Pathogen detection in mosquito excreta for improved surveillance

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HOT NORTH Improving Health Outcomes in the Tropical North
menzies school of health research

Overview

- our team
- mosquitoes & diseases
- mosquito-borne disease surveillance
- lab component & preliminary results
- field component
- future work

Our team

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Photo: Jim Gathany

Photos: Stephen Doggett

Mosquitoes

- ~ 3,500 species globally
- ~ 300 species in Australia

Species of medical importance

Worldwide

- *Anopheles* spp.: malaria
- *Aedes aegypti*: dengue, Zika, Chikungunya, Yellow fever

Australia

- *Culex annulirostris*: Japanese encephalitis, Ross River fever, Murray Valley encephalitis
- *Aedes aegypti*: dengue

Mosquito-borne diseases

- ~ 700 million infections/year
- > 1 million death per year
- currently limited vaccines available

Mosquito-borne disease surveillance

- to monitor mosquito vector populations
- to establish arbovirus/pathogen activity
- to detect virus/pathogen activity before an outbreak occurs

Methods

- sentinel animals
- mosquito collections, pooled by species

Saliva of infected mosquitoes & FTA® cards

- FTA® cards are filter cards (nucleic acid preservation cards) which deactivate viruses but preserve RNA
- cards are coated in honey & placed in mosquito traps
- mosquitoes taking a honey-meal expel saliva
- saliva used for disease detection by eluting viral RNA followed by PCR

FTA® cards

Exploiting mosquito sugar feeding to detect mosquito-borne pathogens

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MVEV
RRV
BFV
WNV_{KUN}
Edge Hill virus
Stratford virus

VECTOR/PATHOGEN/HOST INTERACTION, TRANSMISSION

Expectoration of Flaviviruses During Sugar Feeding by Mosquitoes (Diptera: Culicidae)

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VECTOR-BORNE DISEASES, SURVEILLANCE, PREVENTION

A Simple Non-Powered Passive Trap for the Collection of Mosquitoes for Arbovirus Surveillance

SCOTT A. RITCHIE,[1,2,3] GILES CORTIS,[4] CHRISTOPHER PATON,[1] MICHAEL TOWNSEND,[1] DONALD SHROYER,[5] PAUL ZBOROWSKI,[1] SONJA HALL-MENDELIN,[6] AND ANDREW F. VAN DEN HURK[6]

FTA® cards

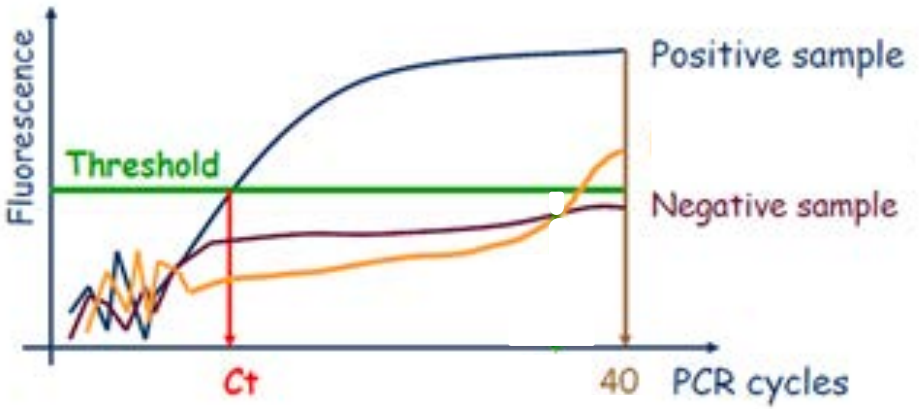
Advantages:

- no cold chain required
- viral RNA preserved at least 28 days
- greater sensitivity than sentinel animals
- less expensive than processing pools of mosquitoes

Photo: Paul Zborowski

Limitations:

