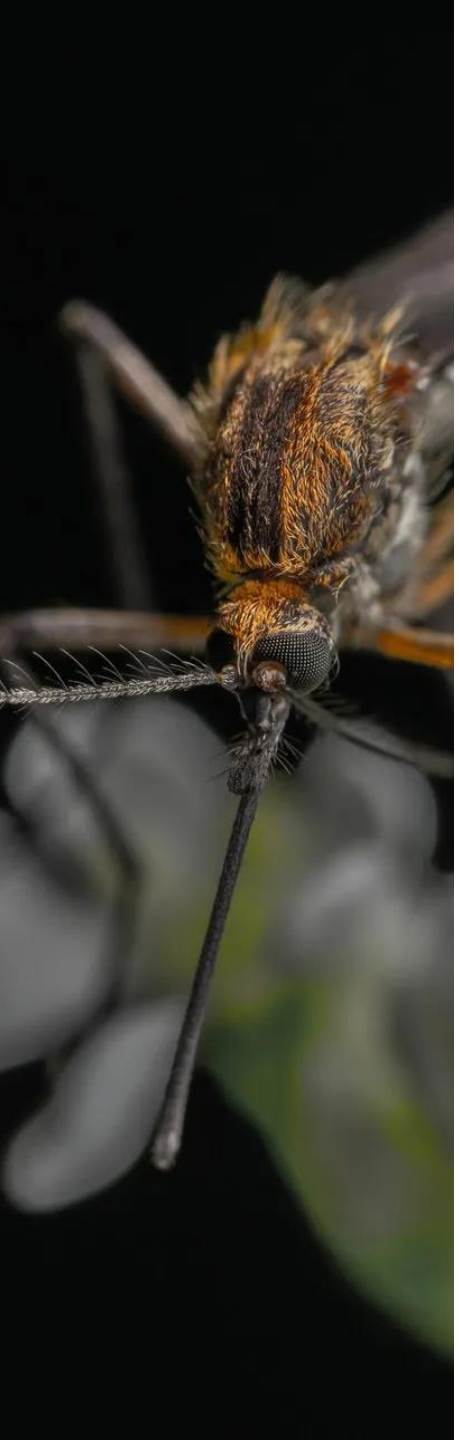
**Institut Européen des Membranes**
UMR5635 CNRS-Université de Montpellier-ENSCM


Montpellier, 14/02/2023



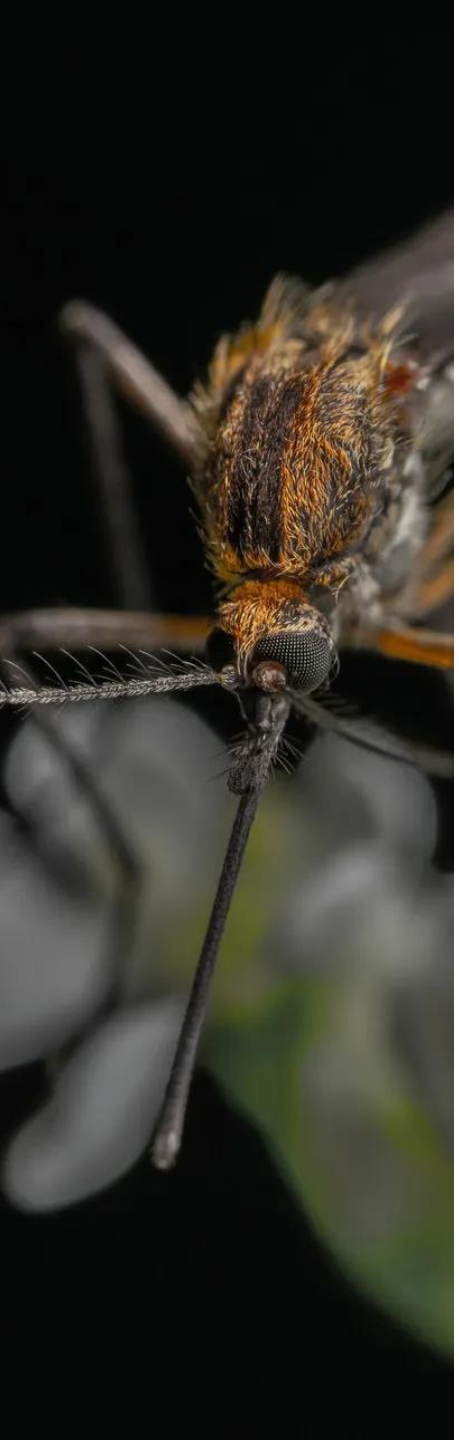
# Biological control of mosquito vectors : why and how?

- Major arthropod vectors of human disease worldwide
- Insecticide-based strategies = **resistance**



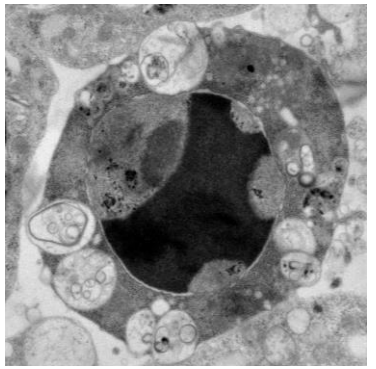
# Biological control of mosquito vectors : why and how?

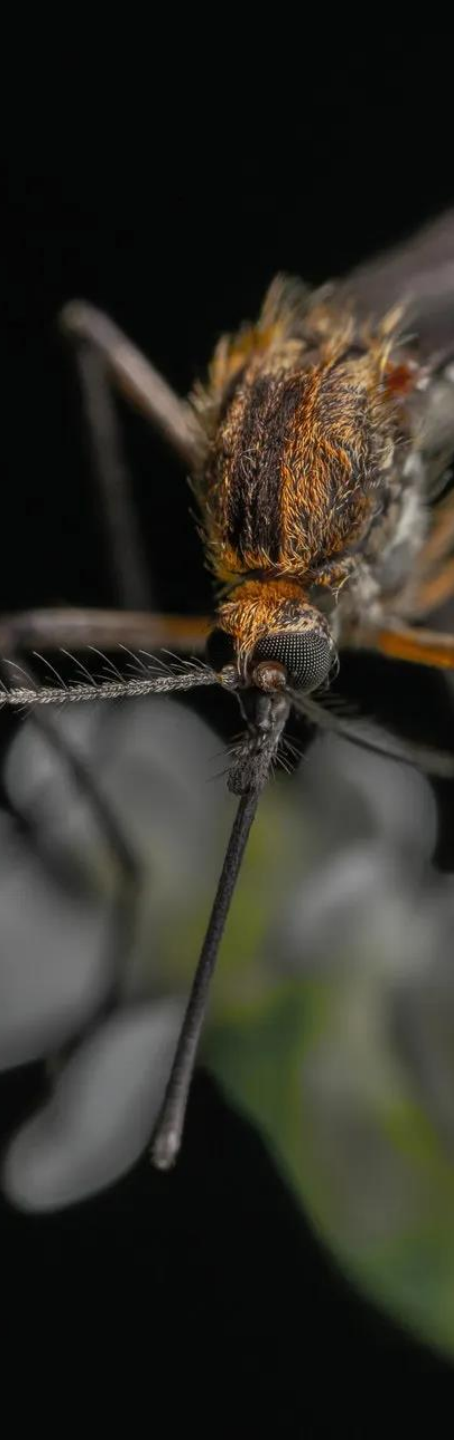
- Major arthropod vectors of human disease worldwide
- Insecticide-based strategies = **resistance**
- Alternative methods respectful of the environment
  - SIT and boosted SIT
  - Predators (copepods, larvivorous fishes, *toxorhynchites* larva…)
  - Sex pheromones
  - Traps
  - *Wolbachia*
  - Fungi
  - Bacteria (*Bacillus sp.*)
  - And viruses….



# Densoviruses : Small highly infectious viruses

- These viruses owe their name to the cellular pathology observed during infection : **Densonucleosis**





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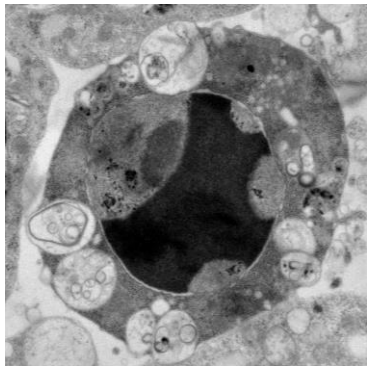
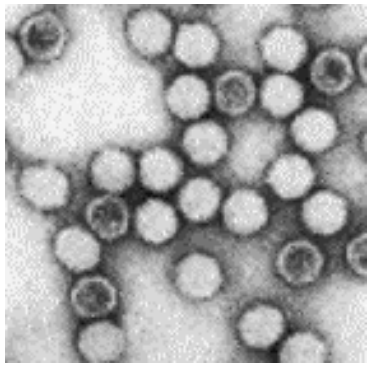
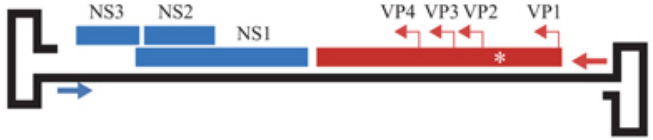


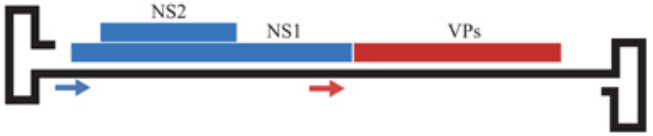
- Non-enveloped viruses with an icosahedral capsid ≈ 20 nm
- Genome : ssDNA ≈ 4 kb
- Structure of genome depending on the genus

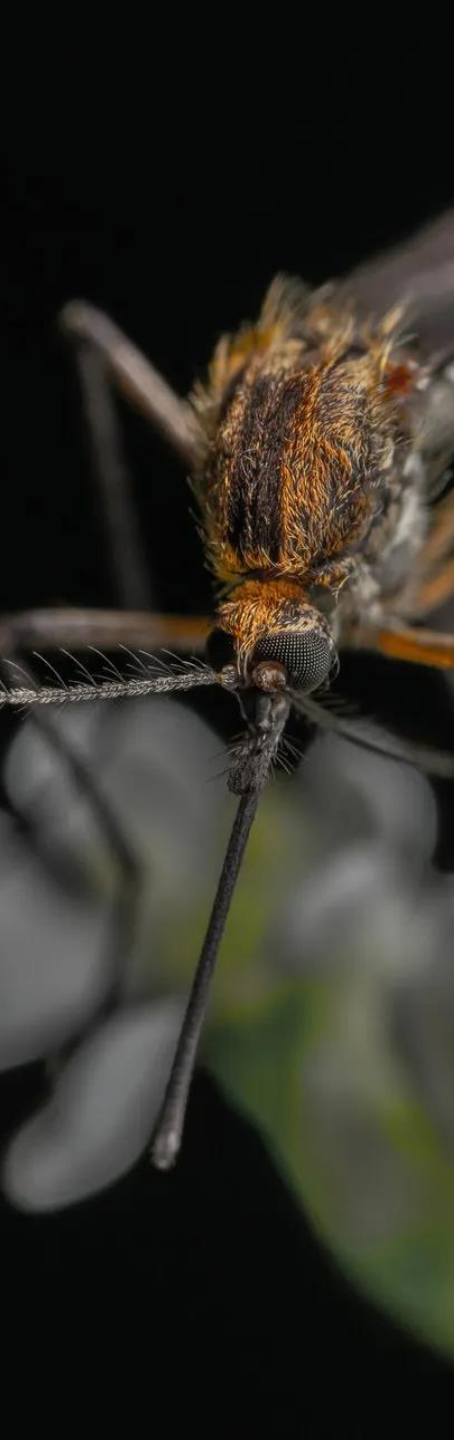


Ambidensovirus



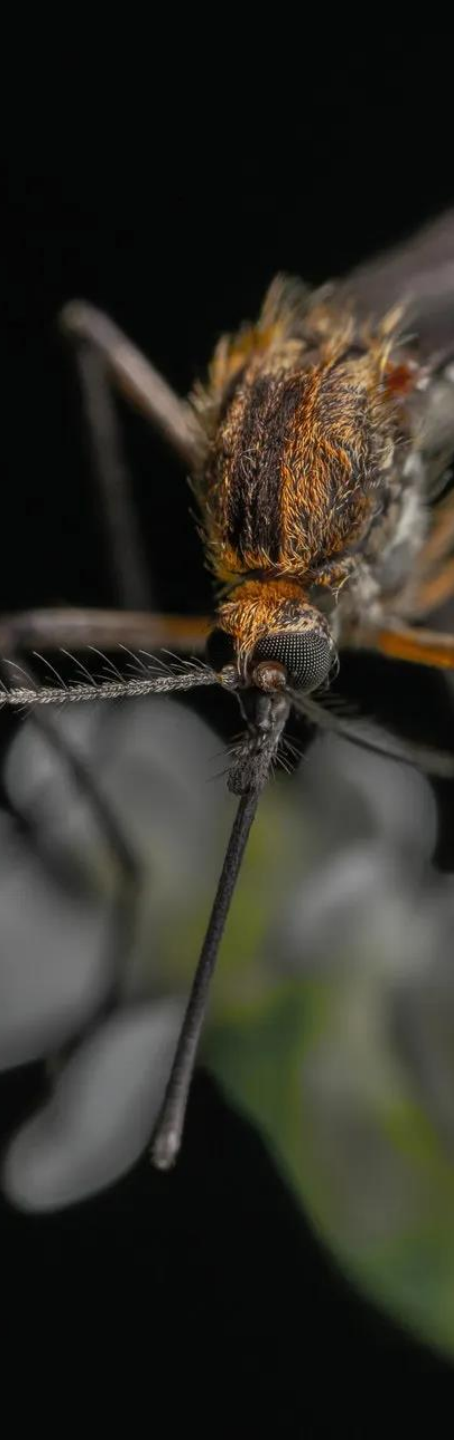

Brevidensovirus





# Densoviruses : Interest as biological agent

- Small genome : easy to manipulate
- Restricted host range
- Infectious in the larval stages



# Densoviruses : Interest as biological agent

- Small genome : easy to manipulate
- Restricted host range
- Infectious in the larval stages
- Ex. of use :

  - GmDV vs *Galleria mellonella*, wax moth (1965)

  - SfDV & CeDV vs *Sibine fusca* & *Casphalia extranea* (70's, 80's)

  - AaeDV vs *Aedes aegypti* (80's) (viroden)

# Parvovirus family

***Parvovirinae***

- Protoparvovirus
- Amdoparvovirus
- Bocaparvovirus
- Aveparvovirus
- Tetraparvovirus
- Dependoparvovirus
- Copiparvovirus
- Artiparvovirus*
- Loriparvovirus*
- Erythroparvovirus

***Densovirinae***

- Scindoambidensovirus*
- Miniambidensovirus*
- Blattambidensovirus*
- Hemiambidensovirus*
- Aquambidensovirus*
- Protoambidensovirus*
- Pefuambidensovirus*
- Iteradensovirus

***Hamaparvovirinae****

- Ichthamaparvovirus*
- Penstylhamaparvovirus*
- Brevihamaparvovirus*
- Hepanhamaparvovirus*
- Chaphamaparvovirus*

https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/parvoviridae

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- Ichthamaparvovirus*
- Penstylhamaparvovirus*
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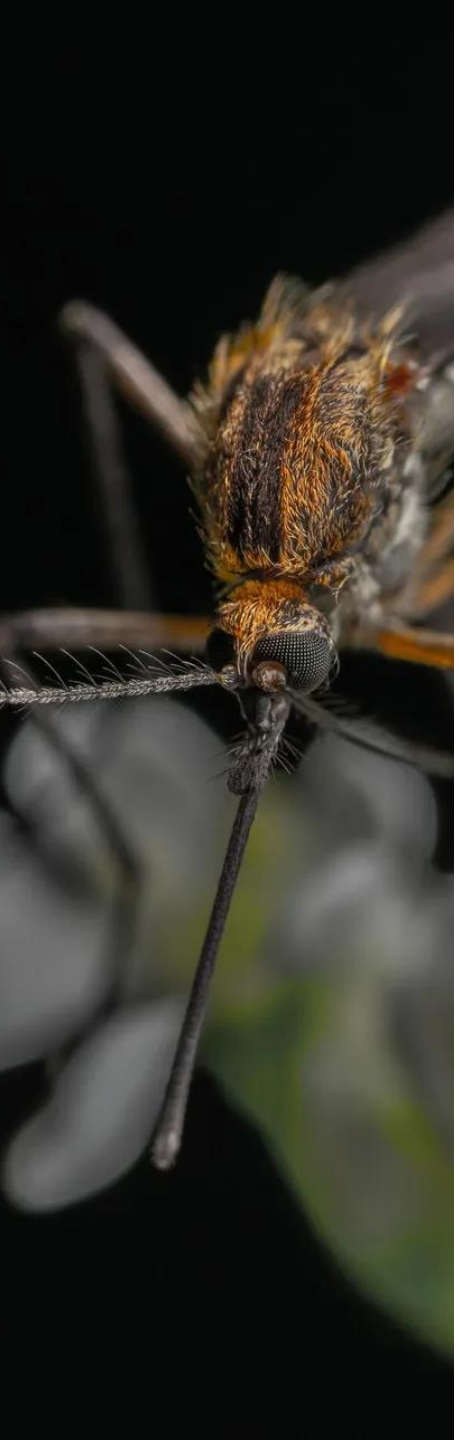
- Hepanhamaparvovirus*
- Chaphamaparvovirus*

