Could Viral Co-infection of Mosquitoes Impact Vector Control?

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Importance of Vector Control

- Mosquito-borne disease responsible for several million deaths worldwide
- Hundreds of million of cases of illnesses
- Vectors of pathogens that cause human and veterinary illnesses
- Example of viruses and diseases spread by mosquitoes
  - Zika virus (ZIKV)
  - La Crosse virus (LACV)
  - West Nile virus (WNV)
  - Dengue virus (DENV)
  - *Plasmodium falciparum, P. vivax, P. ovale* (species that cause malaria)

Current Vector Control Strategies

- Integrated Mosquito Management –AMCA
  - Surveillance, mapping & rational setting of action thresholds
  - Physical control through manipulation of mosquito habitat
  - Larval source reduction and adult mosquito control
  - Monitoring for insecticide efficacy and resistance

Vector Control Strategies cont..

- WHO recommends Integrated Vector Management (IVM):
  - Advocacy, social mobilization, regulatory control for public health and empowerment of communities.
  - Collaboration within the health sector and with other sectors through the optimal use of resources, planning, monitoring and decision-making.
  - Integration of non-chemical and chemical vector control methods, and integration with other disease control measures.
  - Evidence-based decision making guided by operational research and entomological and epidemiological surveillance and evaluation.
  - Development of adequate human resources, training and career structures at national and local level to promote capacity building and manage IVM programs

Current Trends in Arboviruses

- Global re-emergence of arboviruses
  - ZIKV - 2016 Florida, Texas
  - CHIKV - 2014 Florida
  - WNV - 1999 New York
  - DENV - 2009 Florida (5% of Population)
  - DENV - 2005 Texas
  - YFV (Yellow Fever virus) - 2016 South America
- Emergence has become rapid and geographically extensive

Arbovirus Trends cont..

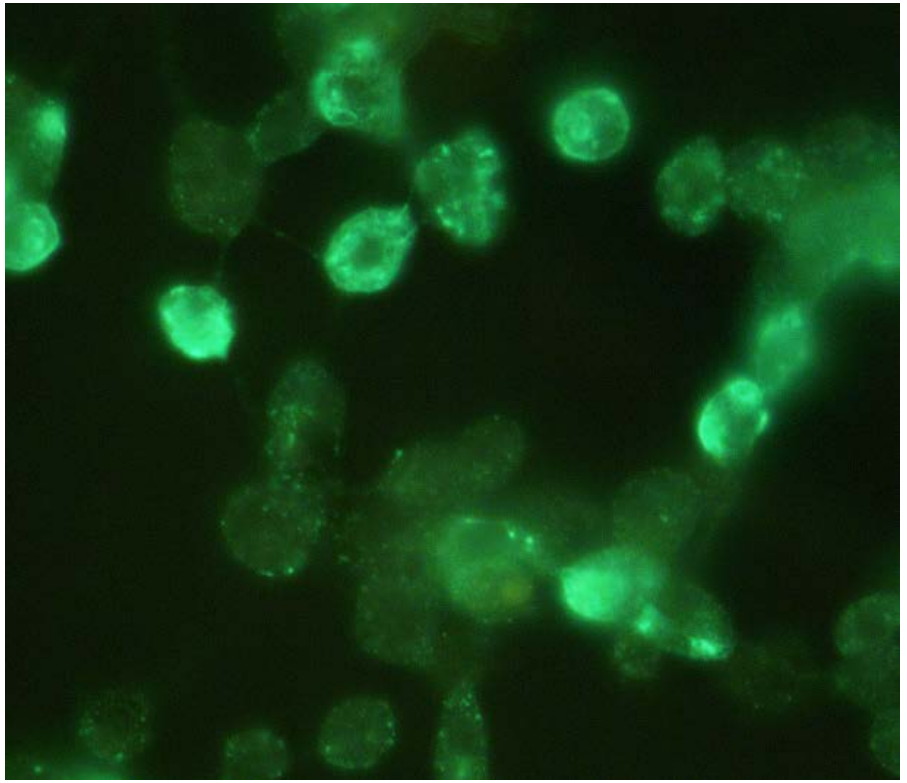
- Viral Co-infections
- Arboviruses found co-circulating in same geographic areas
- Clinical manifestations may be similar
  - Difficulty in telling difference between infections
  - Only 1 virus' symptoms may be recognized
- Human cases of co-infection reported
  - January 2014, New Caledonia, 2 patients tested positive for ZIKV/DENV
  - 2016, Haiti, 1 patient tested positive for ZIKV/DENV

Could One Virus be Used to Suppress Another?