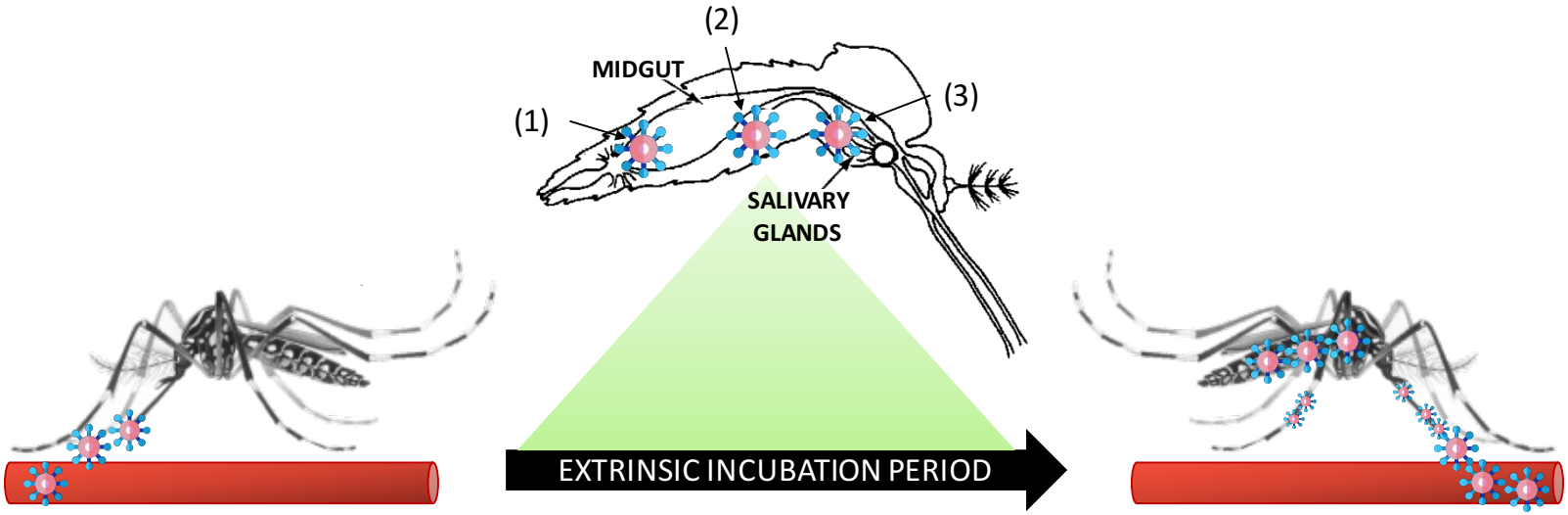
- mosquitoes only expel small quantities of saliva (~4.7 nl) and virus
- therefore C*t* values obtained by RT-PCR can be high (>35 cycles)



Cycle threshold (C*t*) values

C*t* values = the number of cycles needed for a fluorescent signal to cross the threshold

- positive detection only **after the extrinsic incubation period (EIP)**

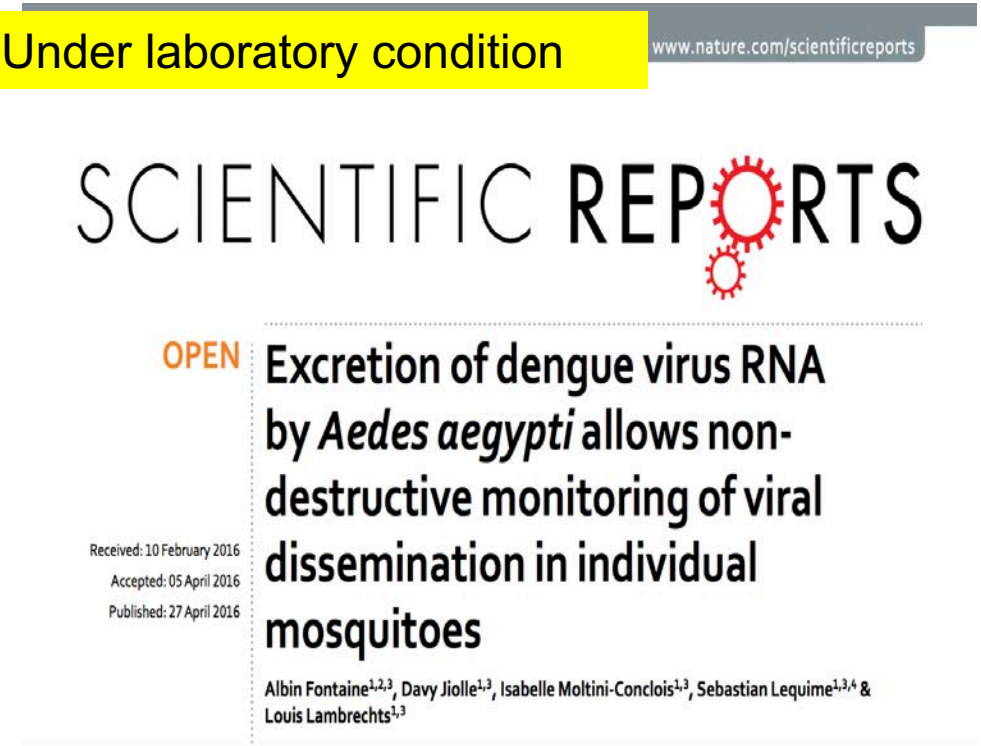


…from 2 to 14 days

EIP = The interval between the acquisition of an infectious agent by a vector and the vector's ability to transmit the agent to other susceptible vertebrate hosts.