*Adeno-associated viruses…*

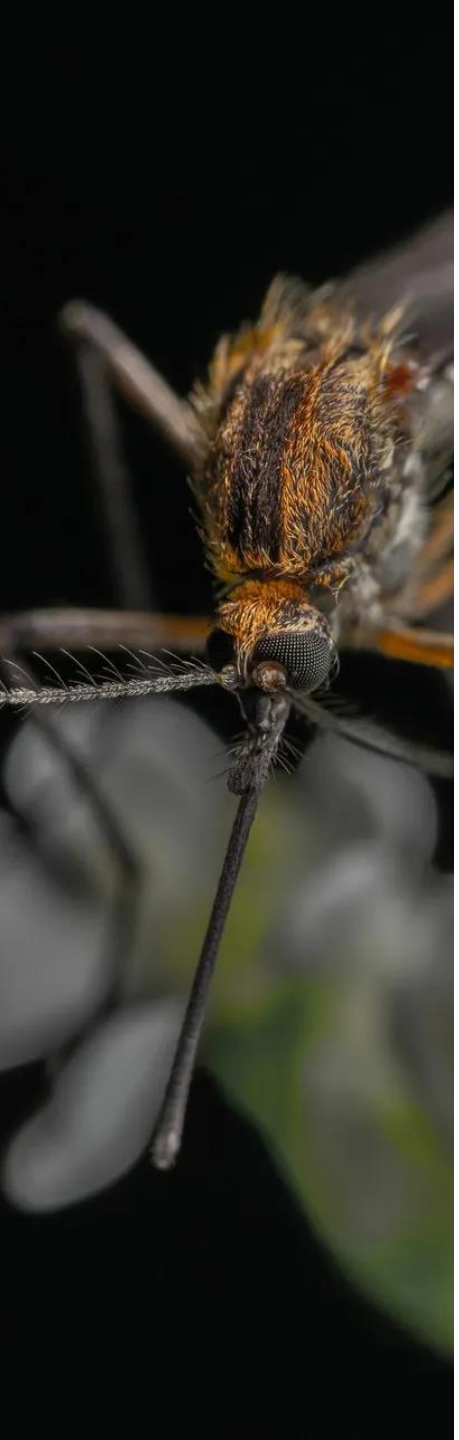
| | VERTEBRATE HOSTS / INVERTEBRATE HOSTS / VERTEBRATE & INVERTEBRATE HOSTS |
|---|---|
| *Hemipteran scindoambidensovirus 1* | Planococcus citri densovirus |
| *Hymenopteran scindoambidensovirus 1* | Solenopsis invicta densovirus |
| *Orthopteran scindoambidensovirus 1* | Acheta domestica densovirus |
| *Orthopteran miniambidensovirus 1* | Acheta domestica mini ambidensovirus |
| *Blattodean blattambidensovirus 1* | Blattella germanica densovirus 1 |
| *Hemipteran hemiambidensovirus 1* | Dysaphis plantaginea densovirus 1 |
| *Hemipteran hemiambidensovirus 2* | Myzus persicae densovirus 1 |
| *Asteroid aquambidensovirus 1* | Sea star associated densovirus |
| *Decapod aquambidensovirus 1* | Cherax quadricarinatus densovirus |
| *Dipteran protoambidensovirus 1* | Culex pipiens densovirus |
| *Lepidopteran protoambidensovirus 1* | Galleria mellonella densovirus |
| *Blattodean pefuambidensovirus 1* | Periplaneta fuliginosa densovirus |
| *Lepidopteran iteradensovirus 1* | Bombyx mori densovirus 1 |
| *Lepidopteran iteradensovirus 2* | Casphalia extranea densovirus |
| *Lepidopteran iteradensovirus 3* | Helicoverpa armigera densovirus |
| *Lepidopteran iteradensovirus 4* | Papilio polyxenes densovirus |
| *Lepidopteran iteradensovirus 5* | Dendrolimus punctatus densovirus |
| *Syngnathid ichthamaparvovirus 1* | Syngnathus scovelli chapparvovirus |
| *Decapod penstylhamaparvovirus 1* | Penaeus stylirostris penstyldensovirus |
| *Dipteran brevihamaparvovirus 1* | Anopheles gambiae densovirus |
| *Dipteran brevihamaparvovirus 2* | Aedes albopictus densovirus 2 |
| *Decapod hepanhamaparvovirus 1* | Fenneropenaeus chinensis hepatopancreatic densovirus |
| *Carnivore chaphamaparvovirus 1* | Cachavirus |
| *Chiropteran chaphamaparvovirus 1* | Desmodus rotondus chapparvovirus |
| *Galliform chaphamaparvovirus 1* | Turkey parvovirus 2 |
| *Galliform chaphamaparvovirus 2* | Chicken chapparvovirus 2 |
| *Galliform chaphamaparvovirus 3* | Chicken chapparvovirus HK |
| *Rodent chaphamaparvovirus 1* | Mouse kidney parvovirus |
| *Rodent chaphamaparvovirus 2* | Rat parvovirus 2 |
| *Ungulate chaphamaparvovirus 1* | Porcine parvovirus 7 |

https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/parvoviridae



# Mosquito Densoviruses : 16 strains in 3 species

| Species | Strain | Origin | Host range (natural population and/or experimental infection) |
|---|---|---|---|
| *Dipteran brevihamaparvodensovirus 1* | Aedes albopictus densovirus 1 **AalDV1** | C6/36 cell line (China) | ? |
| | Aedes albopictus densovirus 4 **AalDV4** | C6/36 cell line (Thaïland) | ? |
| | Aedes aegypti densovirus 1 **AaeDV1** | *Ae. aegypti* laboratory colony (Ukraine) | *Ae. aegypti (per os, larvae) Ae. albopictus, A. cantans, A. caspius, A. geniculatus, A. vexans, Cx. pipiens, Culista annulate* |
| | Aedes aegypti densovirus 2 **AaeDV2** | *Ae. aegypti* lab colony and natural population (India) | *Ae. aegypti* |
| | Culex pipiens pallens densovirus **CppDV** | *Cx pipiens pallens* Natural population (China) | *Cx. pipiens quinquefasciatus, Cx. tritaeniorhynchus, An. sinensi, An. vagus* |
| | Anopheles gambiae densovirus **AgDV** | Sua5B cell line (USA) | *(per os, larvae) An. Gambiae, (injection, adults) An. Gambiae, An. Arabiensis* |
| *Dipteran brevihamaparvovirus 2* | **Aedes albopictus densovirus 2 AalDV2** | **C6/36 cell line (France)** | **(per os, larvae) *Ae. aegypti, Ae. albopictus , Cx. pipiens quinquefasciatus, An, Gambiae, O. detritus, O. caspius* (injection, adults) *Ae. metallicus*** |
| | Aedes albopictus densovirus 3 **AalDV3** | C6/36 cell line (Peru) | ? |
| | Haemagogus equinus densovirus **HeDV** | GML-HE-12 cell line | ? |
| *Dipteran protoambidensovirus 1* | Culex pipens densovirus **CpDV** | *Cx. pipiens* lab colony and natural populations | *Cx. pipiens* |
| Non classified | Aedes Thailand densovirus **AthDV** | *Ae. albopictus* & *Ae. aegypti* lab colony (Thailand) | Ae. aegypti (per os, larves) Ae. albopictus, An. minimus S.L. |
| | Aedes albopictus densovirus 5 **AalDV5** | ? (China) | ? |
| | Aedes albopictus densovirus 6 **AalDV6** | *Ae. albopictus* natural population (China) | *Ae. Albopictus* |
| | Toxorhynchites amboinensis densovirus **TaDV** | TRA-284 cell line | ? |
| | Mosquito densovirus BR/07 **MDV** | C6/36 cell line (Brazil) | ? |
| | Aedes albopictus densovirus 7 **AalDV7** | *Ae. albopictus* natural pop. (China) | *Ae. albopictus (Foshan strain, 1981), Ae. aegypti (Haikou in Hainan Province, fiel), Cx. quinquefasciatus (Guangzhou strain, 1993)* |



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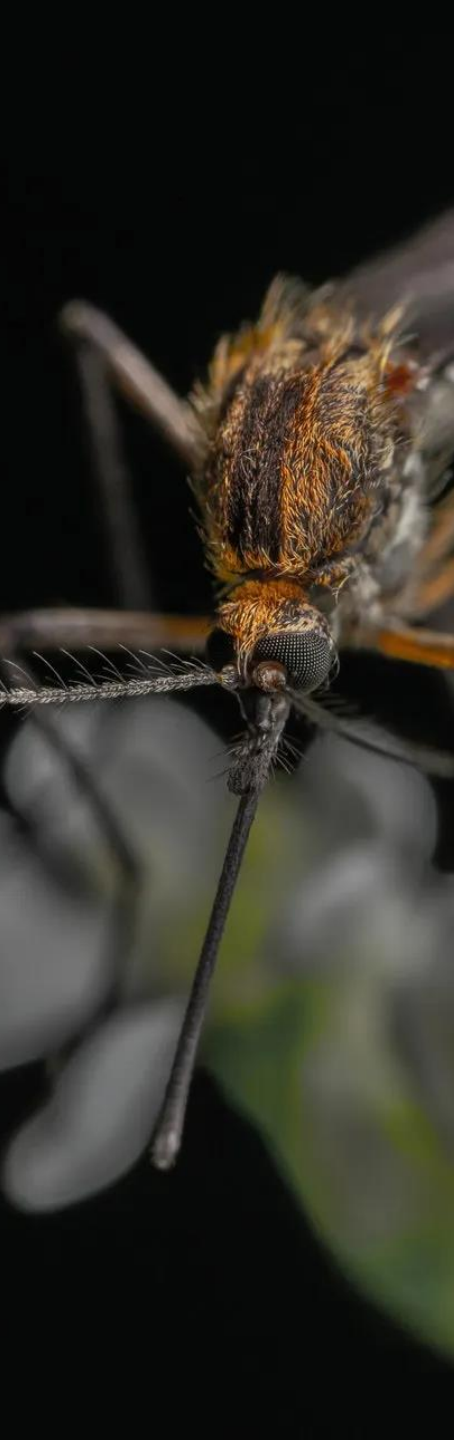
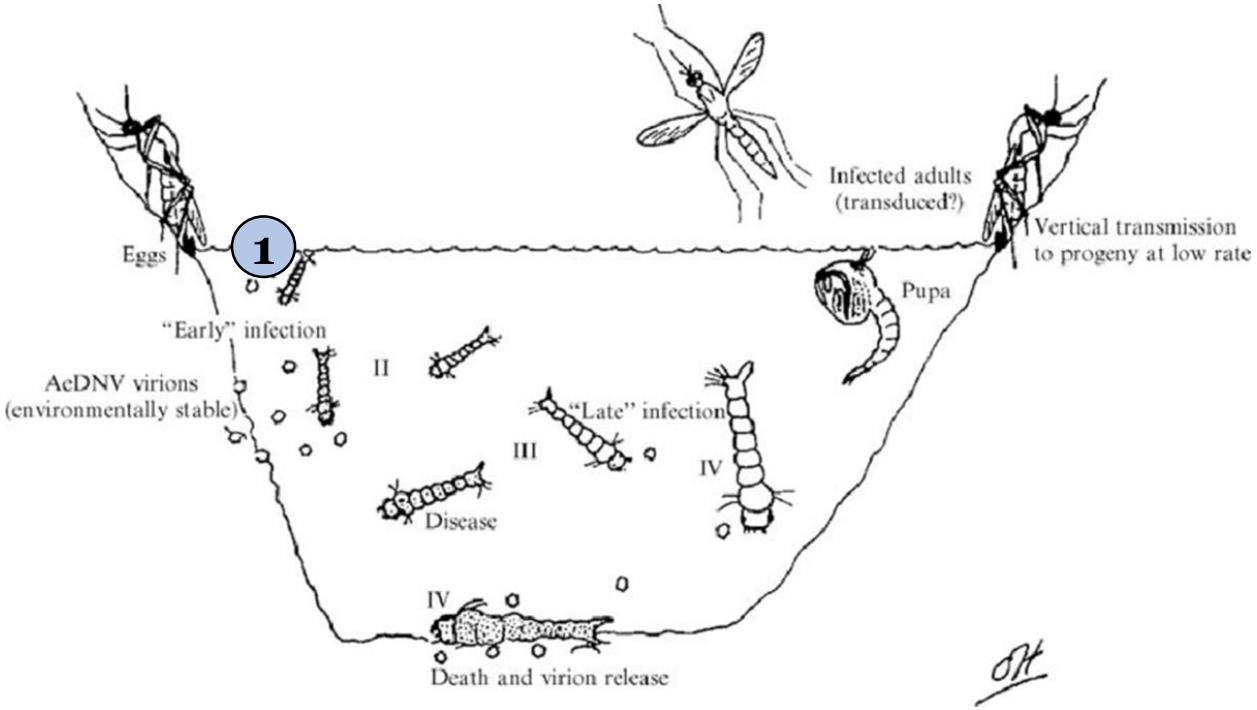


# Mosquito Densoviruses : Life cycle

## Life cycle of *AaeDV*

Carlson et al . (2006)

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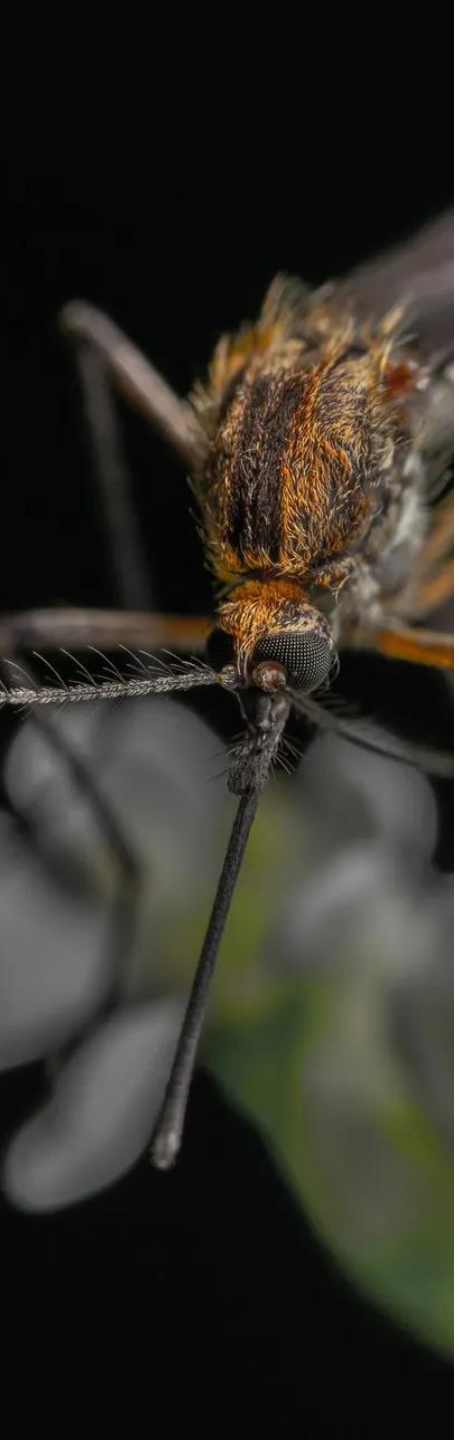

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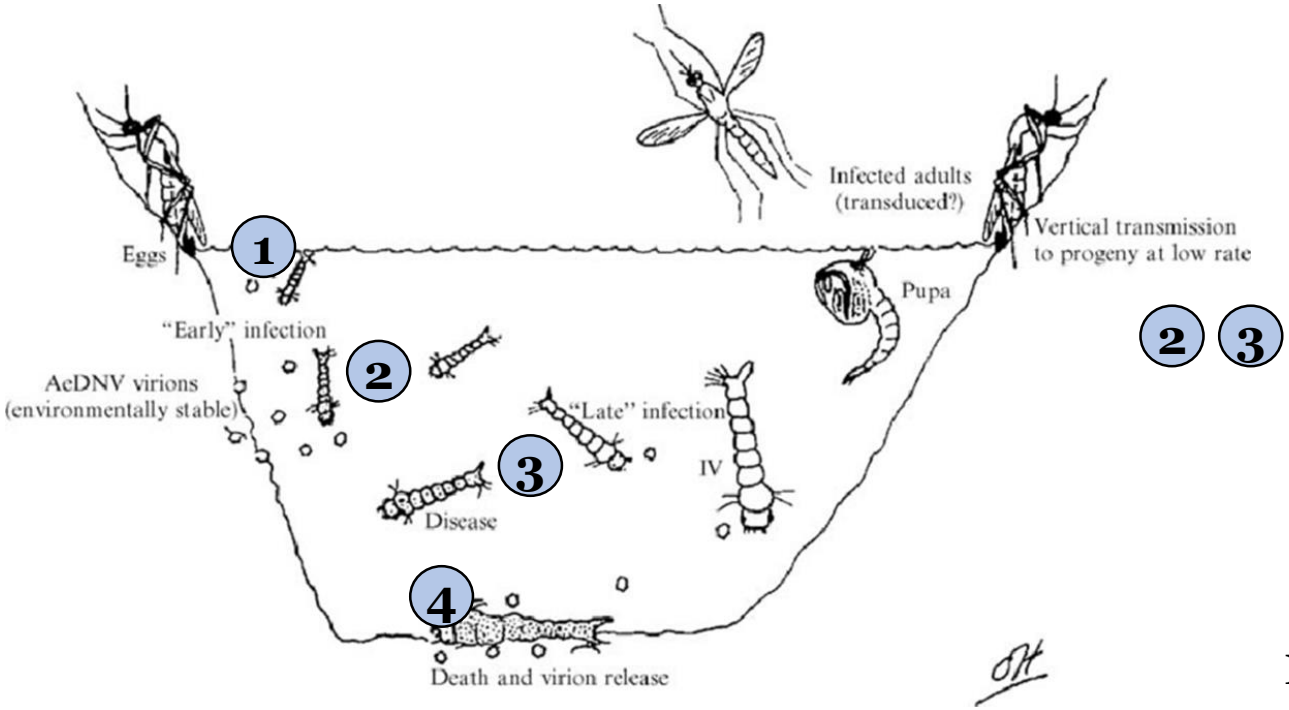


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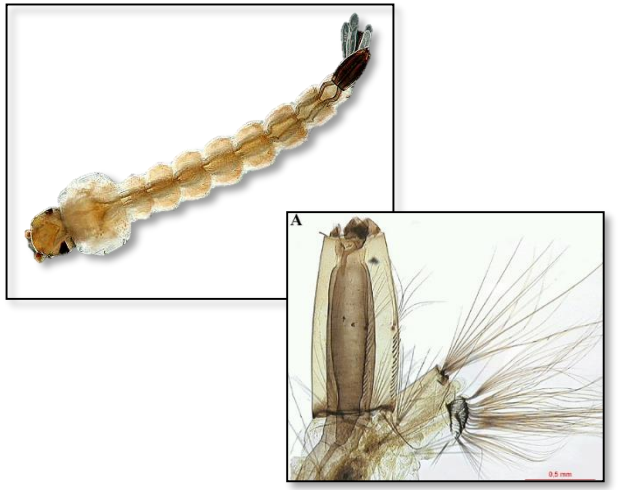


