A Centre for Infectious Diseases and Microbiology - Public Health (CIDM-PH), and Marie Bashir Institute for Infectious Diseases & Biosecurity (MBI) publication

The Broad Street Pump

What can Australia learn from the introduction of chikungunya virus to North America?

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The emergence of mosquito-borne pathogens in new geographic regions is of great concern. While debate has surrounded the role of a changing climate in the potential spread of mosquitoes and mosquito-borne pathogens¹, there is little doubt that increasing globalisation, especially cheap, rapid international travel, provides an easily exploitable route for pathogens.

Chikungunya virus (CHIKV) is a mosquito-borne virus that causes potentially severe illness and has been identified as an emerging pathogen of concern². While the burden of disease caused by CHIKV may be less substantial to that posed by dengue viruses (DENV) internationally, outbreaks have occurred in many parts of the world. Until recently, only imported cases had been reported from the Western Hemisphere but since the first reports of CHIKV detection among residents of the French side of St. Martin in the Caribbean surfaced in December 2013, there have been over 570,000 cases in Caribbean, Central America and South America.^{3,4}

International travel, not a changing climate triggered this outbreak. There was already the mosquitoes present in the region capable of transmitting CHIKV, all that was needed for an outbreak to occur was the introduction of the virus. Infected travellers provided the spark to ignite the outbreak.





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What can Australia learn from the introduction of chikungunya virus to North America? (continued from page 1)

With the current outbreak in the Americas, the number of infected While some endemic mosquito species have been shown to transtravellers visiting or returning to North America is dramatically mit CHIKV in the laboratory 15, it is still Ae. aegypti and Ae. albopicincreasing. In the eight years to 2013, an average of 28 people per tus that pose the greatest risks. Aedes albopictus, given the potenyear infected with CHIKV entered the U.S. while in the first eight tial establishment in some of Australia's major metropolitan remonths of 2014, there have been over 600 imported cases.⁵ gions is a major concern. While authorities are being warned to Where the presence of suitable vectors of CHIKV are present, and consider the risks of urban mosquito-human-mosquito or enzohigh volumes of travellers from regions of endemic/epidemic otic transmission cycles of CHIKV in the Western hemisphere¹⁶, CHIKV activity overlap, the risks of local outbreaks increase.⁶

There are only two mosquito species associated with the spread of may also play a role in the transmission of endemic mosquito-CHIKV in North America, the container-inhabiting Aedes aegypti borne viruses including Ross River virus. 17 and Aedes albopictus. Aedes aegypti has been responsible for locally acquired cases of dengue in some southern regions of North America. However, this mosquito is limited in its distribution. Aedes albopictus, on the other hand, has a greater distribution; being more tolerant of temperate climates. Aedes albopictus has been responsible for some outbreaks of chikungunya virus elsewhere in the world and has been identified as a potential driving factor in the spread of dengue activity internationally.8 Could Ae. albopictus drive an explosive outbreak of CHIKV in North America? Fortunately, the scenario looks unlikely. Genetic studies have indicated that the current strain of CHIKV circulating in the Western Hemisphere is less prone to explosive outbreaks of disease driven by Ae. albopictus. 9 However, with these two mosquito species present in areas where infected travellers have the potential to introduce the virus, there is the potential for local cases. So far, there have been a number of locally acquired cases of CHIKV in Florida. 10

What does this mean for Australia?

We are fortunate in that only one mosquito species, Ae. aegypti, effective at transmitting CHIKV is present in mainland Australia and the distribution of this species is limited to Far North Queensland. However, Ae. albopictus has been detected in the Torres Strait and it is predicted that this species, should it be introduced into mainland Australia, could establish itself in major metropolitan regions. 11,12,13 The introduction, spread and establishment of Ae. albopictus may predispose many metropolitan regions in Australia to a risk of CHIKV. There is already evidence that Australian travellers infected with CHIKV and DENV are returning at increasing rates. 14 Could these travellers introduce Ae. albopictus to mainland Australia? Could infected travellers kick off clusters of locally acquired cases of CHIKV in Australia?

