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Arboviruses

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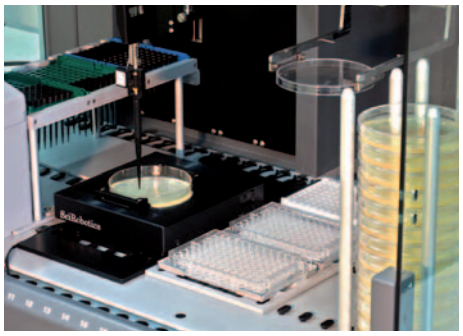

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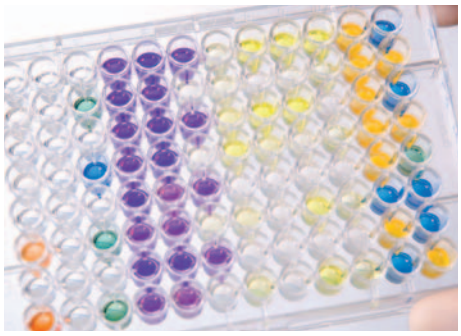

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Cover image: Female (left) and male (right) Aedes aegypti *mosquitoes (photograph by Perran Ross).*

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Roy Robins-Browne
President of ASM

Dear fellow microbiologists

I suspect that this is my last contribution to *Microbiology Australia* in my present role.

Let me start by saying that it has been a tremendous honour and a privilege to serve you as your President. In the past few years I have learned much about how our Society works and hopefully have contributed something towards its progress. Most importantly, I have worked with some truly talented people who have guided and assisted me along the way. Foremost amongst these are members of the Executive Committee: Jon Iredell (whom my voice recognition software identified as 'John Ideal'), Dena Lyras, Cheryl Power, Jack Wang, Kate Seib and Rebecca LeBard.

In July, Jon, Dena and Jack will be stepping down from their current roles and we need to thank all three for their dedication to the Society and their selfless contributions. Dena will not be leaving the Exec, however, as she is due to take over from me as President.

It is reassuring to know that ASM's future is in such good hands, together with those of Kate and Rebecca as our new Vice Presidents for Scientific Affairs and Communications, respectively,

I also want to thank all the other members of Council (our State Branch Chairs and Chairs of Standing Committees), as well as the members of these committees and the members of the Editorial Board of *Microbiology Australia*. As a Society we are also indebted to those members who represent ASM on National and State Advisory Committees and Boards. Special thanks are due to our scientific advisors: Linda Blackall, Heidi Drummer, Tom Riley, Mark Schembri and Deb Williamson, for their wisdom and support. ASM is fortunate to have such an amazingly talented group of people to call upon.

I would like to single out for special thanks Kara Taglieri from ASN Events for keeping me in line, and ensuring that (almost) everything I had to do happened on time.

This would not be a complete communication from me if I didn't remind you of our forthcoming Annual Scientific Meeting in Brisbane – a great place to visit in midwinter. Weather aside, the Local Organising Committee under the skilled leadership of Kate Seib has compiled fantastic scientific and social programs, which are certain to inform and delight all those who attend. If you haven't already registered, please do so at: http://asmmeeting.theasm.org.au/.

Lastly, I would like to thank all ASM members for your support and wish you and our Society all the very best for the future.

Arboviruses

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Arboviruses are established as important causes of human and animal disease within Australia, as well as being high on the list of important emerging and exotic risk to Australia. They have been an integral part of the Australian ecological environment and evolved with it, adapting to our environment, to our arthropods, to our birds and to our mammals.

Most of what we know about human infections with these viruses has been described since the European settlement, but they must have been present and have infected humans and animals for extensive periods of time prior to that. Their ecology is influenced by multiple factors that modify human behaviour, animal populations, mosquito behaviour, and the environment. As such, we have seen, and expect to continue to see, changes in the epidemiology of the arbovirus infections within Australia, and ongoing risks of introduction of new arboviruses. The challenge is to predict, prevent and mitigate both current and future arbovirus threats to human and animal health, without damaging our environment and ecosystems. One Health in action!

In the past century we have seen a host of new influences on arbovirus infections: emergence of new global threats like Zika virus; re-emergence of old enemies like dengue, chikungunya and yellow fever; explosive growth of human travel and migration; expansion of human populations within Australia; international movement of goods and insect vectors; and natural and man-made environmental and climatic changes. Unfortunately, that has not been accompanied by vaccines or antiviral agents for our Australian viruses so far, so our strategies for prevention of infections still rely on control of vector populations and avoidance of mosquito exposure.

This issue of *Microbiology Australia* addresses the impact of these viruses on human and animal health, the multidisciplinary approach to understanding them and their control, and dealing with those that are future threats to Australia. Improved methods for detecting and characterising these viruses and their vectors have expanded our understanding of patterns and drivers of spread. We still have some way to go in understanding the ecology of our endemic arboviruses, predicting our susceptibility to exotic viruses, tracking and controlling the mosquitoes, preventing and treating infections, and in understanding the complex interactions between the viruses, the insect vectors, the animal amplifiers, and the human or animal immune systems.

As you will see from this issue, these are important and fascinating viruses. In Australia, we are blessed with a great depth of expertise and knowledge about arboviruses and arboviral infection, and in developing cross-sectoral interactions. There is plenty more for us to do, and progress depends on a One Health approach nationally and with our international partners!