2 3 Primary infection site = anal papilla

↓

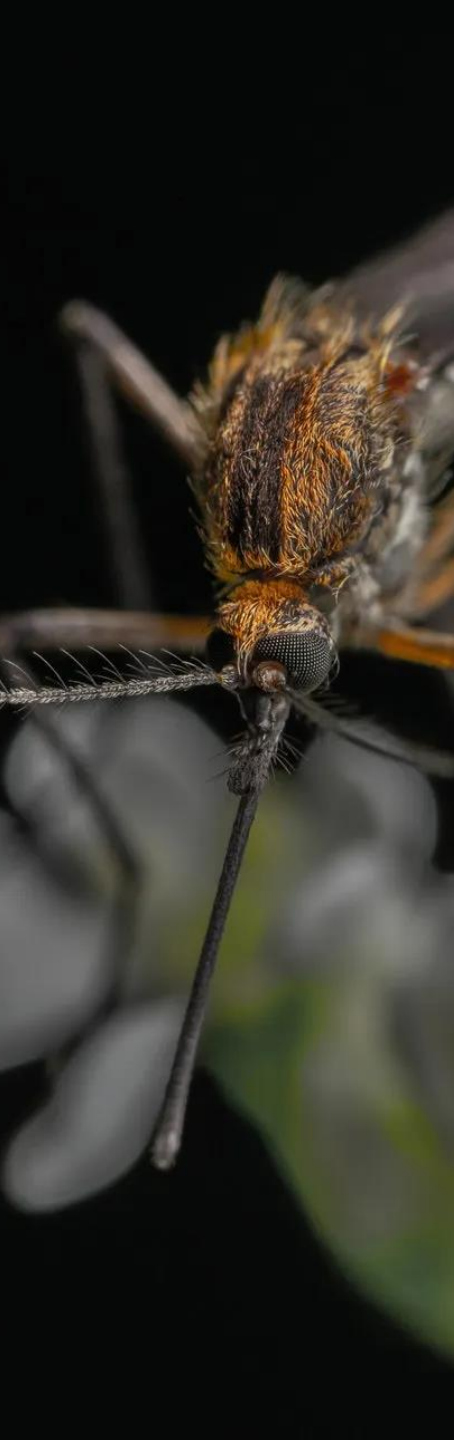
Fat body

↓

Hypoderm / nervous system / muscles

↓

4 Death



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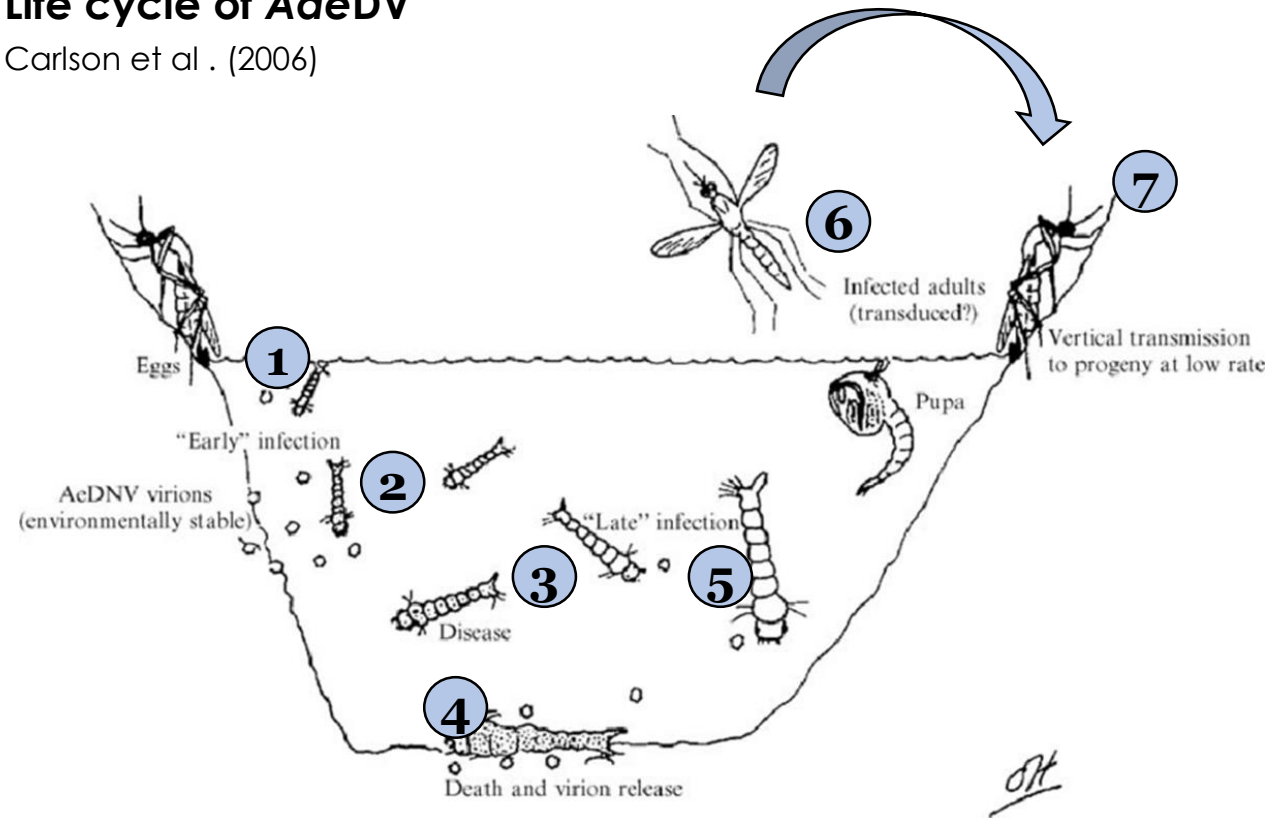


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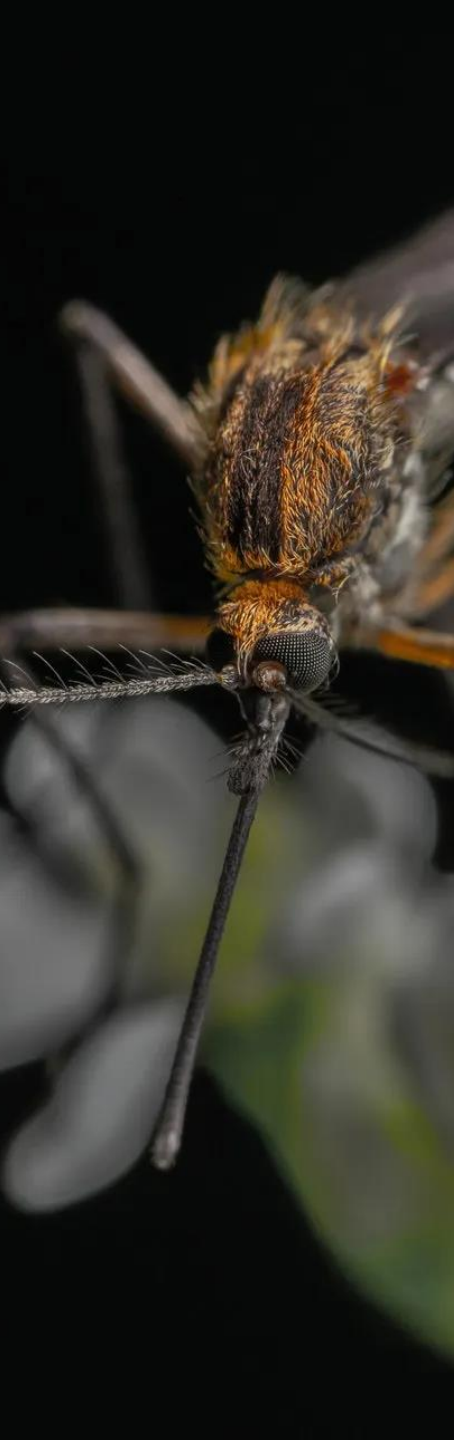
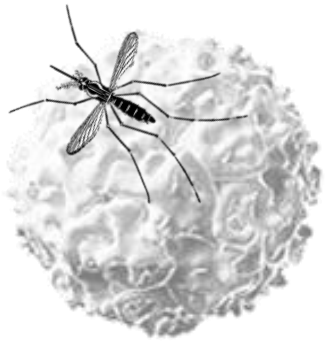
6 ⬇

Survival to infection

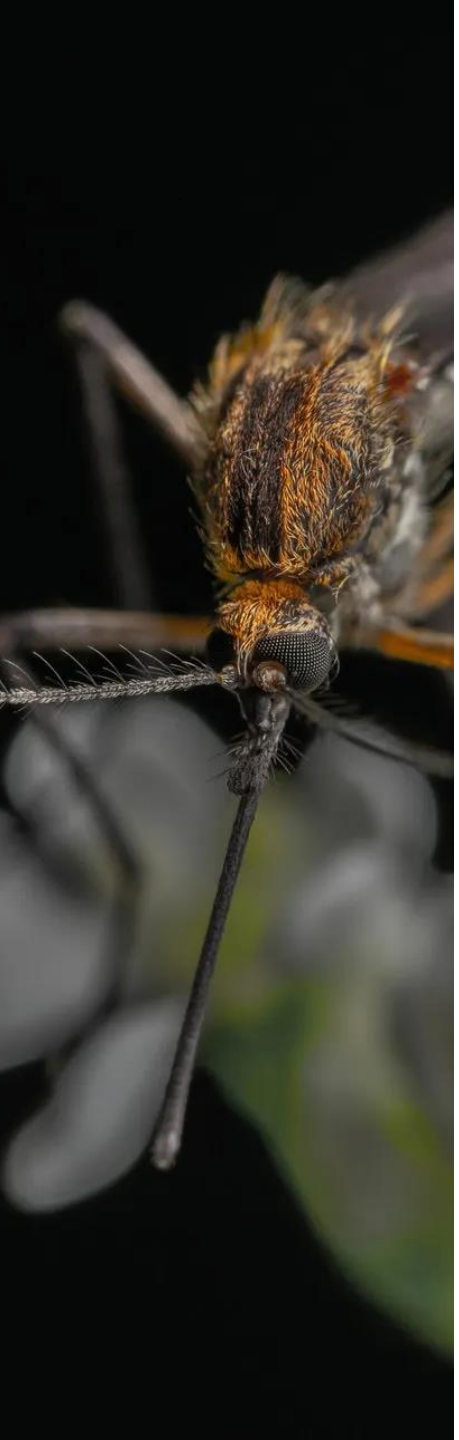
- ↘ lifetime (dose dependant)
- ↘ fecundity
- ↘ qt eggs
- ↘ eggs viability



# Our global project : biological agent ?



**Projects** : Revolinc, Bioviral, Fibi, Biobeads, Yden, DensoTIS



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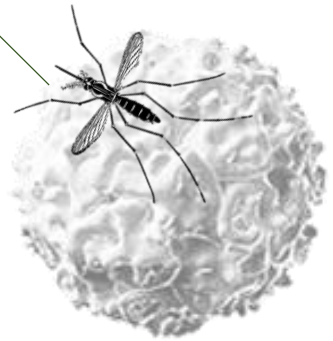
In the laboratory and in the field



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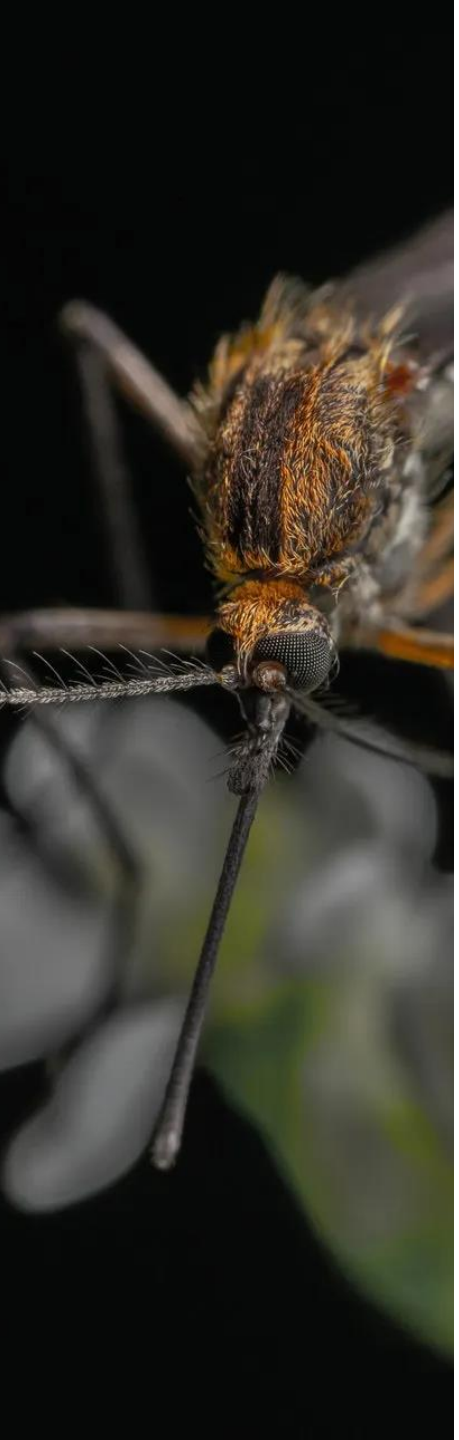

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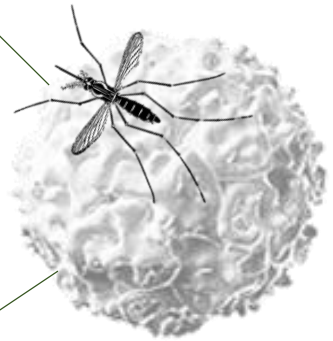
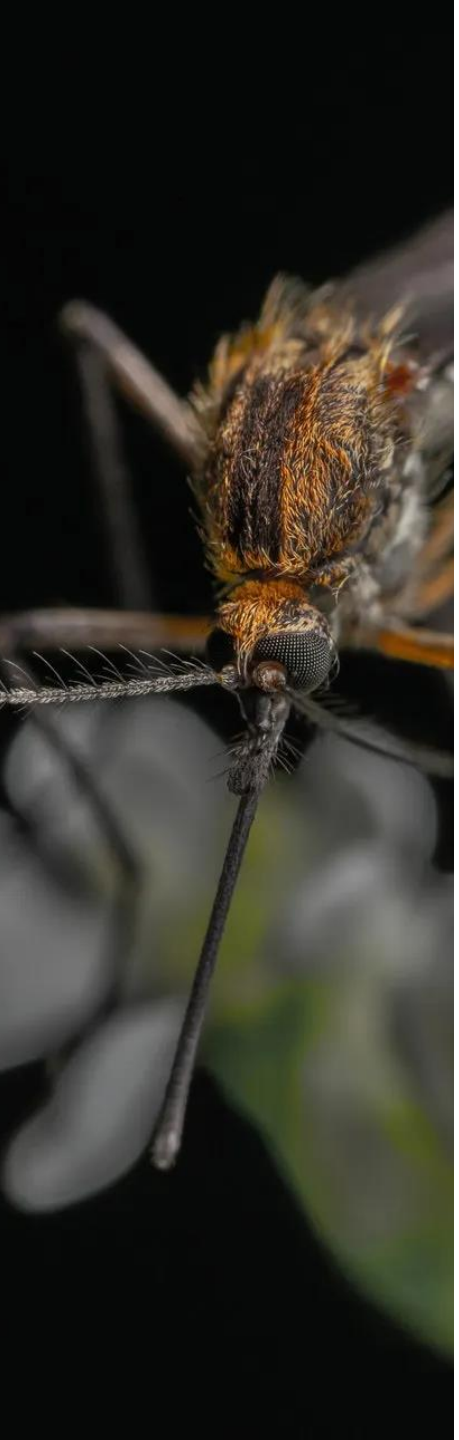


**EVALUATION OF INFECTIVITY**
- ❑ Host range
- ❑ Environmental impact

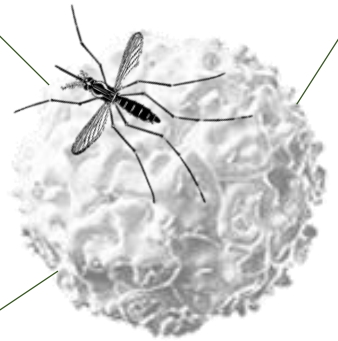
**Projects** : Revolinc, Bioviral, Fibi, Biobeads, Yden, DensoTIS



# Our global project : biological agent ?

**EFFICACY**
In the laboratory and in the field

**PRODUCTION**
From lab to industry



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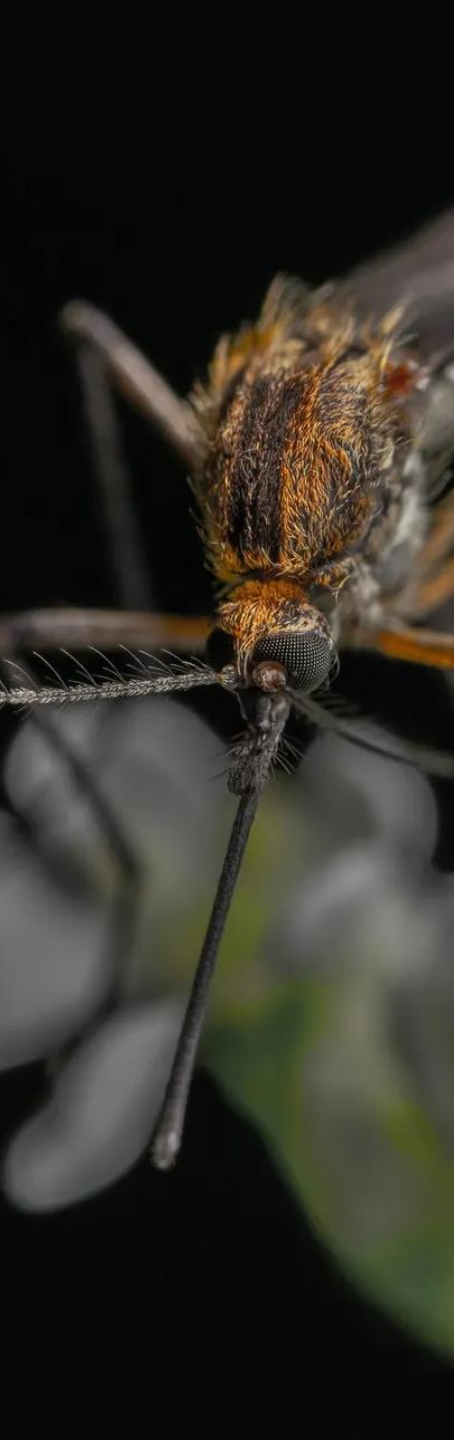