should Australian authorities be planning similarly? Notwithstanding the potential role of this mosquito in CHIKV transmission, it

It is critical that Australian authorities consider how best to undertake surveillance and control of Ae. albopictus. In North America, authorities already had strategies in place to address the risks posed by this mosquito. However, for the majority of Australian metropolitan regions, management of endemic and exotic container-inhabiting mosquitoes is in its infancy. While strategic plans are in place for Far North Queensland where occasional outbreaks of Ae.aegypti driven locally acquired dengue occur, these plans may not be easily transferrable to more heavily populated southern cities. Health authorities and local governments in SE Queensland are mindful of the risk but what about NSW, Victoria and WA? Perhaps authorities elsewhere should be mindful of the need to respond should Ae. albopictus "sneak in" through the back door of southern metropolitan cities.



Dr Cameron E Webb Email: cameron.webb@health.nsw.gov.au Follow Cameron on Twitter: @mozziebites

What can Australia learn from the introduction of chikungunya virus to North America? (continued from page 2)

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An assessment of new approaches to arbovirus surveillance in NSW

Cheryl S Toi¹, John Haniotis¹, Cameron E Webb^{1,2}, John Clancy¹ and Stephen L Doggett¹ ¹Department of Medical Entomology, CIDMLS, Pathology West – ICPMR Westmead, New South Wales ²Marie Bashir Institute for Infectious Diseases and MicrobiologyUniversity of Sydney, New South Wales

Until recently, the arbovirus surveillance in NSW has relied on cell culture of homogenates from mosquitoes with definitive identification by FC-ELISA. While a reliable method, the turnaround time is relatively slow. A new approach to arbovirus surveillance has been proposed to reduce laboratory turnaround times. Rather than testing the mosquitoes, nucleic acid preservation cards (FTA), onto which trapped mosquitoes may have expectorated virus, are tested using molecular assays. To complement this laboratory method has been newly developed "passive traps" that allow for longer deployment in the field than the traditional "once per week" approach using EVS traps. During the 2012-2014 surveillance period, we compared the two approaches to both mosquito collection and arbovirus detection. Preliminary investigations indicated that the use of FTA cards in EVS traps deployed for a single night per week provided as efficient approach to arbovirus detection. Ross River and Barmah Forest viruses were all detected from FTA cards. Further work is required to ensure appropriate quality assurance of molecular methods and to determine the implications of new approaches as an early warning system for the issue of public health messages.

11th Mosquito Control Association of Australia Conference, Mandurah, Western Australia, 7-10 September 2014

Medical Entomology in Australia, 1964—2014

Stephen L Doggett

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Australia has had a rich history in the field of Medical Entomology, in both practical and applied research, particularly over the last 50 years, which has placed the nation on the world stage. Innovations in taxonomy has seen the change from the classical taxonomists, including Marks, Dobrotworsky and Lee (mosquitoes), Kettle, Dyce and Debenham (Ceratopogonidae) and ticks (Roberts), to the development of proteomics (including Bryan, Sweeney and Foley on mosquitoes), to modern molecular based taxonomy (Beebe again on mosquitoes). The emphasis of research over the last 50 years in Medical Entomology has focused on mosquitoes due to their nuisance biting and propensity to transmit infectious agents. This includes the mapping of malaria vectors (Sweeney and colleagues), mosquito biology (notably Russell & Kay), investigations into mosquito borne disease, surveillance and vector competence (most notably Marshal, Mackenzie & colleagues, Cloonan, Russell & colleagues, and van den Hurk), saltmarsh mosquito management (Dale and Easton in runnelling, Whelan and Muller in practical control), constructed wetlands design to minimise vectors (Russell and Webb), repellents (Frances), and to modelling (Williams). In more recent years, many of the research initiatives relating to Dengue and the control of its main vector, Aedes aegypti, have been led by Ritchie and colleagues, with the development of such innovations as lethal and biodegradable ovitraps, and new surveillance technology. More recently, Gates Foundation money has been provided to Scott and colleagues to investigate the use of the intracellular bacteria, Wolbachia, to minimise Dengue transmission. Outside of mosquitoes, researchers such as Tovey have investigated dust mites, while various workers have examined ticks in their impact upon human morbidity (Graves on rickettsia, Russell et al. on Lyme disease, van Nunen on mammalian meat allergies). Geary has led the way in providing a world class pathology service and was the first to employ disinfected maggots for wound debridement. Finally, I have proudly worked with a team who were the first in the world to develop an industry standard to combat the global bed bug resurgence. This presentation will focus on the Medical Entomology researchers, their research and achievements, during the 50 year history of the Australian Entomological Society.