NEW: USING EXCRETA INSTEAD OF SALIVA

Under laboratory condition



Results

Saliva

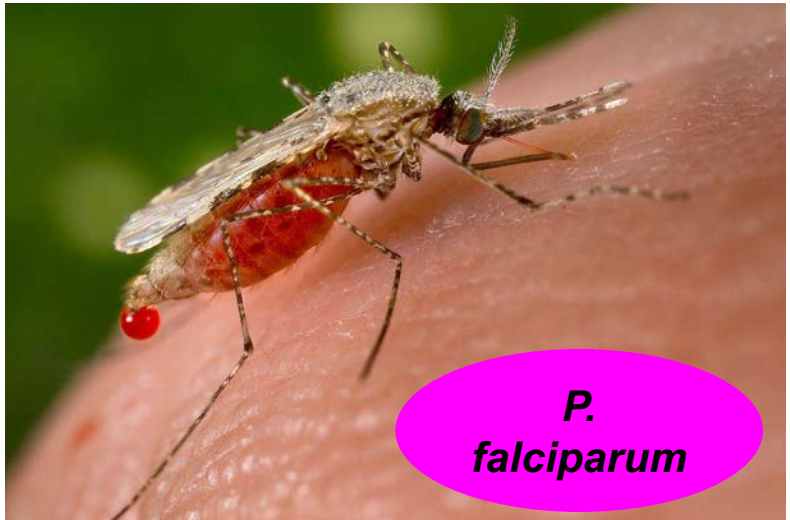
Sensitivity: 33%

Excreta

Sensitivity: 89%

Disease detection with excreta

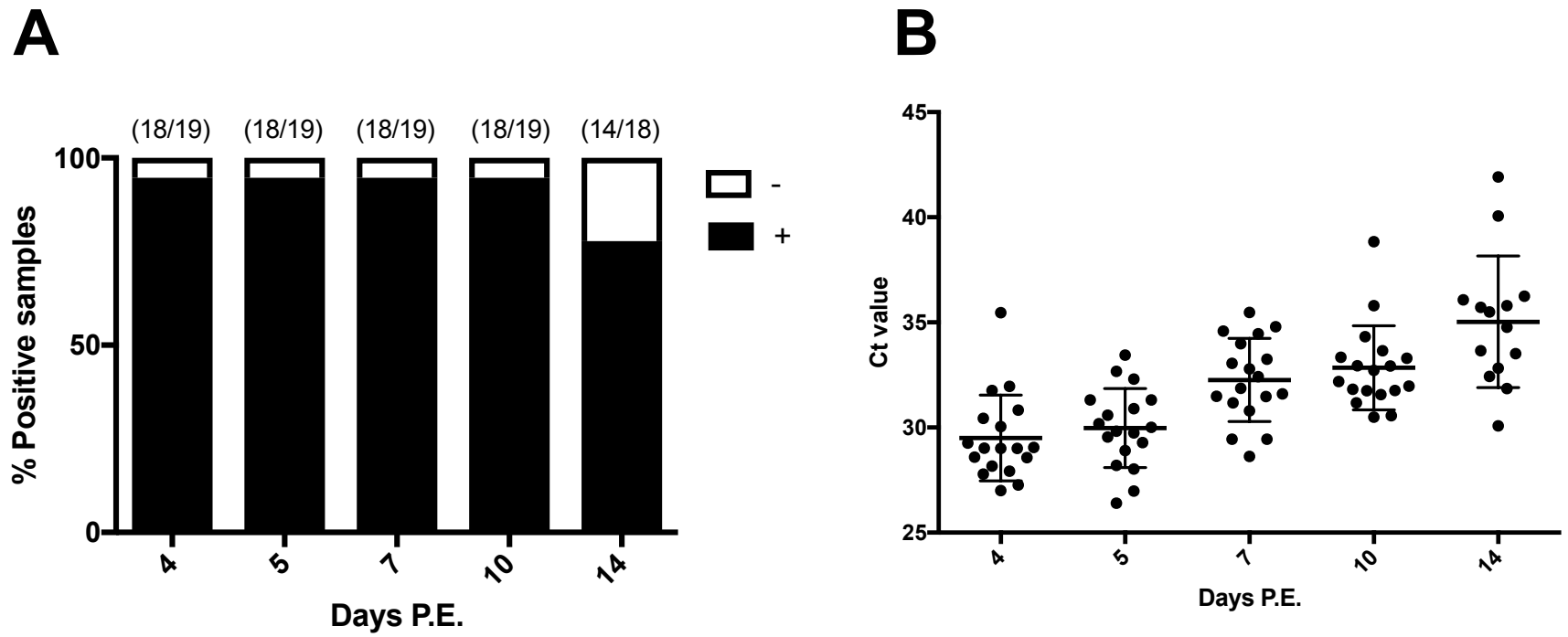
- can these pathogens be detected?
- how long after exposure to infectious blood-meals?