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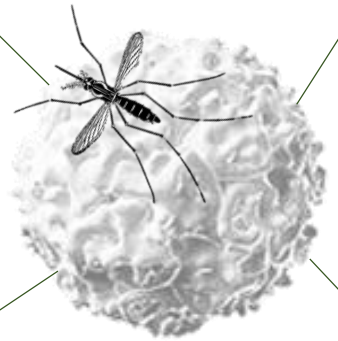
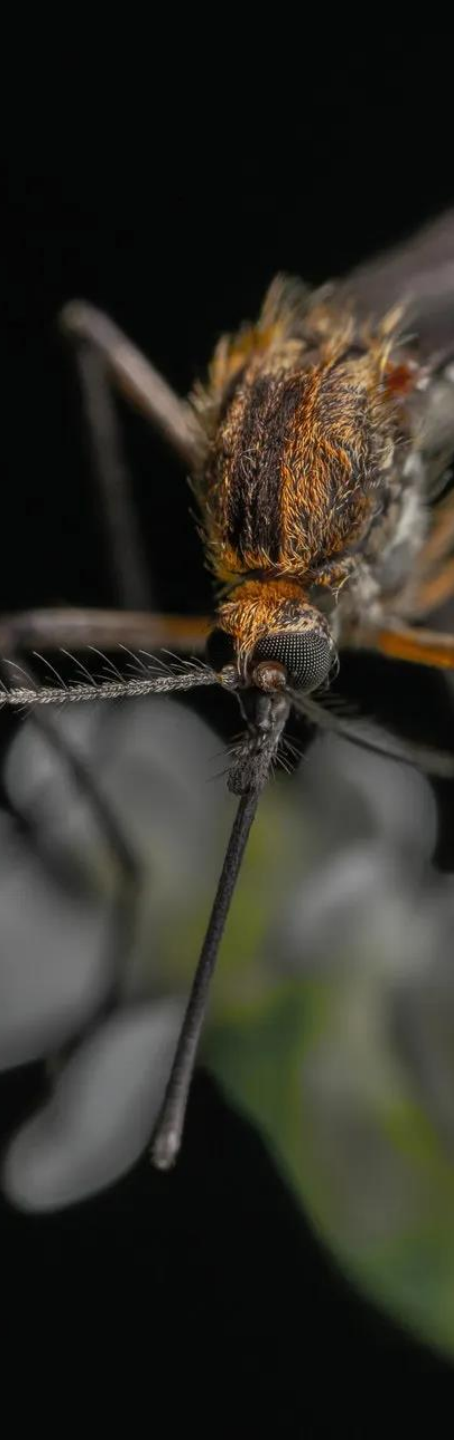
**PRODUCTION**
From lab to industry



**EVALUATION OF INFECTIVITY**
- ❑ Host range
- ❑ Environmental impact

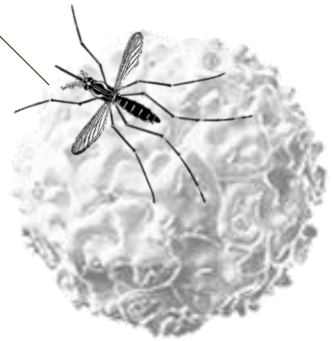
**FORMULATION**
Optimize use and adapt to the strategy

**Projects** : Revolinc, Bioviral, Fibi, Biobeads, Yden, DensoTIS



# Our global project : biological agent ?

**EFFICACY**
In the laboratory and in the field



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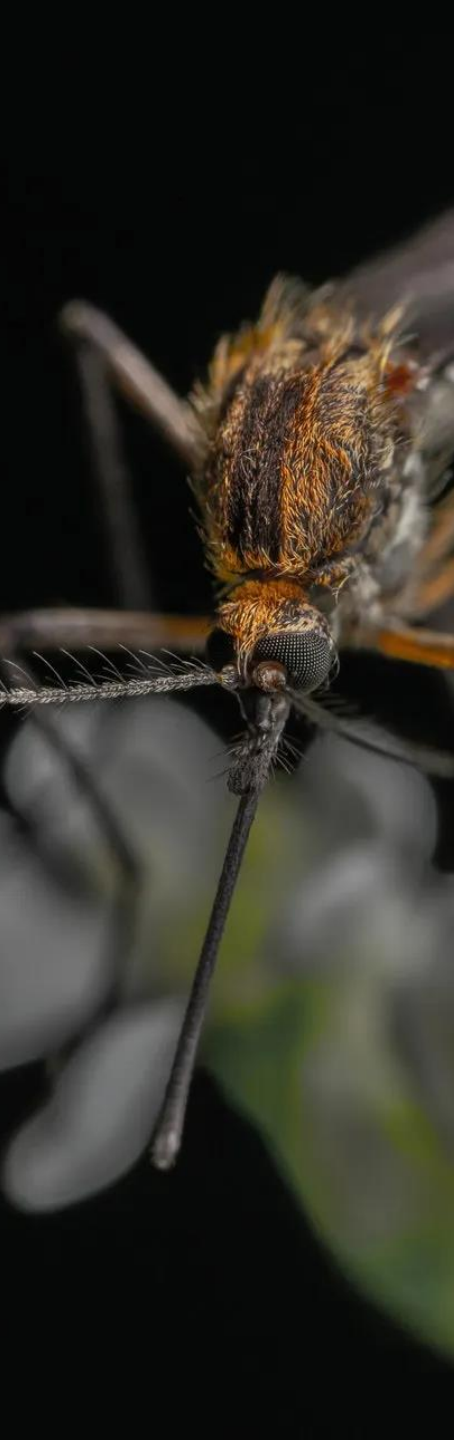
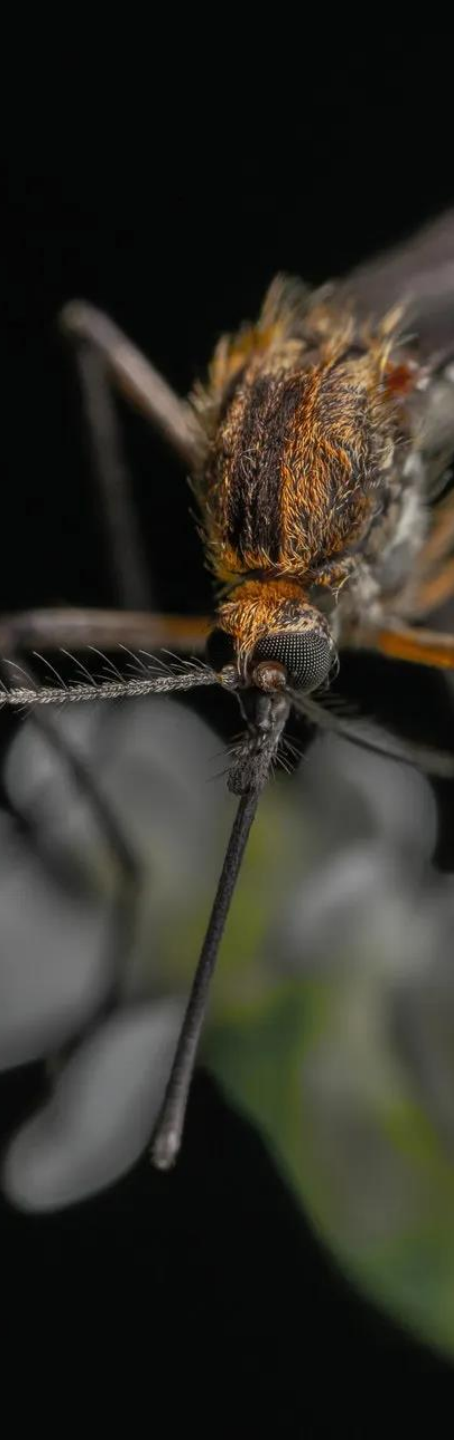


# Infectivity of AaIDV2 on *Aedes* vectors

| Species | Strains | Origin | Lab colonization | Status |
|---|---|---|---|---|
| ***Ae. aegypti*** | BB | French Polynesia | 1980 | Susceptible[1] |
| | SBE | Benin | 2008 | Susceptible[1] |
| | LHP | Vietnam | 199 ? | Resistant[2] (*kdr*) |
| ***Ae. albopictus*** | MTP | France | 2016 | Susceptible[1] |
| | LR | Overseas France | 2016 | Resistant[2]* |



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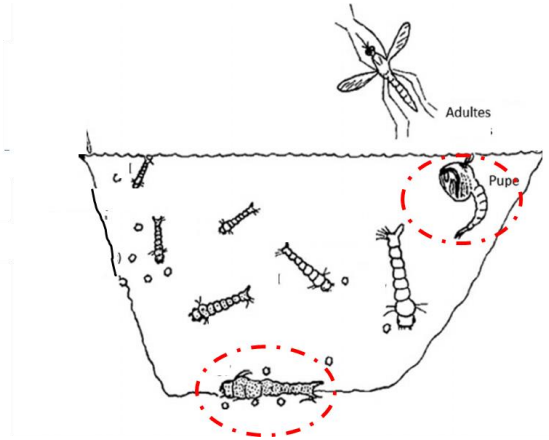


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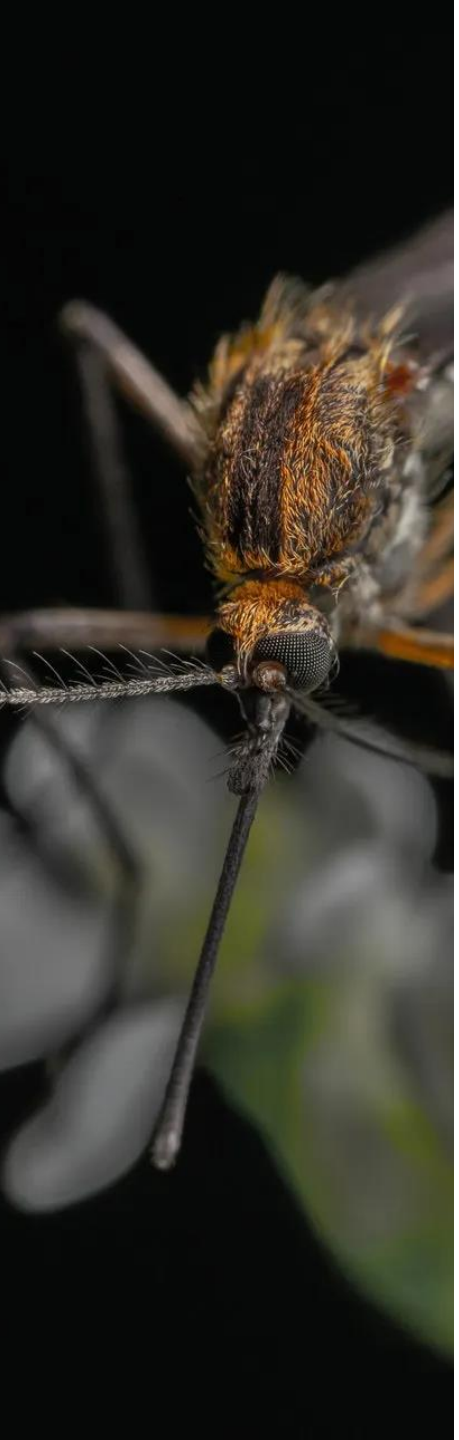

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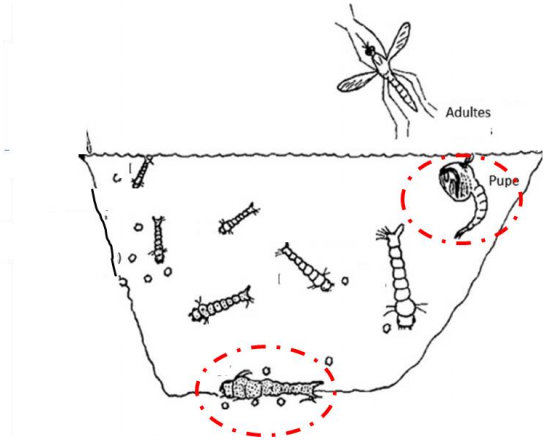


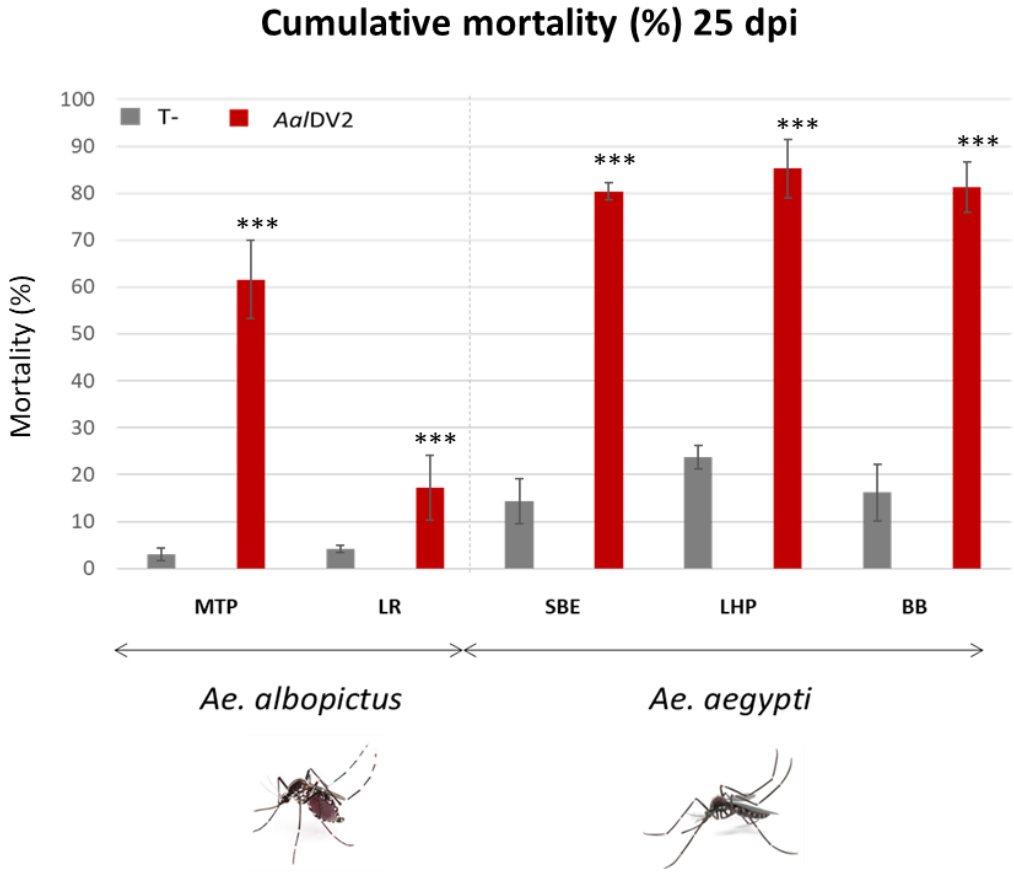


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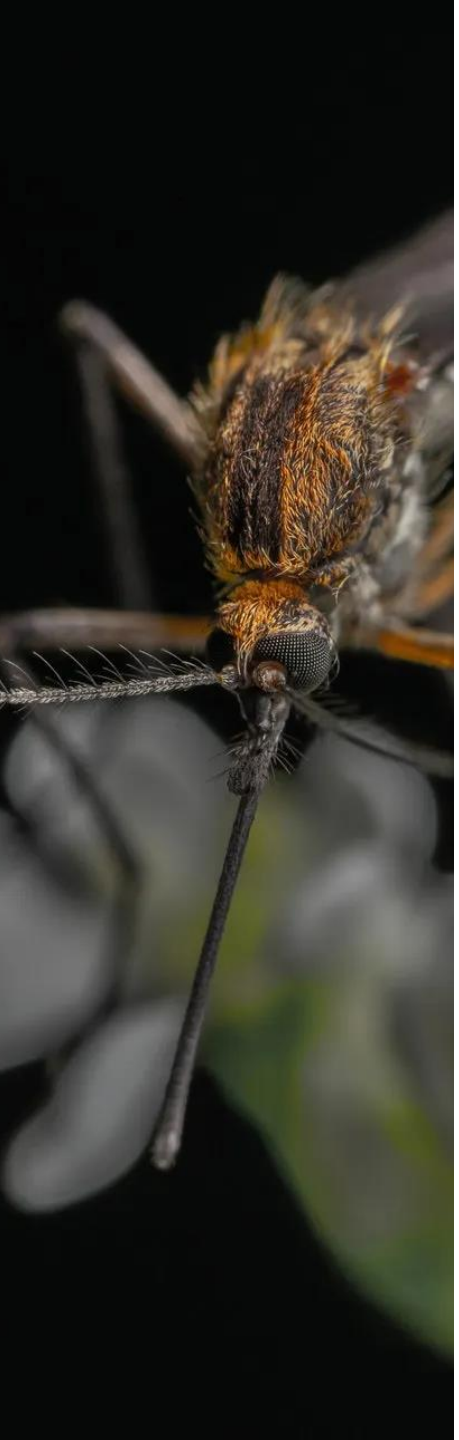