Annual Conference of the Australian Entomological Society, Canberra, ACT, 28 September – 1 October 2014



Stephen Doggett (Medical
Entomology, Pathology West –
ICPMR Westmead) and Prof Chow
-Yang Lee (Urban Entomology
Laboratory, Universiti Sains
Malaysia) discuss new strategies
for the management of bed bugs
at the 8th International
Conference on Urban Pests,
Zurich, Switzerland.



Dr James Logan (The London School of Hygiene & Tropical Medicine), David Lilly (University of Sydney) and Frances McKim (Pest Magazine) inside the converted WWII bunkers housing the School's mosquito colonies.

Implications of insecticide resistance in Cimex lectularius (Hemiptera: Cimicidae), in Australia

David G. Lilly¹, Myron P. Zalucki², Christopher J. Orton³, Richard C. Russell¹, Cameron E. Webb¹ and Stephen L. Doggett¹

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Insecticide resistance in bed bugs (*Cimex lectularius* L. and *C. hemipterus* F.) has been nominated as a major factor in the pest's resurgence. Various studies across Europe, Asia and North America have demonstrated resistance to the pyrethroids, carbamates and, to a lesser extent, the organophosphates. Resistance has been suspected in Australia, with anecdotal reports of poor product performance. Laboratory studies applying insecticides via topical application have demonstrated insecticide resistance in a previously suspected-resistant field strain of bed bugs, with substantial differences in LD_{50} values when compared to a susceptible strain. The resistance factors for each compound were: permethrin = 1.4 million, deltamethrin = 430,000, bendiocarb = 240, pirimiphos-methyl = 2.8, imidacloprid = 2.7. Thus resistance was confirmed in the field-collected strain with the pyrethroids and carbamates, but not the organophosphates or neonicotinoids (the small number being unrelated to resistance). With demonstrated resistance in bed bugs, professional pest management operations require the development of new strategies to combat the pest. Regulatory authorities must similarly consider the implications of resistance to multiple insecticide groups when registering new products utilizing either existing or new modes of action. One of the gaps in our current knowledge is an investigation of the drivers of this resistance and further research is required to elucidate the breadth, magnitude and mechanisms of the resistance observed.

8th International Conference on Urban Pests, Zurich, Switzerland, 20 - 23 July 2014

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Detection of knockdown resistance (kdr) in Cimex lectularius and Cimex hemipterus (Hemiptera: Cimicidae)

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Worldwide, there are many reports of pyrethroid resistance in bed bugs (Cimex spp.). Previous studies on the Common bed bug, Cimex lectularius L., have identified two mutations (V419L and L925I) in the voltage-gated sodium channel (VGSC) gene responsible for knockdown resistance (kdr). However, nothing is known on possible kdr mutations in Australian strains of C. lectularius or on the Tropical bed bug, C. hemipterus F. This study aimed to identify the status of kdr mutations in Australian C. lectularius strains, and *C. hemipterus* in Australia and international strains. Samples of C. lectularius were obtained from 24 sites across Australia, while the C. hemipterus were sourced from Australia, as well as Africa, India, Malaysia and Thailand. DNA was extracted, purified and examined for the kdr-related genes by PCR and Sanger sequencing. In C. lectularius field populations, the haplotypes A (neither V419L nor L925I), B (L925I only), and C (V419L and L925I) were found, with most (88%) of the field populations being haplotype B. A novel mutation, 1936F, was identified in an 'Adelaide' strain (initially identified as haplotype A), which may be linked with kdr-type resistance. In C. hemipterus, the V419L and L925I mutations were not detected, however, three novel mutations, M918I (Methionine to Isoleucine), D953G (Aspartic acid to Glycine) and L1014F (Leucine to Phenylalanine), were identified. Samples from Thailand have the three mutations, while samples from Australia and India have both M918I and L1014F mutations. Only the L1014F mutation was evident in the samples from Malaysia and Africa. The M918I and L1014F were assumed to be kdr mutations and contribute to the high pyrethroid resistance in *C. hemipterus*. Further studies are in process to determine the non-kdr type resistance mechanisms.