Photos: Stephen Doggett

Preliminary results RRV

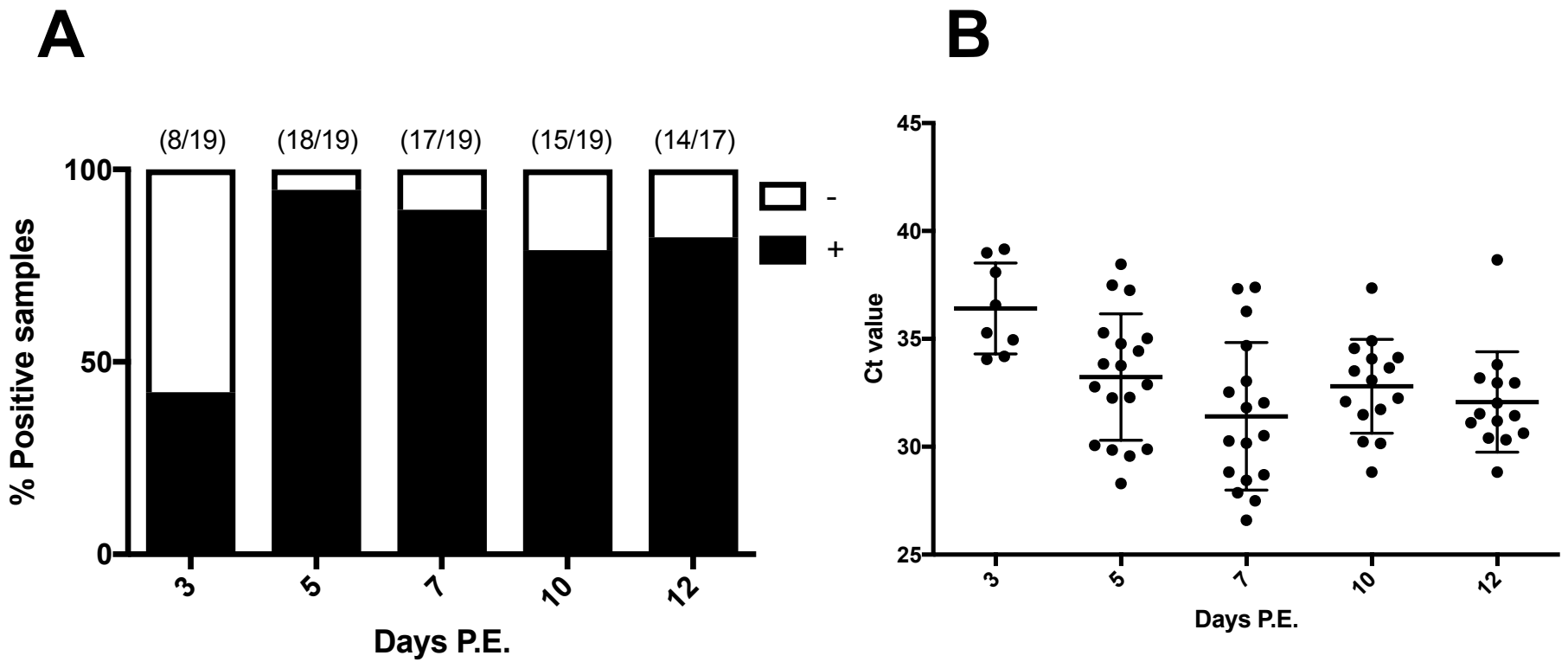
Ae.vigilax infected excreta detected by qRT-PCR



(A) Proportion of positive samples. (B) Mean C*t* value (±SD) for positive samples.

Preliminary results WNV_{KUN}

Cx. annulirostris infected excreta detected by qRT-PCR

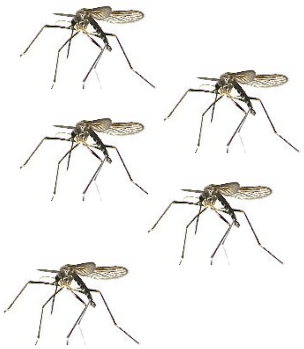


(A) Proportion of positive samples. (B) Mean C*t* value (±SD) for positive samples.