- Mosquito specific viruses
- Birnaviruses found in mosquitoes
- Birnaviridae family
  - CYV - *Culex* Y virus – isolated from *Culex pipiens*
  - ESV – Espirito Santo virus – isolated from biological sample in Espirito Santo Brazil

Espirito Santo Virus (ESV)

- Entomobirnavirus
- ESV, collected from Espirito Santo, Brazil
- Newly observed virus found in C6/36 mosquito (*Aedes albopictus*) cells
- Currently under study to learn about viral interactions within mosquito

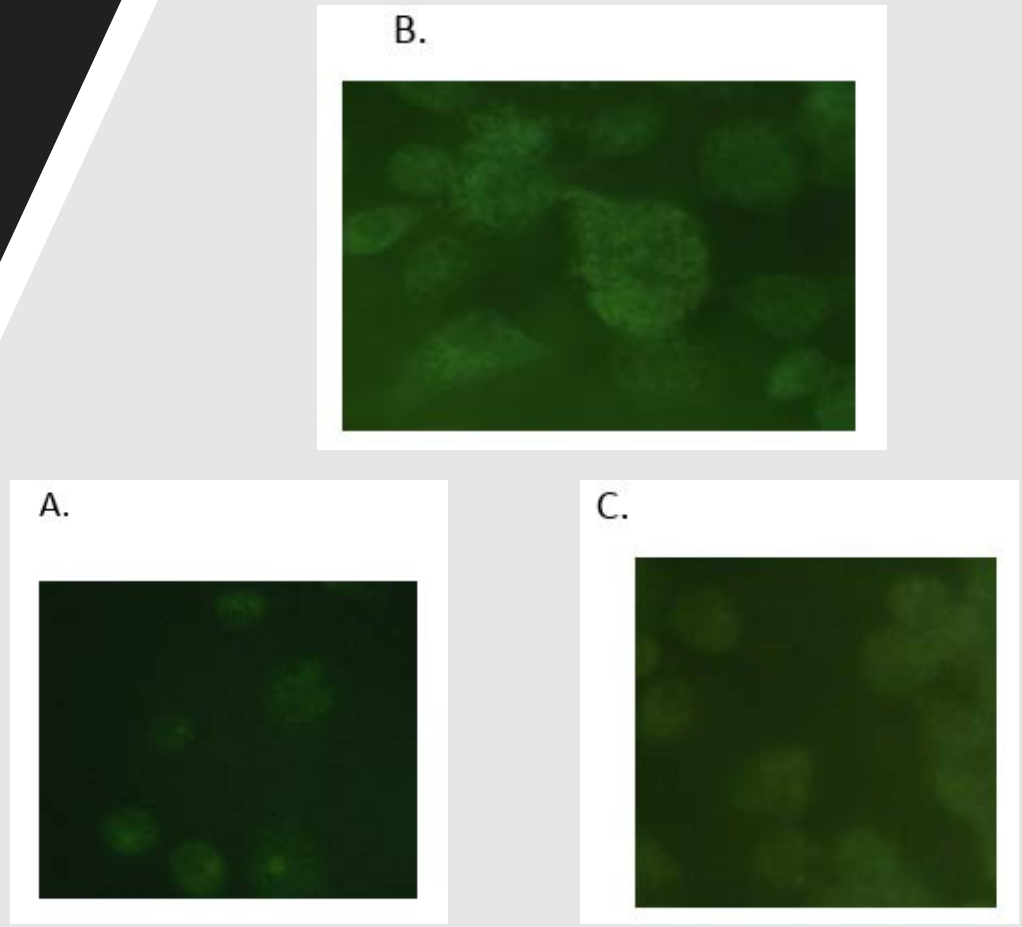


Preliminary and Future testing

- ESV shown to grow in C6/36 insect cells infected with DENV-2
- ESV was not shown to infect mammalian cells
- Studies underway observing effects and differences between co infection of DENV-2 with different levels of virulence
  - C6/36 *Ae. albopictus* cell line
  - Impacts on different mosquito species
    - *Aedes aegypti*
    - *Aedes albopictus*

Immunofluorescence Assay of ESV/DENV Co-infection in C6/36 cells

- A. uninfected C6/36 cells
- B. C6/36 cells infected with dengue virus added to cells after 48 hours
  - Brighter fluorescence due to recognition of dengue virus
  - Appearance of cell structures e.g. endoplasmic reticulum
- C. C6/36 cells co-infected with ESV and dengue virus
  - Less pronounced fluorescence
  - ESV appears to suppress dengue



Preliminary ESV Co-infection in adult Mosquitoes

- Infected with ESV as larvae
- Adult mosquitoes fed DENV-2 blood meal
- Tested at 14 dpi

ESV	**Aedes aegypti** Anna Marie Island, FL	**Aedes albopictus** Lab Colony, LA
Infection Rate (%)	57%	75%
Virus Titer (PFUeq/mL)	2.1 ± 0.7	5.8 ± 0.3
Dissemination Rate (%)	0	33
Non-ESV		
Infection Rate	80%	87%
Virus Titer (PFUeq/mL)	1.5 ± 0.4	6.0 ± 0.7
Dissemination Rate (%)	0	67

Indications of Preliminary Studies

- More in depth studies needed to understand relationship between ESV and DENV
- Effects of different viral concentrations on each virus
- Effects of virus co-infections on mosquitoes of different mosquito species
- Possibilities of inducing antagonistic effects in viruses
- Ability to promote one virus over another

Future Implications

- Using mosquitoes infected with ‘harmless’ viruses to help with current vector control strategies
- *Wolbachia* infected mosquitoes
    - Studies using *Wolbachia* infected mosquitoes to induce pathogen interference and inhibit growth of pathogens such as Malaria
- Insect specific Flavivirus e.g. Palm Creek virus (PCV)
    - Palm Creek virus (Palm Creek Australia) initial studies showed prior infection of mosquito cells, suppressed replication of medically significant West Nile and Murray virus encephalitis
- Oxitec mosquitoes
- Genetically engineered mosquitoes to help with vector control

Questions?