8th International Conference on Urban Pests, Zurich, Switzerland, 20 - 23 July 2014



Kai Dang (Nankai University and Medical Entomology, Pathology West – ICPMR Westmead), David Lilly (University of Sydney) and Stephen Doggett (Medical Entomology, Pathology West – ICPMR Westmead) outside the London School of Hygiene & Tropical Medicine where all three presented the findings of bed bug research as part of the School's 'Bugging you' seminars for the Public Health and Entomology Groups. Funding support from CIDM-PH enabled Kai to present the findings of his collaborative research with Medical Entomology/University of Sydney at the 8th International Conference on Urban Pests, Zurich, Switzerland.

Managing mosquitoes in urban wetlands: Strategic control and social communications

Cameron E Webb^{1,2} and Swapan Paul³

Mosquito management programs in urban wetlands need to find a balance between reducing nuisance-biting and mosquito-borne disease risk with wetland conservation and rehabilitation. Community engagement is critical. The mosquito management program at Sydney Olympic Park has been in place since 1998. The region contains extensive estuarine wetlands that provide ideal habitats for the saltmarsh mosquito, *Aedes vigilax*. The region also has a growing residential and working population as well as attracting millions of visitors each year. A coordinated treatment program associated with a carefully designed surveillance program, including timely notifications to the public and stakeholders, ensures that mosquito treatment is undertaken with minimal or no interruption to the community. Operational efficiencies have ensured that both peaks in mosquito abundance and frequency of wetland treatments have been minimised. Community engagement is a priority, not only with regard to mosquito awareness but broader wetland conservation within the region. With increasing accessibility of online resources via smartphones, local authorities must develop strategies to effectively use social media for communications. In particular, the ability to easily engage with traditional media outlets through a network of social media platforms further increases exposure beyond online communities. Examples from this region will be discussed with implications for state and national community engagement strategies.

11th Mosquito Control Association of Australia Conference, Mandurah, Western Australia, 7-10 September 2014

High phylogenetic diversity of the cat flea (*Ctenocephalidesfelis*) at two mitochondrial DNA markers

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The cat flea, Ctenocephalides felis (Siphonaptera: Pulicidae) (Bouché), is the most common flea species found on cats and dogs worldwide. We investigated the genetic identity of the cosmopolitan subspecies C. felis felis and evaluated diversity of cat fleas from Australia, Fiji, Thailand and Seychelles using mtDNA sequences from cytochrome c oxidase subunit I (cox1) and II (cox2) genes. Bothcox1 and cox2 confirmed the high phylogenetic diversity and paraphyletic origin of C. felis felis. The African subspecies C. felis strongylus (Jordan) is nested within the paraphyletic C. felis felis. The south East Asian subspecies C. felis orientis (Jordan) is monophyletic and is supported by morphology. We confirm that Australian cat fleas belong to C. felis felis and show that in Australia they form two distinct phylogenetic clades, one common with fleas from Fiji. Using a barcoding approach, we recognize two putative species within C. felis (C. felis and C. orientis). Nucleotide diversity was higher in cox1 but COX2 outperformed COX1 in amino acid diversity. COX2 amino acid sequences resolve all phylogenetic clades and provide an additional phylogenetic signal. Both cox1 and cox2 resolved identical phylogeny and are suitable for population structure studies of Ctenocephalides species.