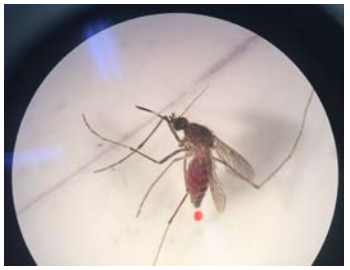
Modifying traps for mosquito excreta collection

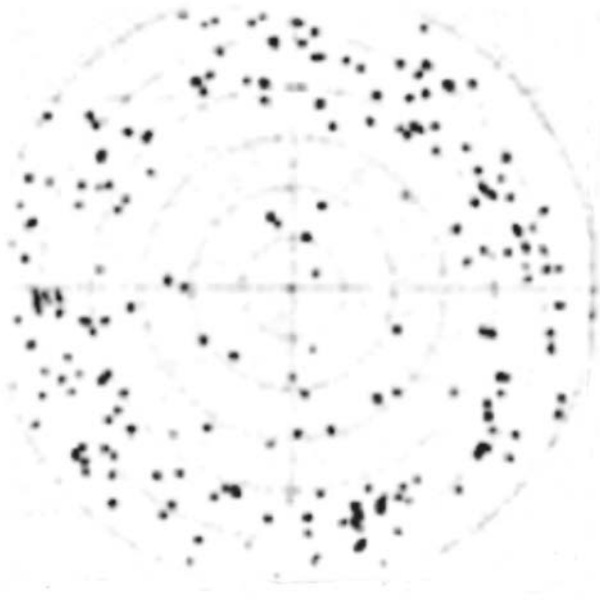
Need to know:

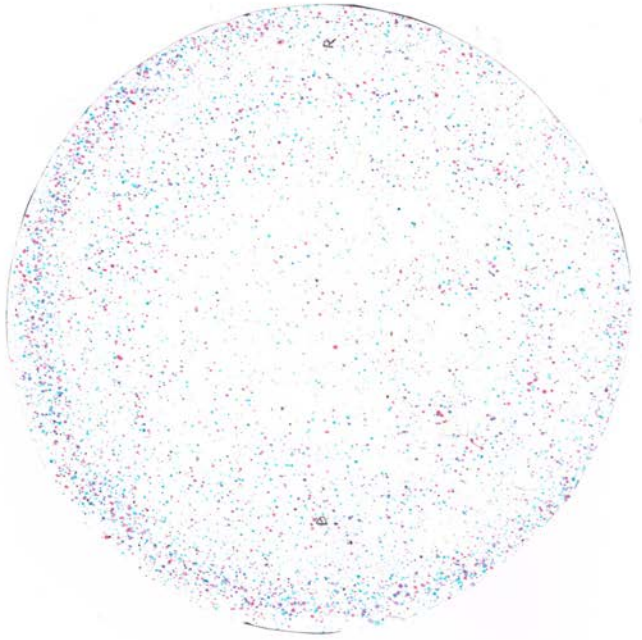
- where in a trap do mosquitoes mainly defecate
- what type of substrate is most suitable to collect excreta
- which feeding substrate & honey concentration causes mosquitoes to feed and defecate the most
- which substrate causes the least mortality



Location of defecation







- from lab experiments: 10 *Ae. aegypti* (container ⌀ 64 mm)
- from field sampling: 1245 individuals (trap ⌀ 180 mm)

Substrate for excreta collection

Parafilm

BBQ liner

Baking paper

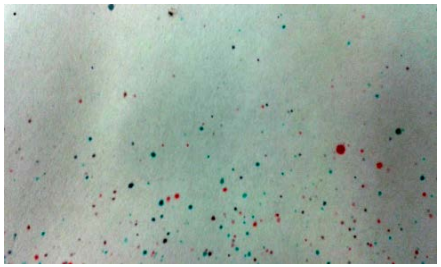
Builders sheet

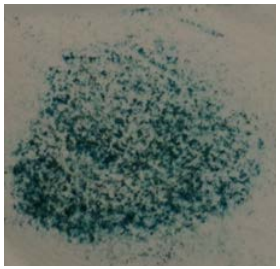
Plastic dividers

Plastic letter files

Result

	easy to remove	observations
Parafilm	✗	sticky, unsuitable for large surface
BBQ liner	✓	black residue, expensive
Baking paper	✗	absorbs excreta, warps/twists, moves
Builders sheet	✗	sticky
Plastic dividers	✓	
Plastic letter files	✓	