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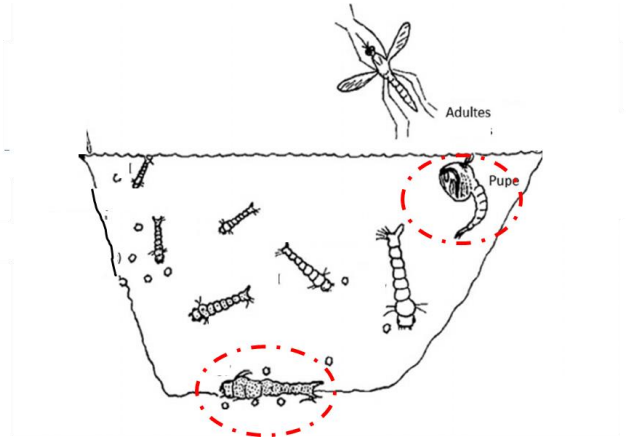
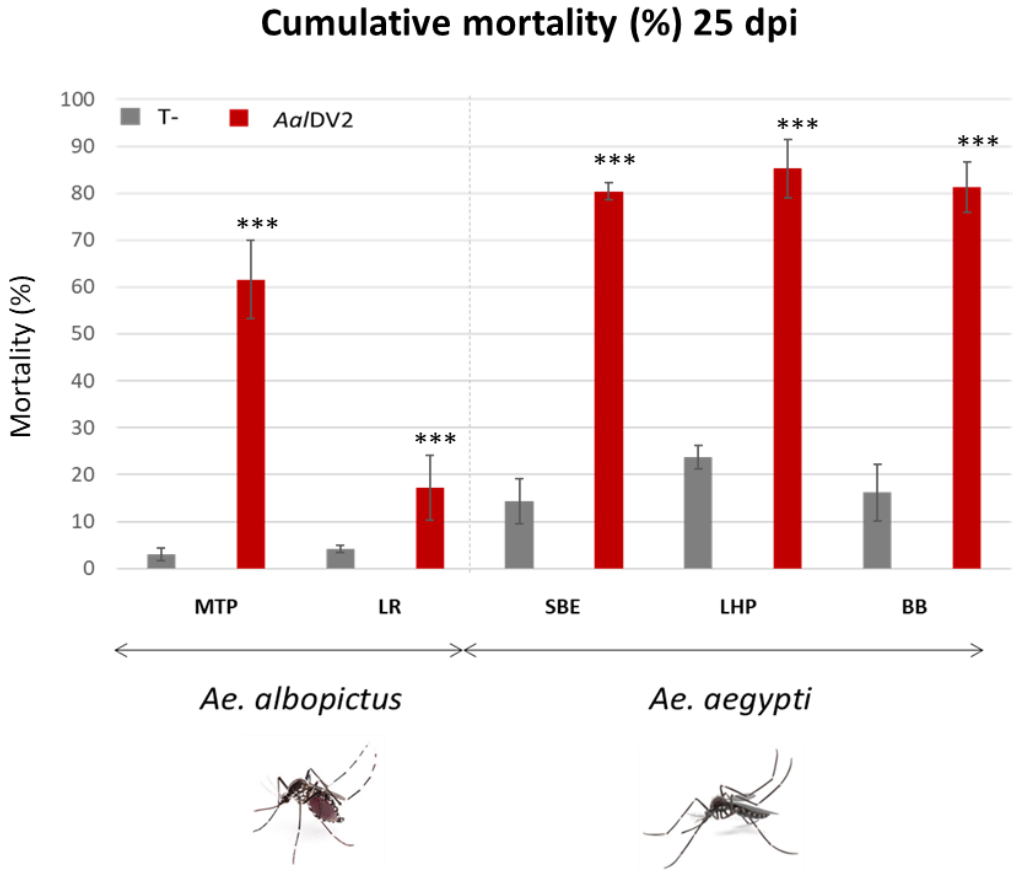



- Mortality at all stages of development: mainly at the pupa stage in *albopictus* and larva in *aegypti*
- 100% of mortality is related to infection

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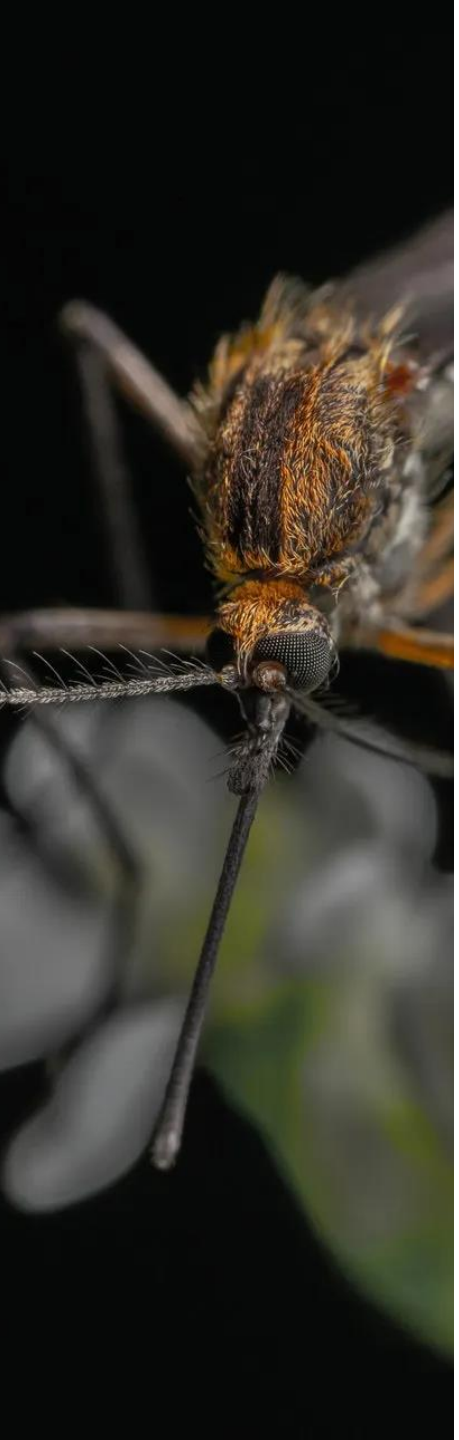

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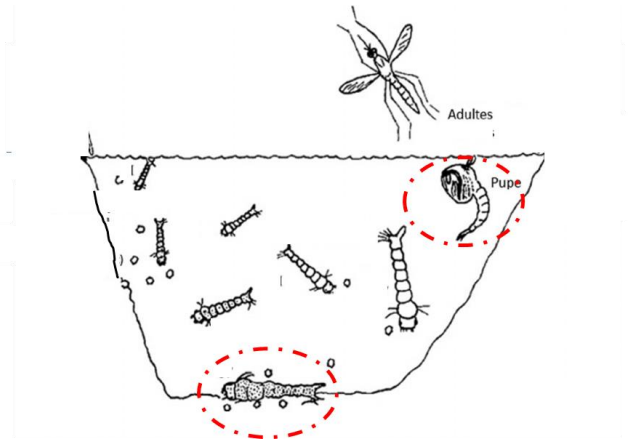
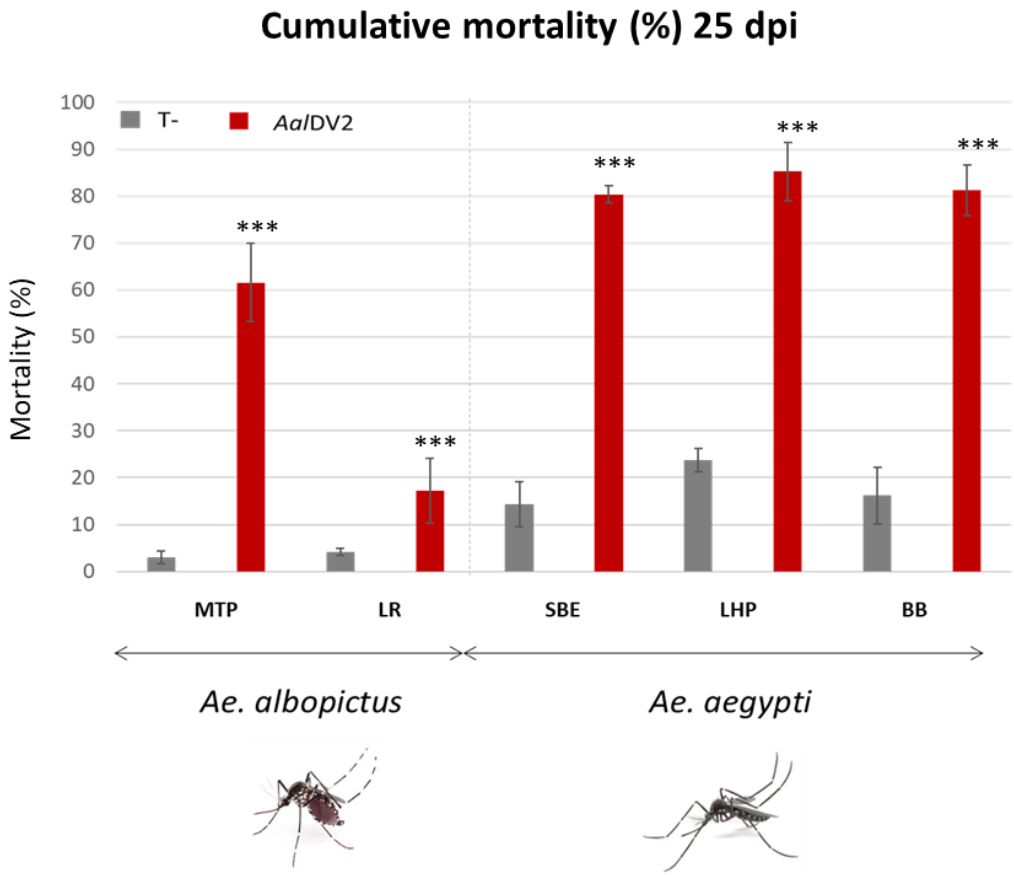



- Mortality at all stages of development: mainly at the pupa stage in *albopictus* and larva in *aegypti*
- 100% of mortality is related to infection
- Loss of dead larvae (by cannibalism or necrophagy ?)

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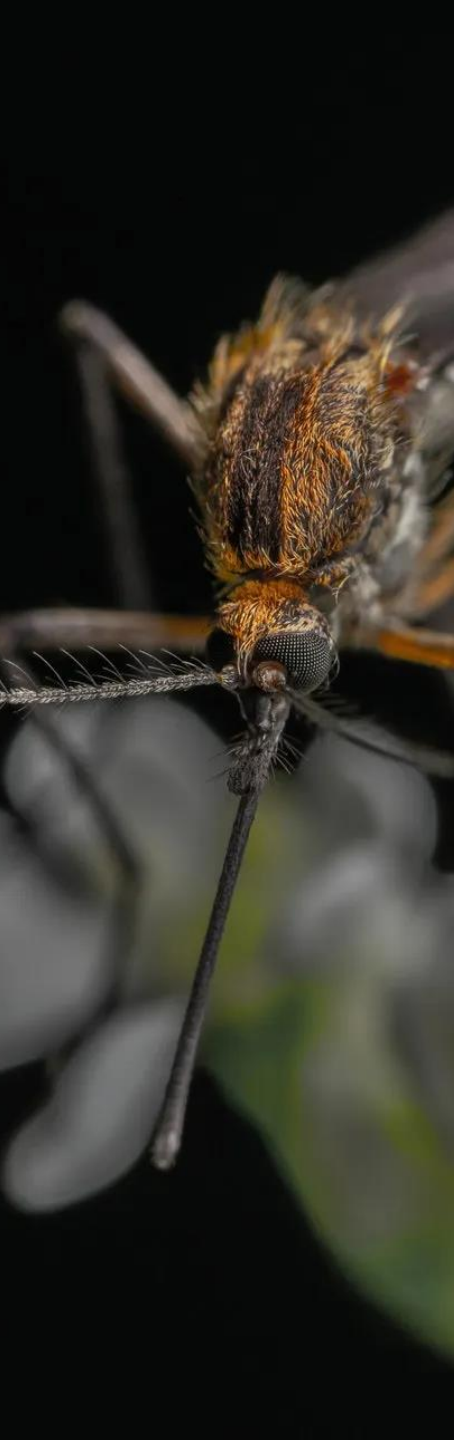

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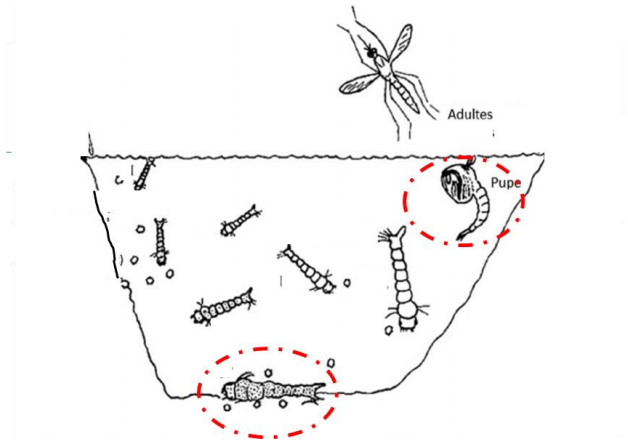
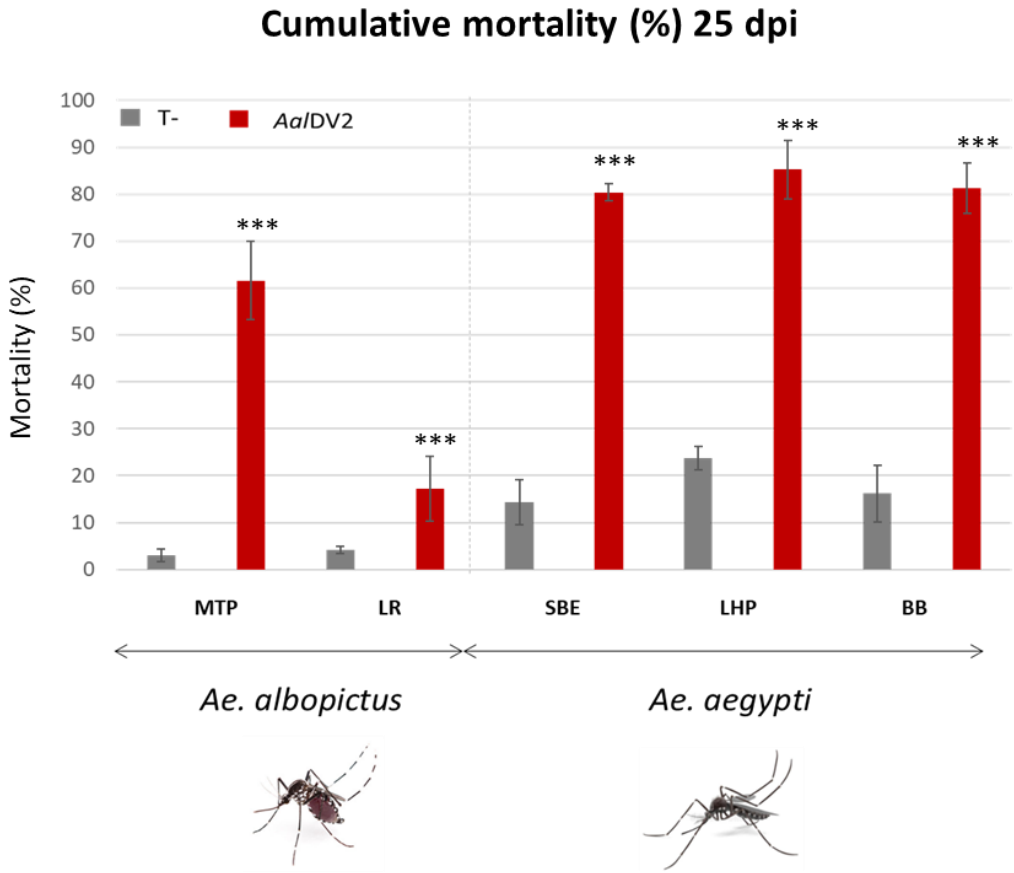



- Mortality at all stages of development: mainly at the pupa stage in *albopictus* and larva in *aegypti*
- 100% of mortality is related to infection
- Loss of dead larvae (by cannibalism or necrophagy ?)
- **High intra and interspecific variability**

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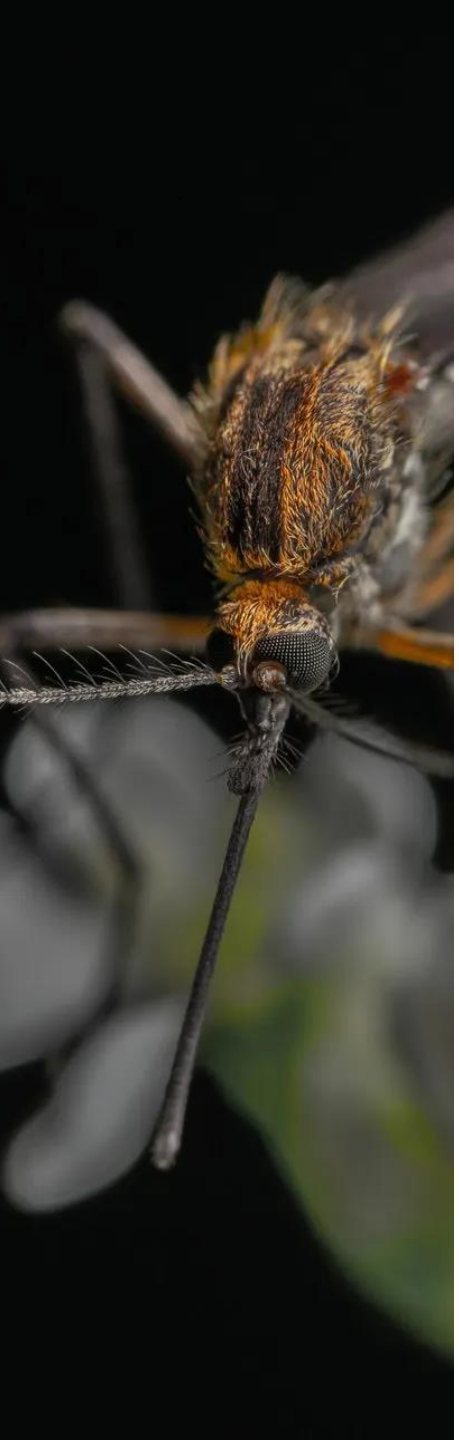

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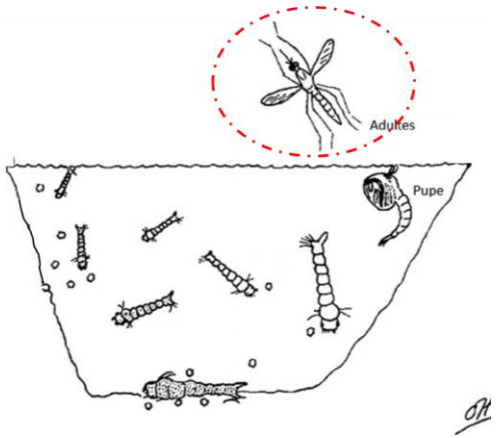