The Australian Society for Parasitology Annual Conference, Canberra, ACT, 30 June – 3 July 2014

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Solving the common mystery of the cat flea and their role in public health risks

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Fleas infest our pets and our homes. We treat them with a variety of substances and yet they persist and occasionally bite people, causing annoyance and sometimes severe skin reactions. Although they're considered pests¹, they're often overlooked in terms of their significance to animal health, their competence as disease vectors and the impacts they make on our everyday lives. However, studies have demonstrated that companion cats and dogs can be commonly infested with fleas carrying bacterial pathogens of significance to human and animal health². There is much more to these irritating insects than meets the eye.

The most common flea encountered in Australia is the cat flea, *Ctenocephalides felis*. Despite their common name, these fleas are found on both cats and dogs. It is often assumed that if a dog has fleas, it must be due to dog fleas (*Ctenocephalides canis*). This is a common misconception. In fact, it appears as if the dog flea is something of a mythical creature in Australia. Despite historical records and anecdotal reports of dog flea infestations, there is no recent literature confirming their presence. Despite historical records and anecdotal reports of dog flea infestations, there is no recent literature confirming their presence. A recent study³ of over 2,500 pets failed to find a dog flea.

Notwithstanding the economic impact of flea control treatments on domestic animals, the cat flea also carries zoonotic pathogens such as Bartonella⁴ and Rickettsia⁵. There may be many cases of underdiagnosed febrile illnesses caused by flea-borne pathogens that fly under the radar due to the presentation of generic fever and flu-like symptoms that rarely warrant further pathological investigation. Of course, the most famous and historically significant pathogen spread by fleas is the plague bacteria, *Yersinia pestis*. Plague is certainly not a thing of the past with recent outbreaks in Madagascar and up to 17 cases reported from North America each year. Although cat fleas aren't considered a primary vector of *Y.pestis*, recent studies in Uganda have suggested that these fleas may play a role in local transmission risk⁶. Considering

the highly ubiquitous nature of fleas in human environments, and tendency of many species to be host generalists, particularly the cat flea, shouldn't we be more concerned or at least more aware of the risk factors?

Given the impact these little parasites have on our lives, it is baffling how little we know about them. The genetic profile of the cat flea is highly understudied and yet within the genetic code lies hidden implications for the evolution of insecticide resistance, disease transmission and the passage of fleas across continents and the global sphere. A study² across 5 states of Australia cat fleas collected from veterinary practices were 100% genetically identical at the mitochondrial DNA. This was a very unusual result as populations of other flea species are generally very diverse. The result was comforting news at the time for the regulation of veterinary pharmaceuticals as the efficacy of flea control products were able to be compared against flea populations across the entire country.

If fleas from Australia are genetically similar but what about elsewhere? When fleas from Australia are compared to those collected from Thailand, Fiji and Seychelles, the results showed that from a global perspective, cat fleas are genetically diverse⁷. The 2013 study yielded a novel second Australian haplotype found in north-east Australia which contradicts the unanimous results from the previous study in 2011. This haplotype was shared with most fleas tested from Fiji, suggesting some recent flea transfer between the two countries. With the rapid emergence of this second haplotype since the previous study, it sparks the question of whether there may be a division of fitness between the two haplotypes. Could this division be resulting in a steady 'invasion' of Australia by the second haplotype?

To investigate the haplotype diversity in this study we developed a novel genetic marker capable of clearly delineating different flea species, subspecies and haplotypes. Previously, genetic studies primarily used a mitochondrial DNA marker called cox2. However, there is an emerging global standard of genetic taxonomy called DNA barcoding, which uses a similar gene called cox1. This method involves storing massive amount of short DNA sequences in an electronic database, accessible to anyone with internet access. Currently the database called Barcode of Life Database or BOLD holds 3 million 'barcodes', 2 million of which are arthropod barcodes. It is surprising given the global significance fleas that the marker has not been optimised before. The 'barcodes' collected from this study are now available on BOLD and can be searched allowing greater dissemination of and accessibility to flea genetic data.