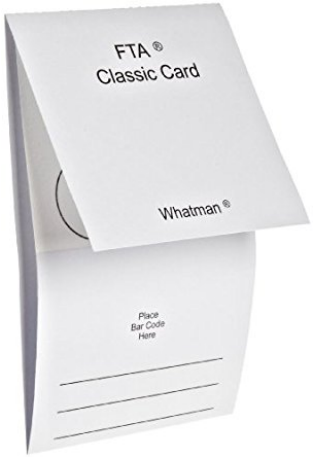




FTA-card

Feeding substrate & honey concentrations to increase defecation

FTA card



thin filter paper

thick filter paper

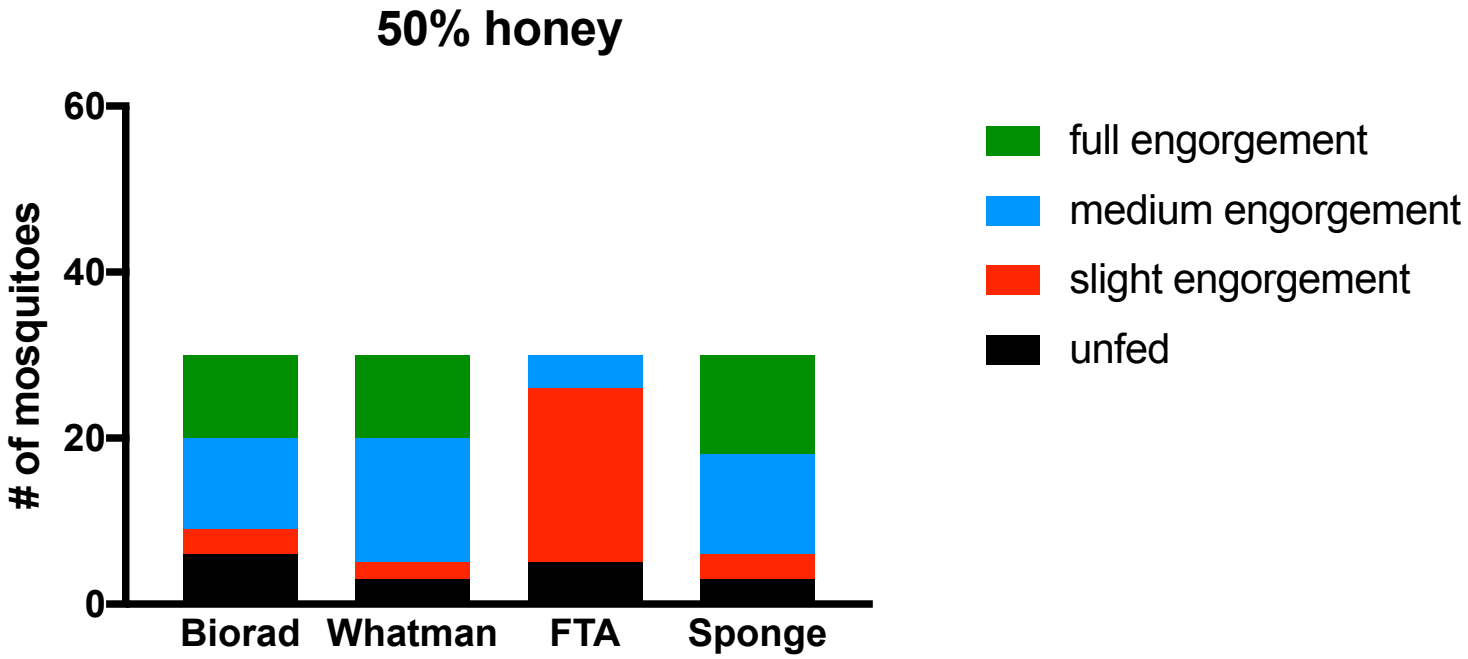
sponge

50% & 100%

Mortality

Honey	BIORAD	WHATMAN	FTA	SPONGE
100%	0	0	10/30	1/30
50%	0	1/30	15/30	0

Engorgement



Amount of excreta

laboratory *Ae. aegypti*

Honey	FTA	BIORAD	WHATMAN	SPONGE
100%	7	10	6	9
50%	5	20	12	19

wild mosquitoes

Species	Excreta counts*
Ae. kochi	21
Ae. vigilax	32
An. farauti	15
Cq. nr.crassipes	8
Cx. annulirostris	23

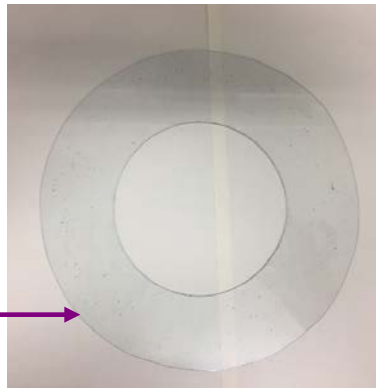
*average counts for one individual

- sponge feeding substrate with 50% honey

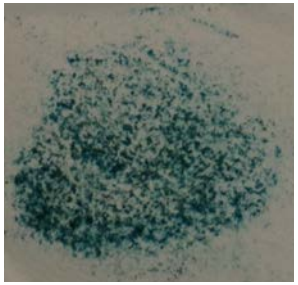
Trap modifications: to collect field mosquitoes & their excreta