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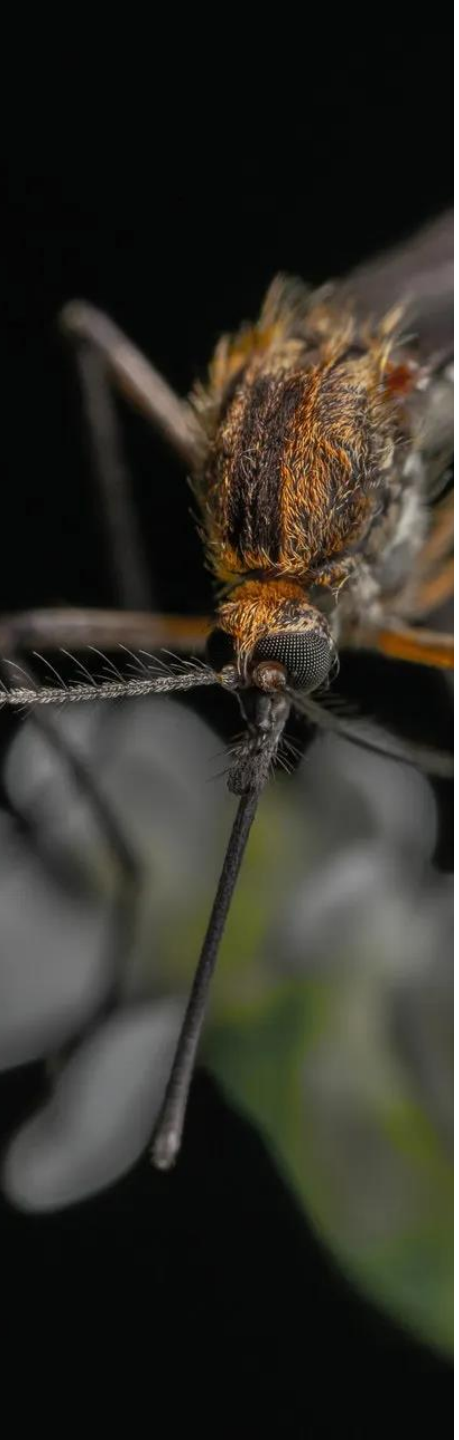

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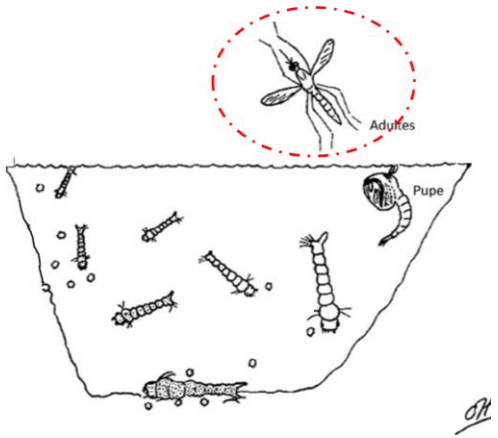
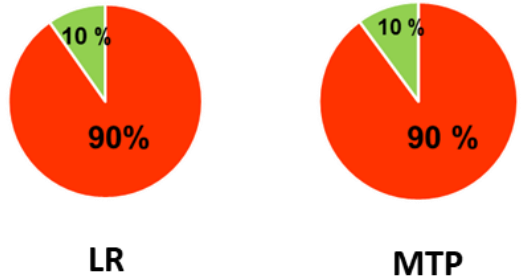
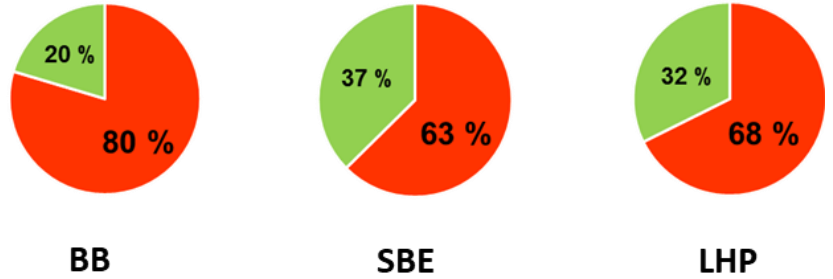



- High rate of contagion among emerging adult survivors

*Ae. albopictus*




*Ae. aegypti*

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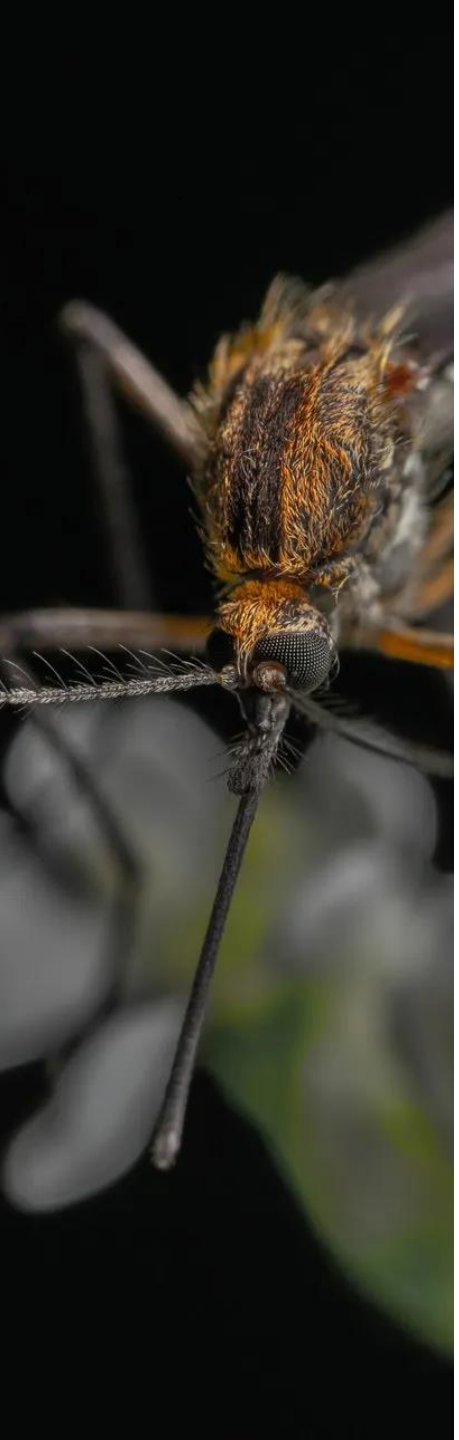

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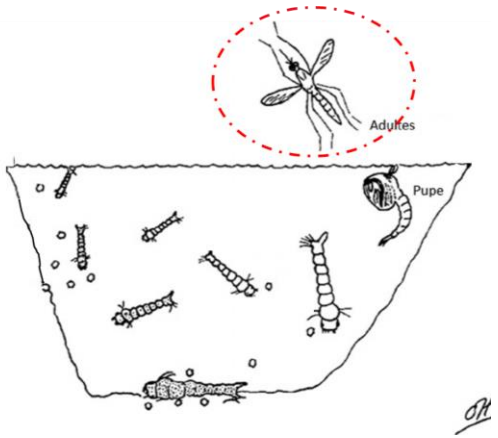



- Sex ratio biased in favor of female

**Tab.2** – Sex ratio (female number / male number) in control and infected groups for each strains of *Ae. aegypti* (LHP, SBE, BB) and *Ae. albopictus* (MTP, LR).

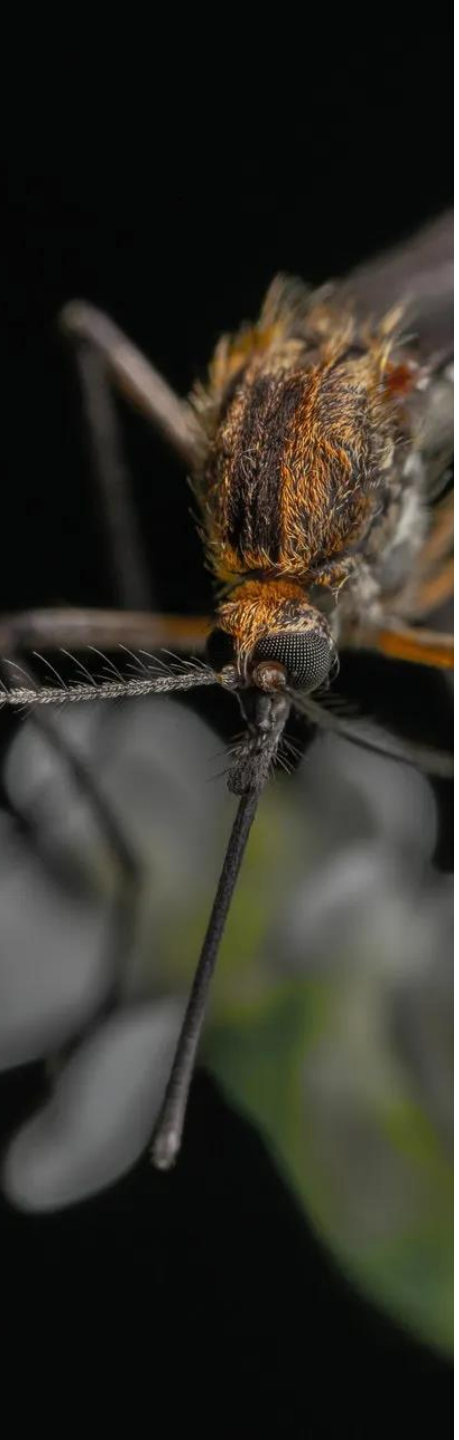
| | | Female | Male | sex-ratio F/M | $\chi^2$, df=1,p-value |
|---|---|---|---|---|---|
| LHP[1] | Control | 117 | 141 | 0.83 | |
| | Infected | 21 | 14 | 1.5 | 2.65, p=0,10 |
| SBE[1] | Control | 135 | 134 | 1.01 | |
| | Infected | 73 | 43 | 1.70 | 5.30, p<0.05 |
| BB[1] | Control | 111 | 138 | 0.80 | |
| | Infected | 38 | 14 | 2.71 | 13.98, p<0.001 |
| MTP[2] | Control | 111 | 115 | 0.97 | |
| | Infected | 32 | 31 | 1.03 | 0.05, p=0.81 |
| LR[2] | Control | 154 | 144 | 1.07 | |
| | Infected | 128 | 124 | 1.03 | 0.04, p=0,83 |

1: *Aedes aegypti*; 2: *Aedes albopictus*

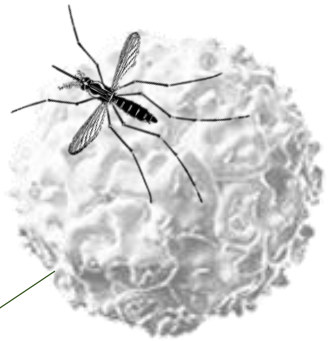

*Ae. aegypti*


*Ae. albopictus*

**Perrin, A., Gosselin-Grenet, A. S., Rossignol, M., Ginibre, C., Scheid, B., Lagneau, C., Chandre F., Baldet T., Ogliastro M. & Bouyer, J.** (2020). Variation in the susceptibility of urban Aedes mosquitoes infected with a densovirus. Scientific reports, 10(1), 1-10.



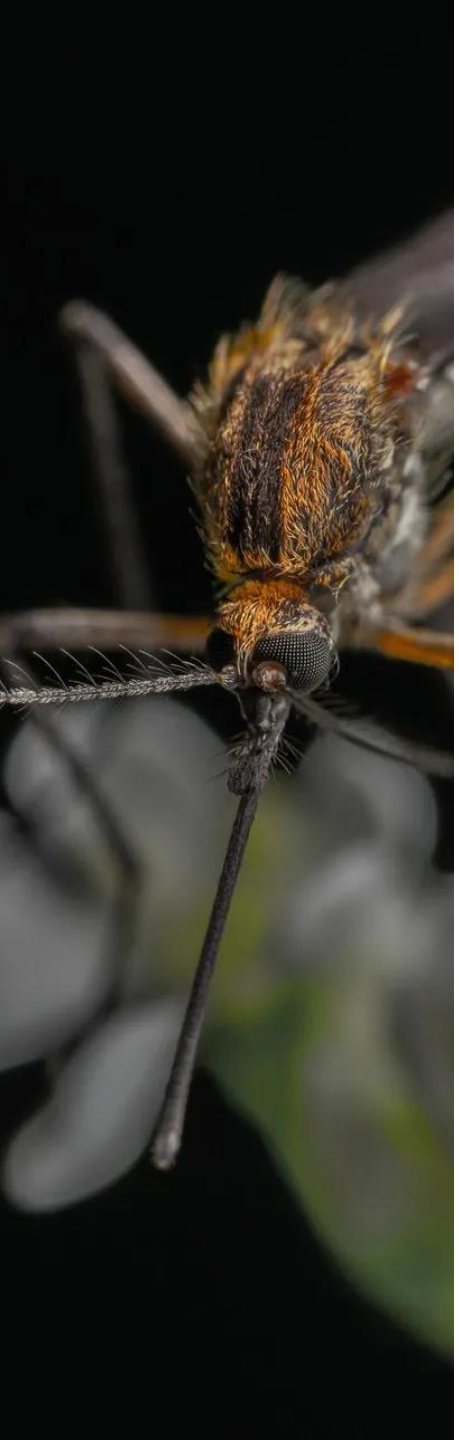
# Our global project : biological agent ?