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Solving the common mystery of the cat flea and their role in public health risks

(continued from page 8)

A change in the genetic makeup of Australia's flea population as discovered recently has implications for the pharmaceutical companies who can no longer apply a blanket approach to flea control efficacy testing. It is hoped that this research may yield greater understanding of the cat flea genetic puzzle that will lead to finding the key to effective control of these tenacious blood-sucking creatures and the diseases they carry.

With recent insights into genetic differences amongst cat fleas internationally, it is critical for the development of surveillance systems to prevent flea-borne disease outbreaks, as well as flea control strategies, that the relationships between fleas, pathogens, companion animals and wildlife are understood⁸. Future studies are also underway to determine the role fleas play in public health risks in Australia. The development of rapid diagnostic tests for flea-borne pathogens will allow investigations into the role *Ct. felis* may play as a vector of zoonotic pathogens.

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UPCOMING EVENTS...

Chasing the tiger and the pursuit of innovative solutions to mosquito borne disease threats

Friday, 12 September 2014
Westmead Millenium Institute, Level 2, Conference
Room C2.20, 176 Hawkesbury Road, Westmead, Sydney
Registration: sydney.edu.au/mbi

Whole Genome Sequencing in Clinical and Public Health Microbiology Symposium

Friday, 10 October 2014

Loewenthall Auditorium, Westmead Education & Conference Centre, Westmead Hospital, Sydney Registration: sydney.edu.au/mbi

MBI Colloquium

Tuesday, 25 November 2014
Auditorium, Charles Perkins Centre, University of Sydney
Registration: sydney.edu.au/mbi

CRE In Critical Infection Colloquium

Tuesday 18 - Friday 21 November 2014 New Law School, Main Campus, University of Sydney Registration: sydney.edu.au/medicine/criticalinfection

CIDM-PH Colloquium

Friday, 5 December 2014

Lecture Theatre 2, Westmead Education & Conference
Centre, Westmead Hospital, Sydney

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Kai Dang is a PhD student currently working with the Department of Medical Entomology (CIDM-PH) as part of an internationally collaborative research project with the Institute of Entomology, Nankai University, Tianjin, China. Kai is currently undertaking molecular research into the genetic drivers of insecticide resistance in bed bugs, a major concern for local authorities in managing the impacts of these nuisance-biting insects. Previous studies on the Common bed bug, Cimex lectularius L., have identified two mutations (V419L and L925I) in the voltage-gated sodium channel (VGSC) gene responsible for knockdown resistance (kdr). However, possible kdr mutations in Australian strains of C. lectularius or on the Tropical bed bug, C. hemipterus F have not been documented.

Kai, in collaboration with Stephen Doggett and Dr Cheryl Toi (Pathology West – ICPMR Westmead) and David Lilly (University of Sydney) aim to identify the status of *kdr* mutations in Australian *C. lectularius* strains as well as local and international strains of *C. hemipterus*. Current research findings have shown that kdr mutations may be driving resistance to commonly used insecticides in local bed bug populations.

Kai grew up in south-west China. After undergraduate studies focused on plant protection science, Kai primarily studied the agricultural pest lace bugs (Family: Tingidae) for his MSc at Nankai University before turning his attention to bed bugs. His project has opened his eyes to the importance of bed bugs as urban pests and hopes to continue working on them in the future, perhaps as part of post-doctoral research. He hopes to take back what he has learned on Australia to help authorities in China raise awareness of bed bugs as a problem and develop better strategies to control these pests.

Kai has enjoyed his time at Westmead and grown to love the people, culture and environment of Australia. While many may complain of air pollution in Sydney, Kai is happy to report he much prefers it to China! Medical Entomology has enjoyed having Kai as part of its team and we wish him all the best for future endeavours after he leaves us later in the year.



CONTACT US

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