CDC light trap

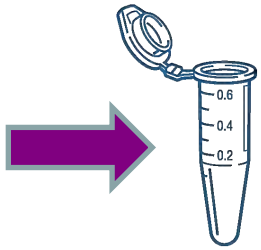
Centers for Disease Control



RNA will be extracted and tested by TaqMan qRT-PCR



FTA card



Trap modifications……

to be continued……..

Passive Box trap

BG Sentinel trap

Collapsible Passive trap

We still need to evaluate:

- if excreta is infectious
- the duration excreta can be left in the field for long term surveillance
- field validation Cairns, Darwin & Brisbane

Acknowledgements

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Stephen Doggett

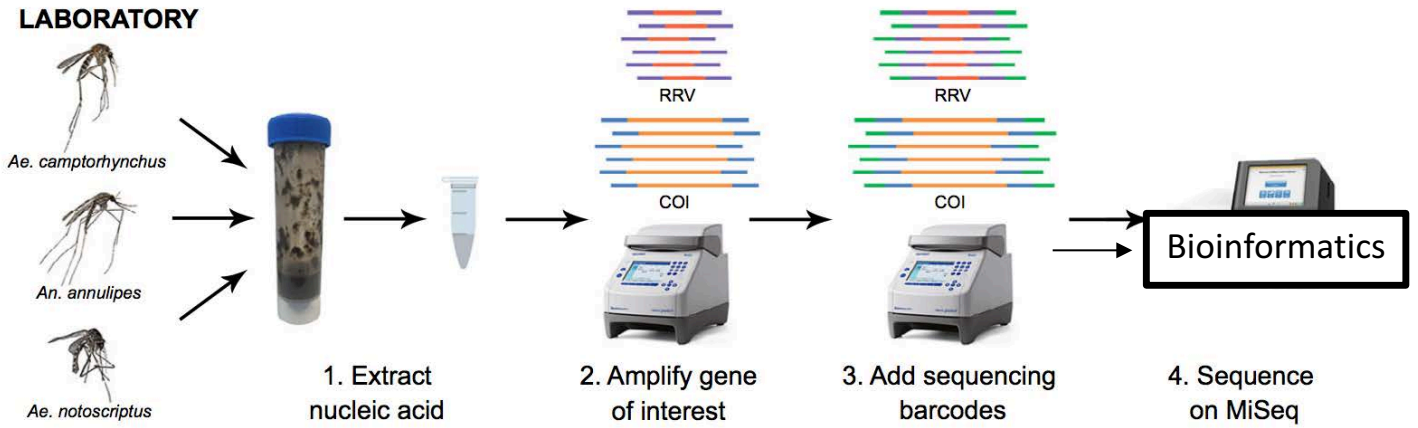
Funding

HOT NORTH ECR Fellowship to Dagmar Meyer

METHODS

3. Next-generation sequencing on mosquito excreta

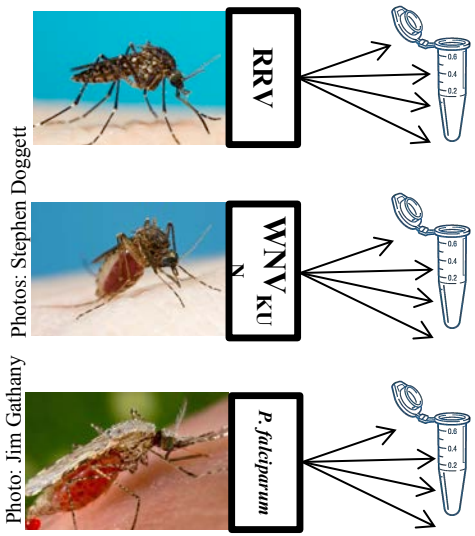
Metabarcoding enables the ID of multiple species and pathogens from sample pools by PCR amplification followed by NGS