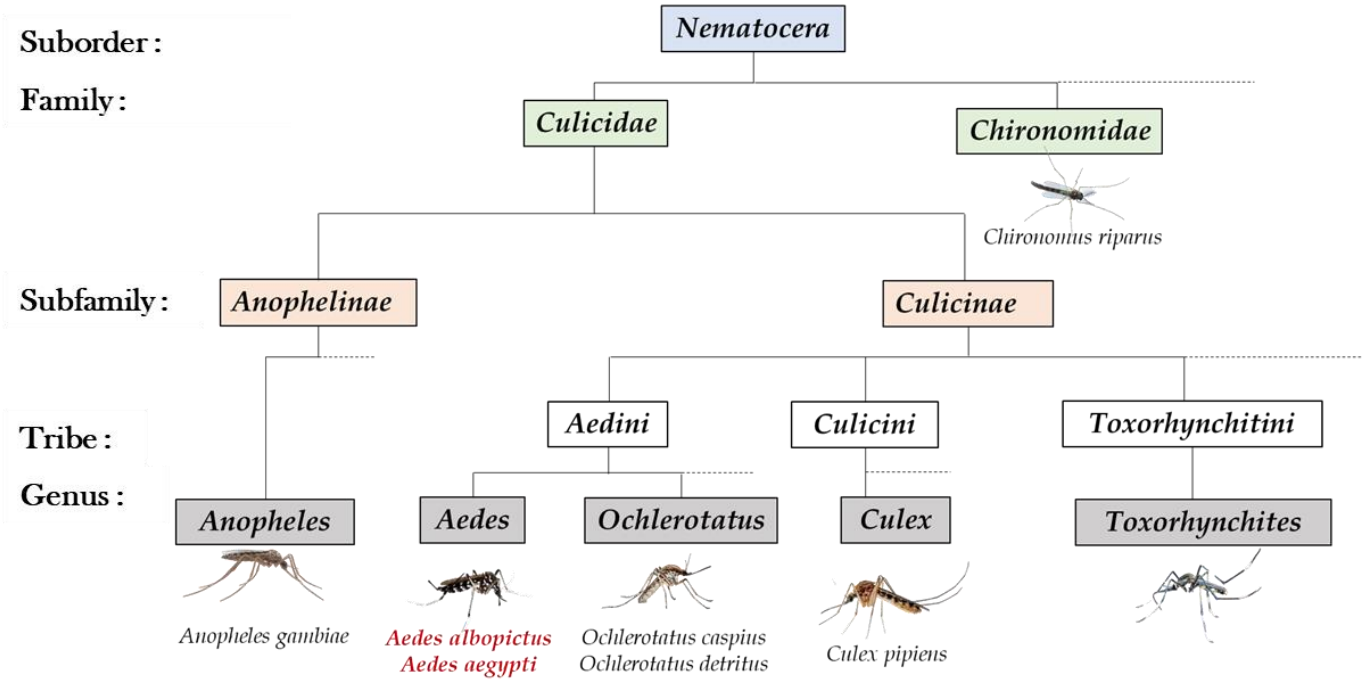
**EVALUATION OF INFECTIVITY**

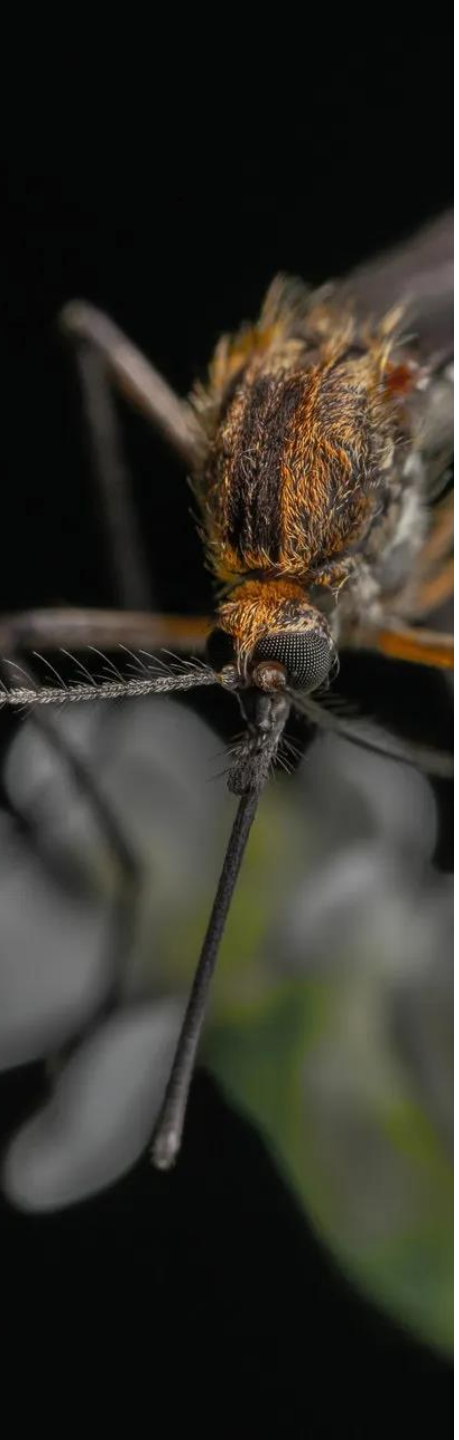
- ❑ Host range
- ❑ Environmental impact

**Projects** : Revolinc, Bioviral, Fibi, Biobeads, Yden, DensoTIS



# Mosquito species susceptible to AalDV2 oral infection





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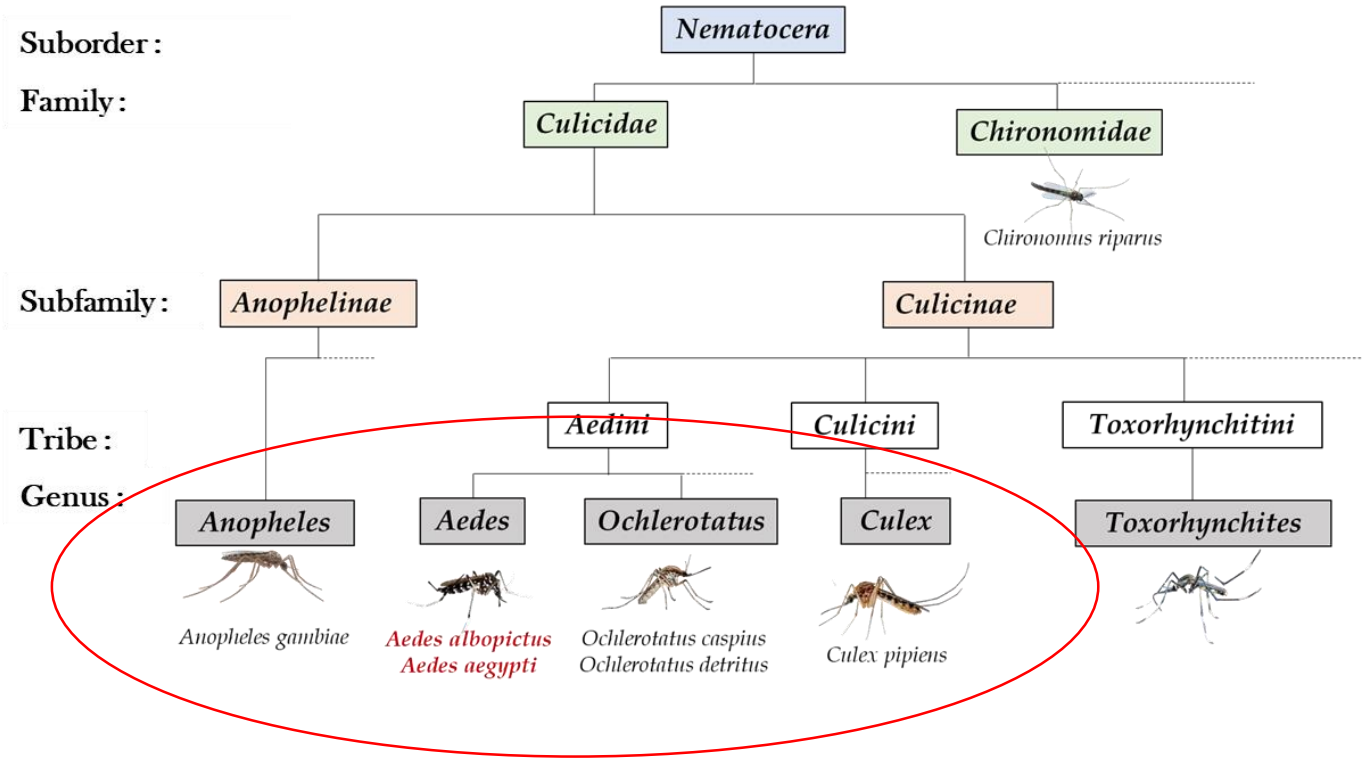

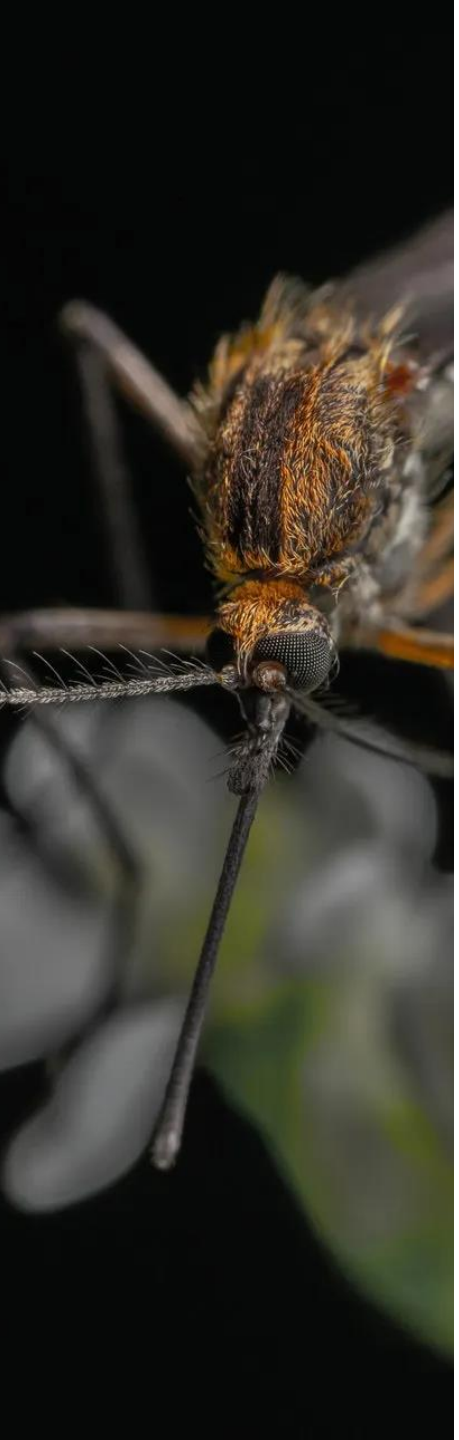

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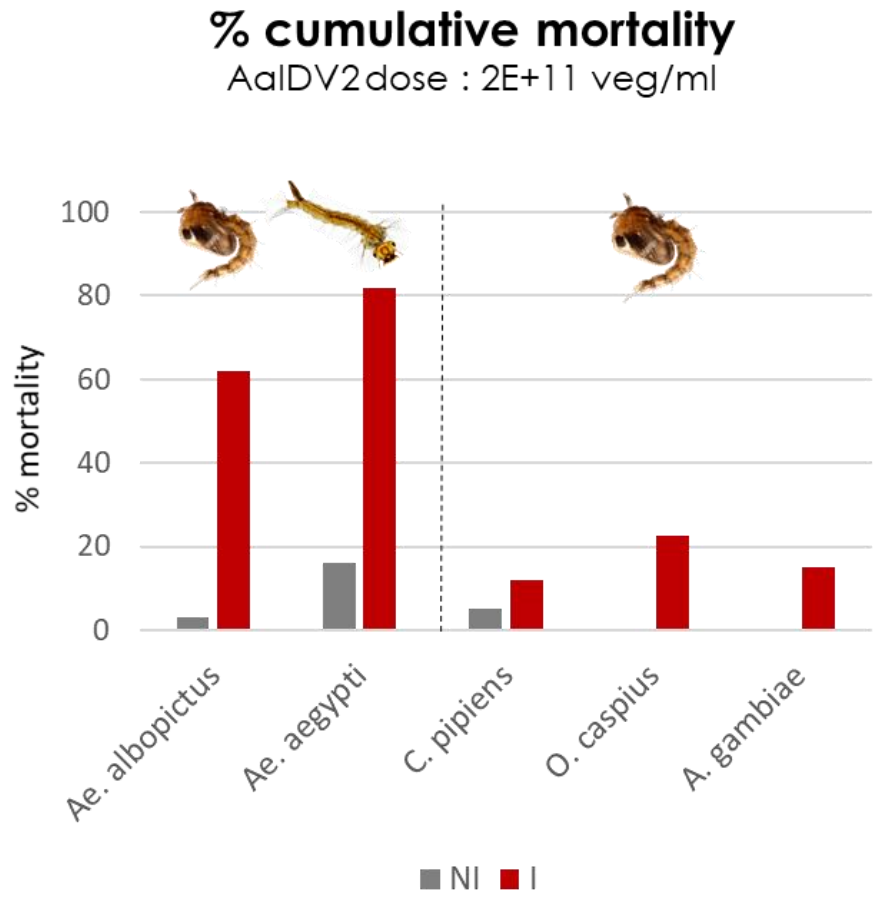

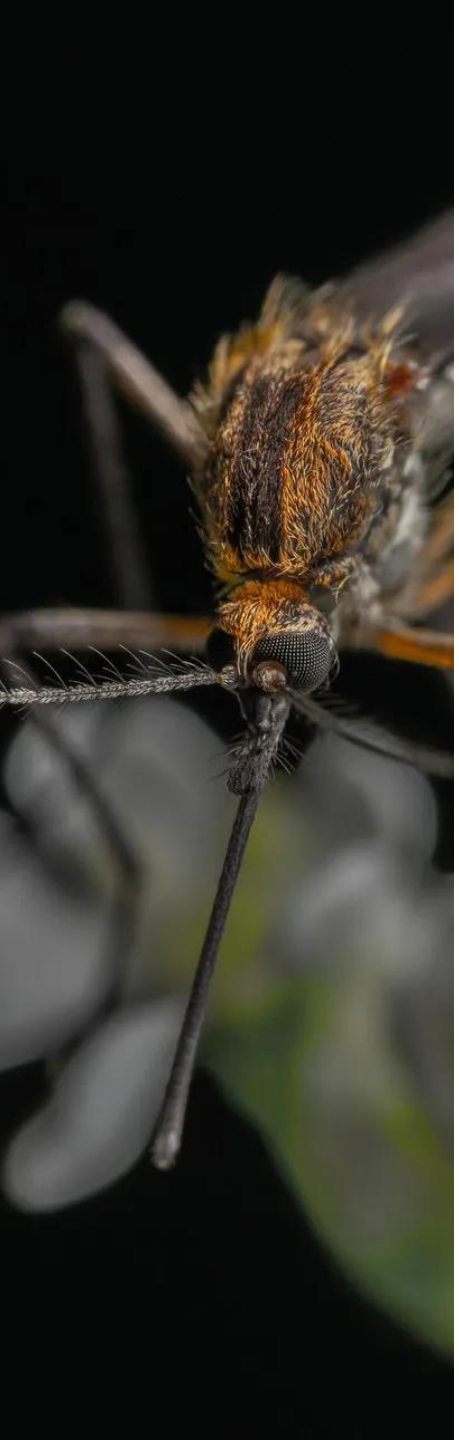

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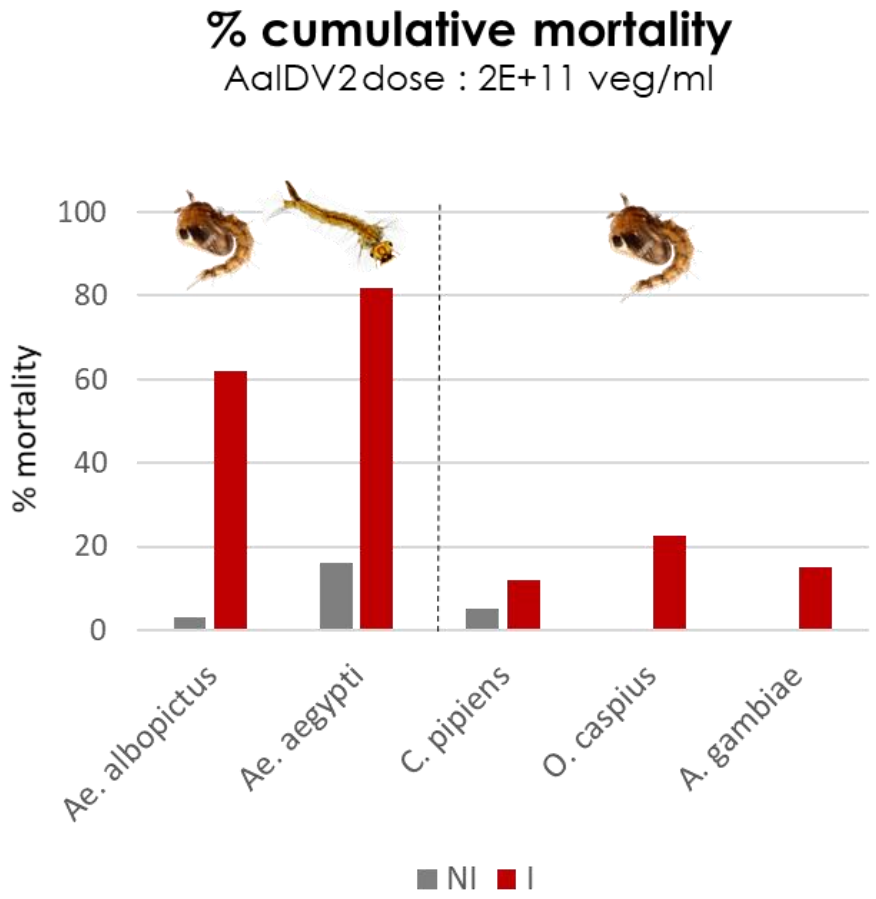
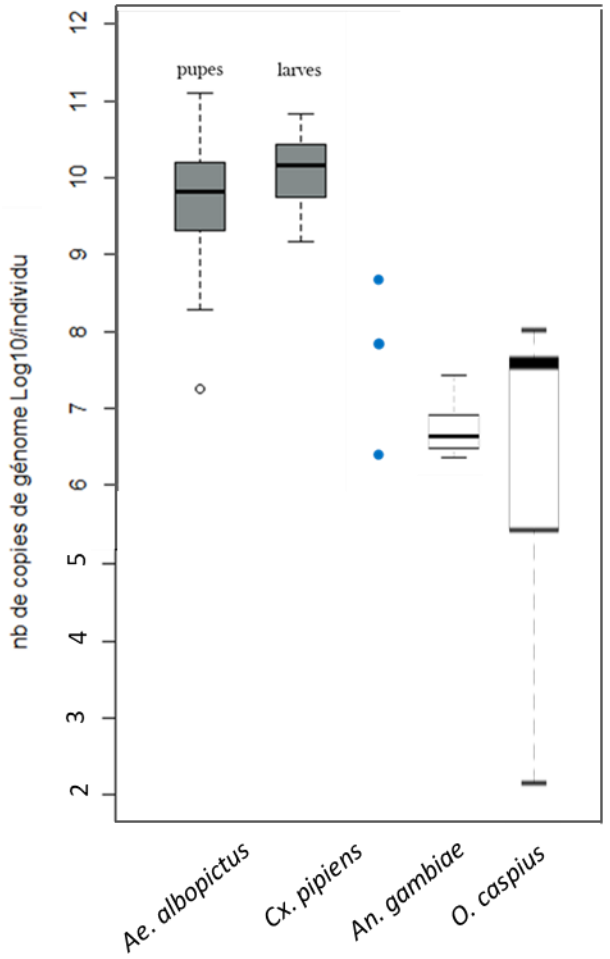
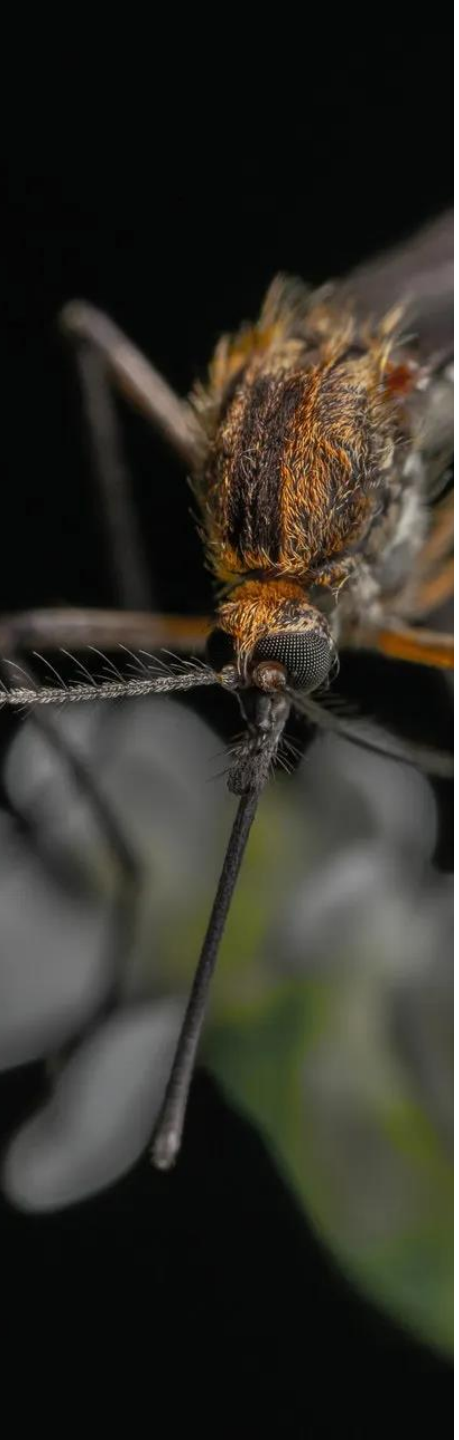



Viral load in the dead larvae or pupae





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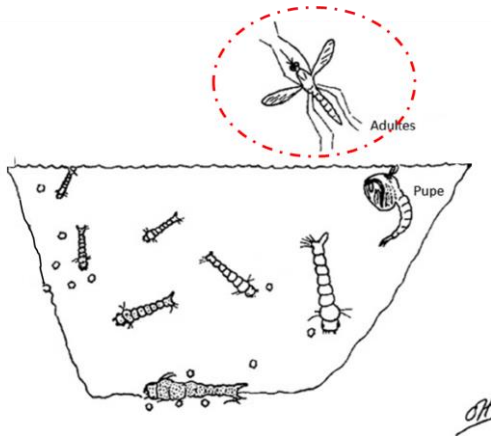

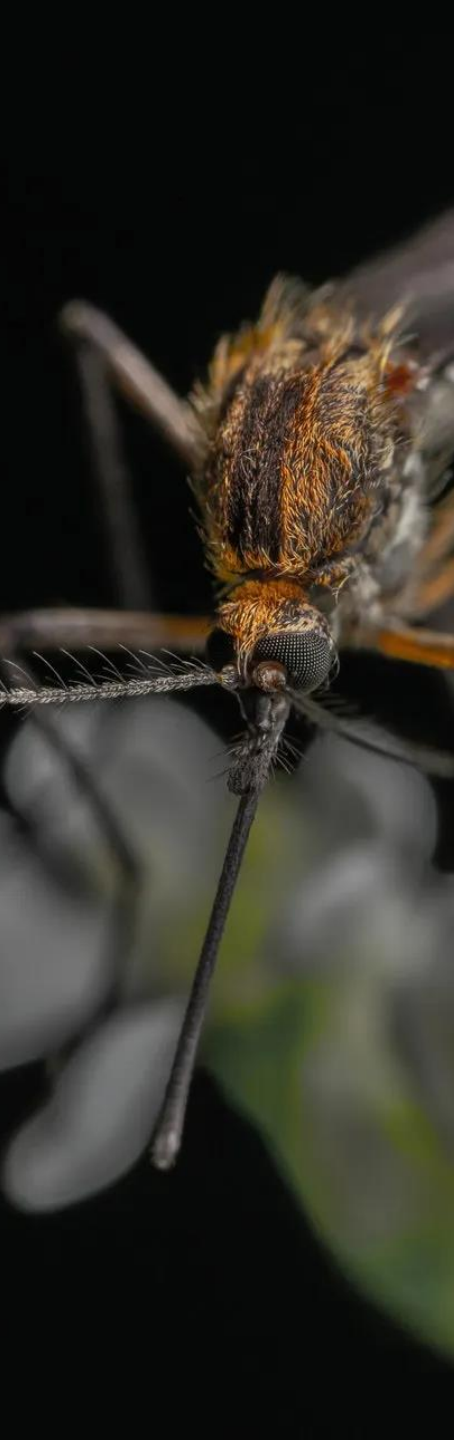

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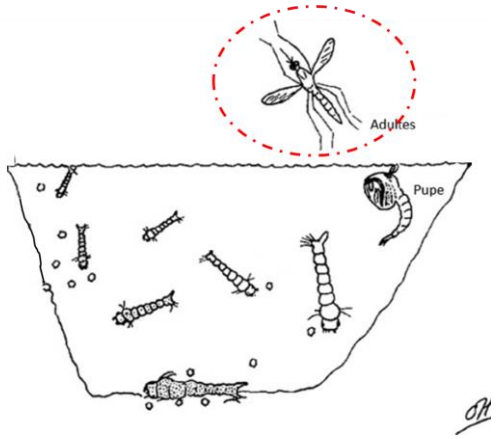
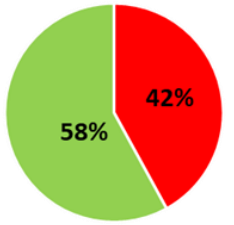
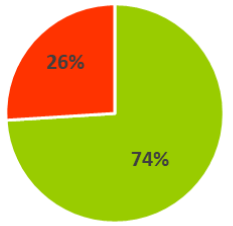


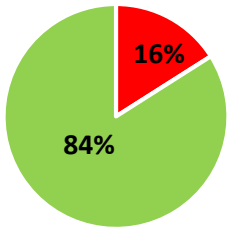

*Culex pipiens*

*O. caspius*

*A. gambiae*



# Environmental impact

**Acute and chronic toxicity tests with AaIDV2 on *Daphnia magna***

# Environmental impact

## Acute and chronic toxicity tests with AaIDV2 on *Daphnia magna*

**Acute toxicity test** = mobility test over 48 hours of exposure to AaIDV2
- No toxicity, but increased mobility of Daphnia

# Environmental impact

## Acute and chronic toxicity tests with AalDV2 on *Daphnia magna*

**Acute toxicity test** = mobility test over 48 hours of exposure to AalDV2
- No toxicity, but increased mobility of Daphnia

**Chronic toxicity test** = measuring of the offspring after 21 days of exposure to AalDV2
- No toxicity
- Increase mobility
- Spawning stimulation

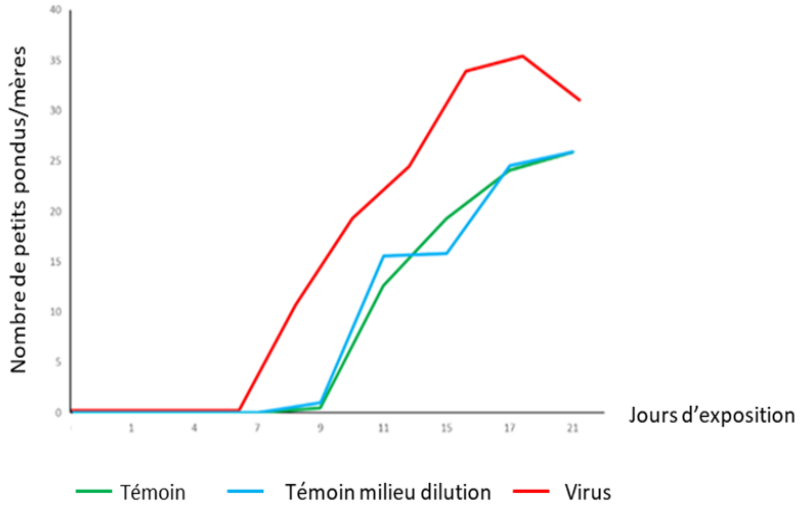
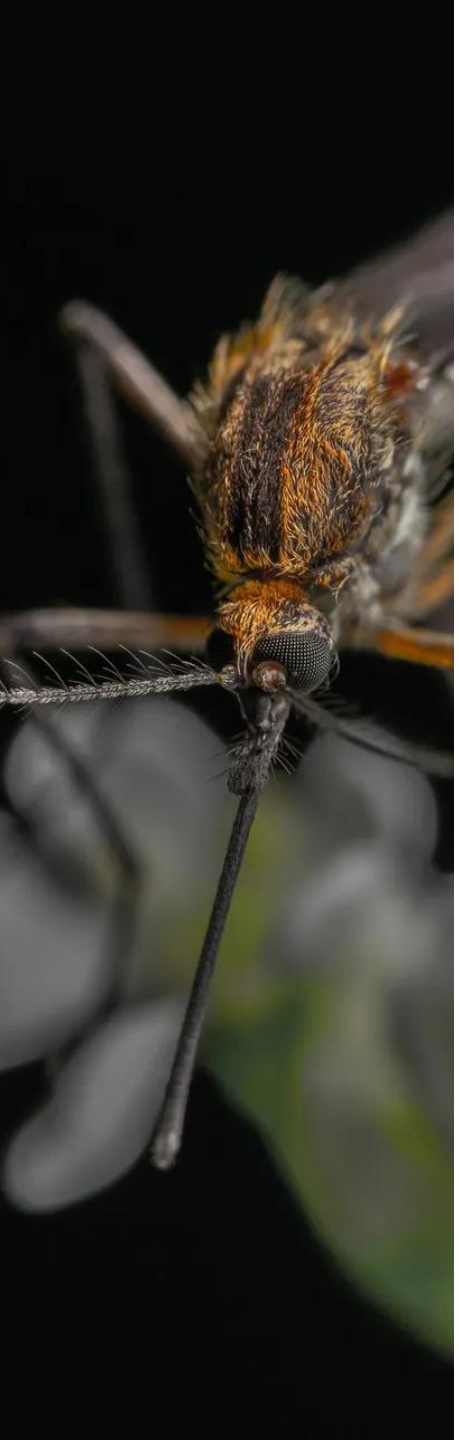
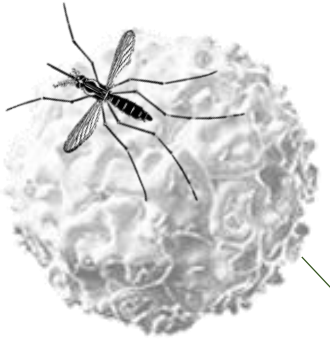



**Fig.26**- Evolution de la ponte (nombre de petits pondus / daphnie) pendant les 21 jours d'exposition de daphnies au densovirus AalDV2 (virus), au milieu de dilution du virus (témoins milieu dilution) et chez les daphnies non exposées (témoins).

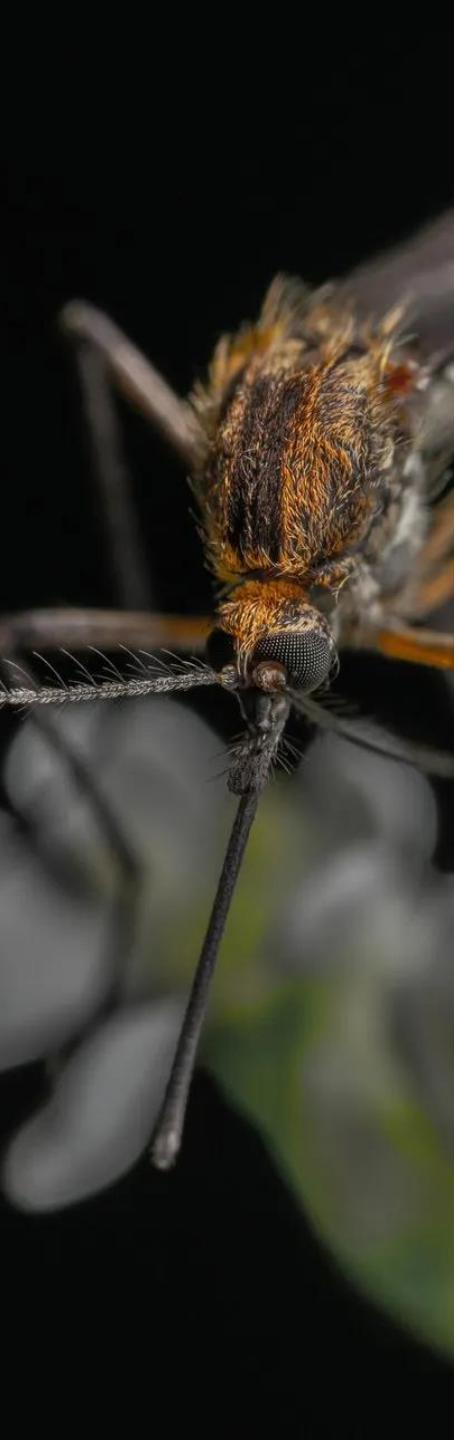


# Our global project : biological agent ?



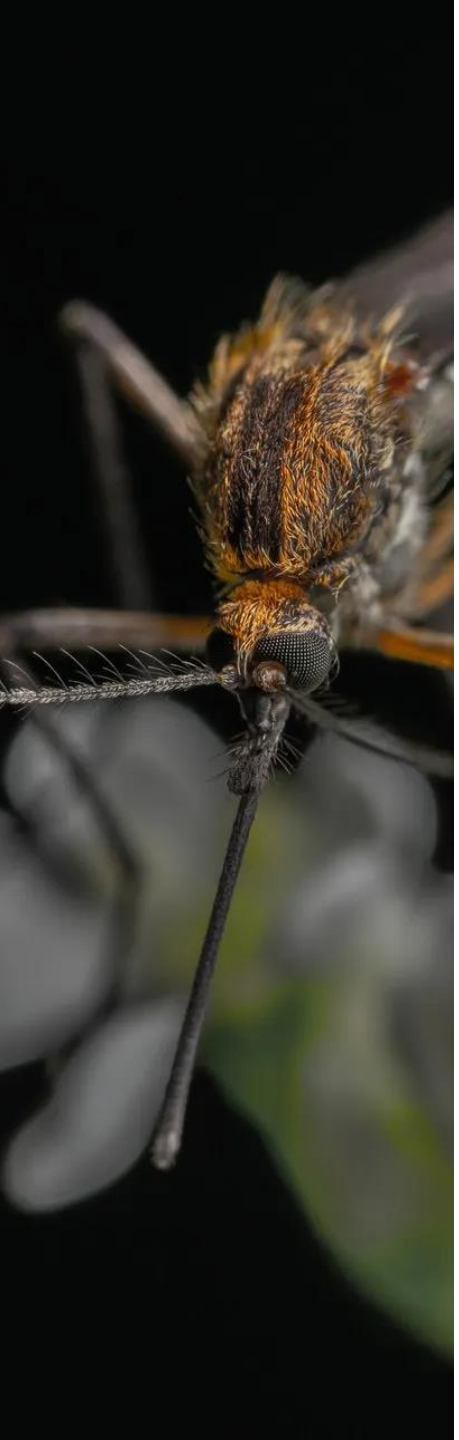
**FORMULATION**
Optimize use and adapt to the strategy

**Projects** : Revolinc, Bioviral, Fibi, Biobeads, Yden, DensoTIS



# Development of a technology of formulation

**Formulation of biocides (viruses, bacteria, nematodes, fungi, pheromone, molecules…)**
Maturing patent



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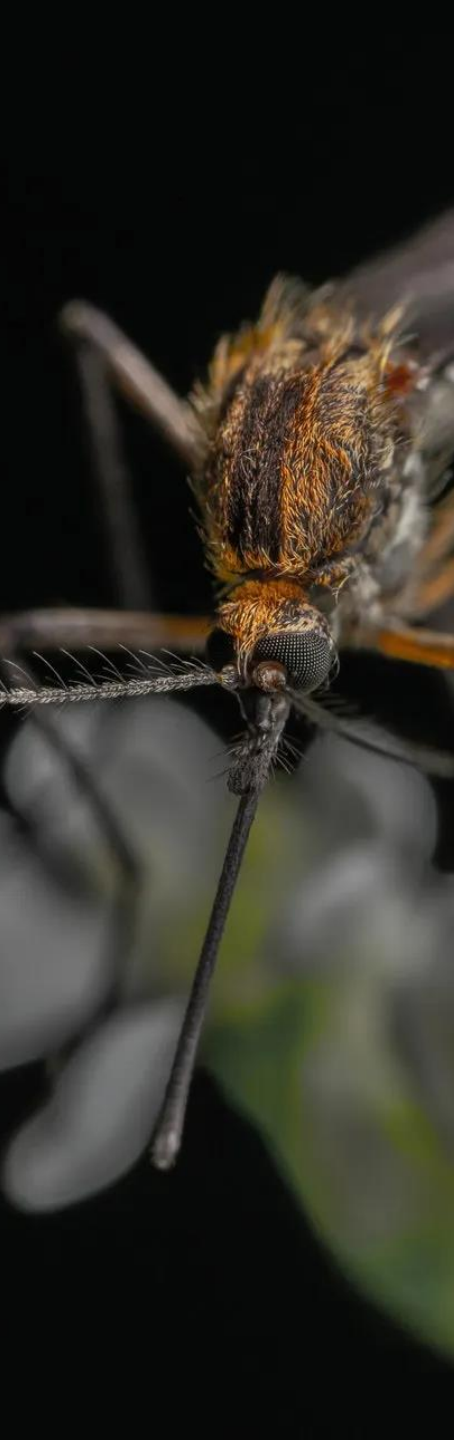
These formulations aim to:

- **Control the release** of the biological control agent
- **Protect** it against environmental conditions (UV, T°C...)
- **Optimize the use** of the virus by adding potentiating agents



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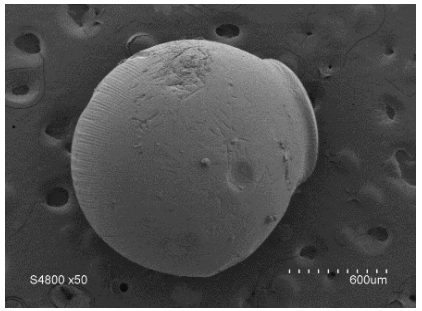
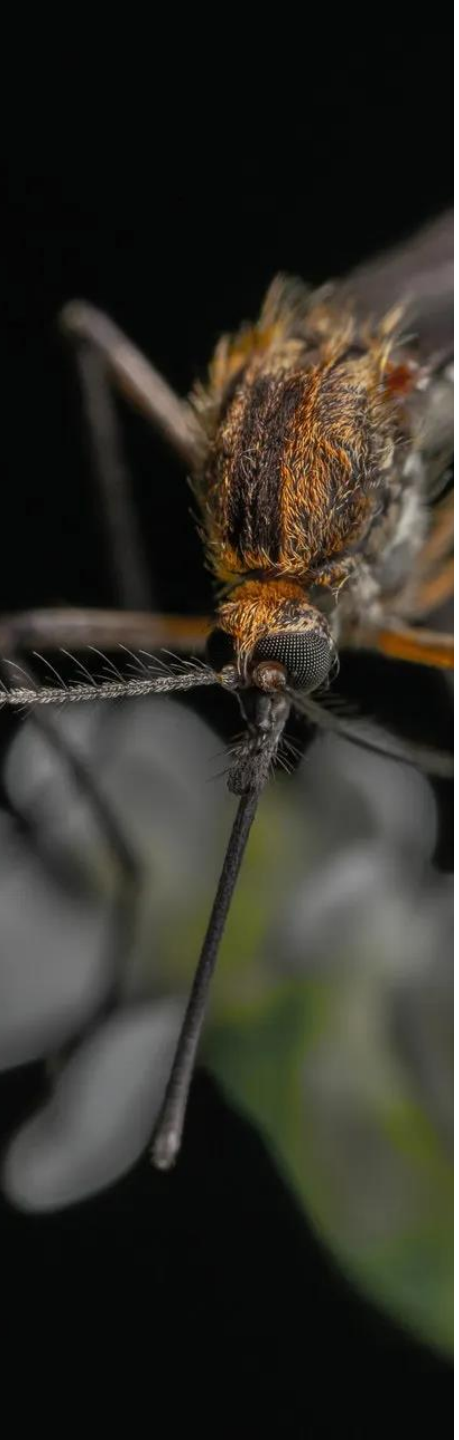
**<u>Our formulations :</u>**

- Size between 100µm to 5mm
- Wet or dry formulation
- Adapte to the strategy : boosted SIT context or directly use in the breeding sites





# To conclude

**Densoviruses as tools for biological control of mosquito vectors: Why not…but :**

**We need :**

- More knowledges about efficiency and host range
- Test the infectivity in the field
- More strains to avoid the use of a single strain (resistance)
- A high level of production compatible with field use and competitive with synthetic products

# Thank you !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