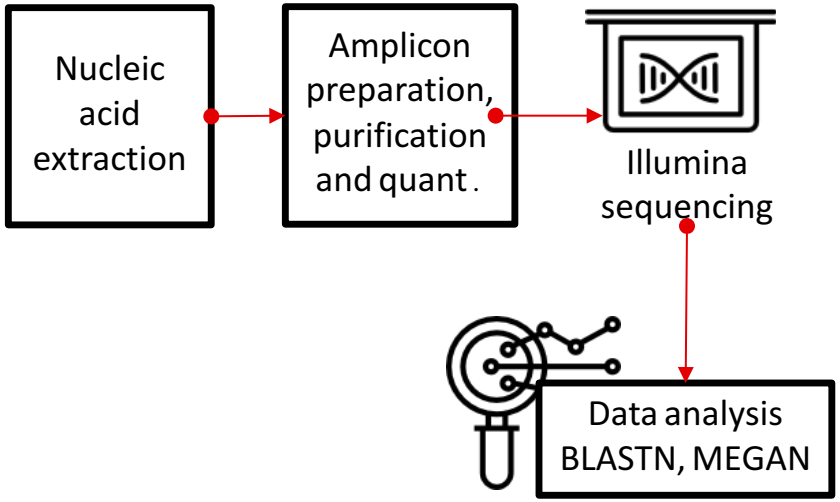
Batovska et al. 2017

…an effective and highly sensitive alternative which in the future could be integrated into surveillance programes.

Based on results from previous experiments…



30 samples



MOSQUITO SPECIES
ARBOVIRUSES
PARASITES
BACTERIA

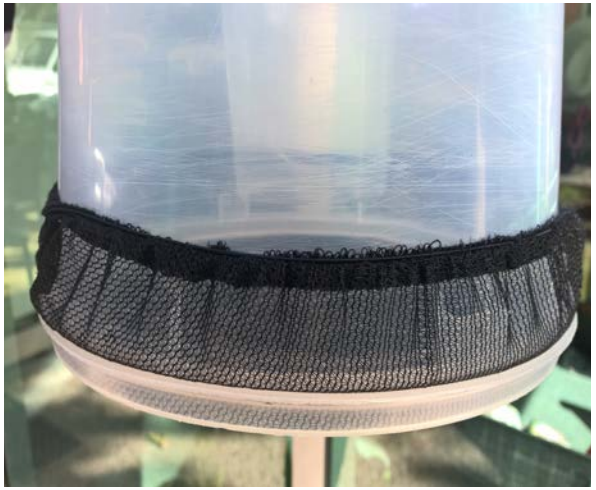
?

Batovska et al. 2017
Hall-Mendelin et al. 2013

1. Next generation sequencing on mosquito excreta

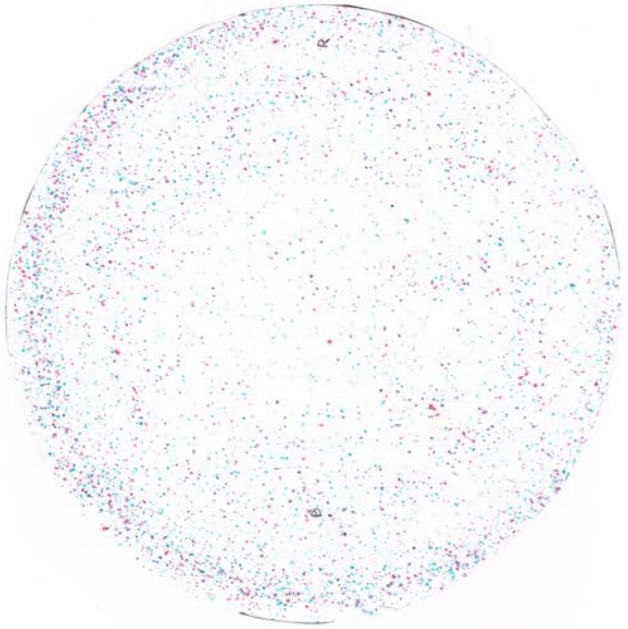
Based on the results obtained in the previous experiments, 10 pooled samples from *Ae. vigilax* infected with RRV, *Cx. annulirostris* infected with WNV_{KUN} and *An. stephensi* infected with *P. falciparum* will be prepared for a total 30 samples. Nucleic acid (including both DNA and RNA) will be extracted from each pool. Amplicons will be prepared, purified and quantified. Samples will be sequenced on an Illumina platform and reads will be quality trimmed. Data will be analysed using bioinformatics packages such as BLASTN and MEGAN[37, 38]. To obtain optimal results, factors such as nucleic acid extraction and analytical thresholds will be evaluated. All the experiments and analysis will be conducted under the guidance of D. Warrilow at QHFSS.

protecting substrate with mesh



Location of defecation: from field sampling

Modified CDC trap



- sampling in mangroves
- Whatman #1 filter paper with 50% honey
- total excreta spots (8109) / wild mosquitoes (1245 individuals)
  = ~7 spots/mosquito