Biography

Clinical Professor David Smith BMedSc, MBBS, FRCPA, FACTM, FASM, FFSc(RCPA) is a graduate in Medicine from the University of Western Australia and trained in Medical Microbiology in Perth. He is a Medical Virologist at PathWest Laboratory Medicine WA at the QE2 Medical Centre in Perth, Australia, where he is a Director of the Arbovirus Research Laboratory. He is also a Clinical Professor in the Faculty of Health and Medical Sciences at the University of Western Australia. Professor Smith serves on a number of state, national and international committees and advisory groups, and is currently Chair of the National Arbovirus and Malaria Advisory Committee. He has a particular interest in public health issues, including mosquito-borne viruses, influenza and other respiratory viruses, and emerging infections.

From the Editorial Team

Microbiology Australia, the official journal of the *Australian Society for Microbiology*, works towards publishing content that is of interest and benefit to ASM members. It does this with contributions and feedback from members, through its Editorial Board, which meets five times each year (usually by teleconference), and through support from CSIRO Publishing. Articles in the thematic issues are usually solicited via Guest Editors who are knowledgeable in the particular topic of the issue. The aim is produce topical informative articles that are readable by the ASM audience who have very broad interests.

As Editor-in-Chief of *Microbiology Australia*, I orchestrate the management of the journal and I also work part time at RMIT University. I am assisted by Rebekah (right) and Hayley (left), who each manage two issues per year, organising the contributions, the reviews and the completion of articles to meet the publication schedule. Between them they may be handling up to four issues at once!

Microbiology Australia's open access policy means that articles and whole issues are downloaded ~300 000 times per annum. The excellence of the articles means that access to them continues long after their original publication: in general, download rates are increasing as years pass and a decade old issue still has almost 20 000 downloads each year. The relevance to student education is huge. I refer my Microbiology students to particular articles and I know of other instances where some issues are 'compulsory reading'.

A few years ago the Editorial Board decided to further increase the profile of *Microbiology Australia* by seeking listings in the major citation indices. Our pathways to listings are progressing well due to the high standard of articles, peer review, adherence to strict journal policies (see http://microbiology.publish.csiro.au/nid/214.htm and http://www.publish.csiro.au/journals/publishingpolicies), including membership of *COPE* (*Committee for Publication Ethics*) and *ICMJE* (*International Committee of Medical Journal Editors*). In December 2015 *Microbiology Australia* was listed with *ESCI* (*Thompson Reuter's Emerging Sources Citation Index*) and in February 2018 with Scopus.

Suggestions for future issues and items of interest to ASM members (e.g. book reviews, new methods, hot topics) are always welcomed.

Dengue introduced by travellers, Australia

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Dengue is a mosquito-borne acute viral infection that can develop into a potentially lethal complication known as severe dengue. It is endemic in more than 100 tropical and subtropical countries where the mosquito vectors, predominantly *Aedes aegypti* and *Aedes albopictus*, are found. Non-immune travellers are at risk of infection and with the rise in international travel and the availability of cheap holiday packages to endemic countries, many of which are popular tourist destinations, there has been a significant increase in spread of dengue viruses.

In recent years dengue outbreaks have occurred in countries where the disease has never been reported or has not occurred for many decades, coinciding with increased global distribution of the primary vectors. In Australia, dengue is currently restricted to northern Queensland where epidemics occur following introduction of virus by travellers; however, dengue is regularly imported to other states and territories where the vectors have not been present for several decades. Recent detection of the vectors at international air and sea ports in Australia is of concern in light of widespread dengue activity throughout the country up until the 1960s. Genetic analysis of dengue viruses (DENV) imported by travellers provides important information on DENV circulating in the region and introduced into Australia.

Epidemiology

Dengue is a mosquito-borne viral disease that is endemic in most tropical and sub-tropical countries. Epidemics were described in Australia, Asia, the Americas, the Pacific and the Caribbean in the 18th and 19th centuries and outbreaks described in central America and the Caribbean in the 17th century are also believed to be dengue[1]. Increased incidence of dengue and emergence of dengue haemorrhagic fever in Southeast Asia in the second half of the 20th century was a consequence of uncontrolled urbanisation, human movement and population growth during the Second World War. The incidence has continued to increase dramatically since that time and dengue is now endemic in more than 100 tropical and subtropical countries, where the principal mosquito vectors *Aedes aegypti* (Figure 1) and *Aedes albopictus* are found. An estimated 390 million dengue virus (DENV) infections have been estimated to occur annually, of which approximately one quarter result in clinical disease[2]. The number and magnitude of dengue epidemics have increased consistently in Southeast Asia and the Western Pacific since 2000 and more than 70% of the global dengue disease risk is currently borne by people who live in this region[3]. Autochthonous dengue transmission has recently been reported in Europe[4], the United States[5] and Japan[6], areas where local outbreaks have not been previously reported or had not occurred for many decades.

Clinical features

Infection with any of the four dengue viruses (DENV-1-4) causes a spectrum of illness ranging from a mild or severe acute flu-like illness known as dengue fever (DF) to severe dengue (previously known as dengue hemorrhagic fever and dengue shock syndrome)[7]. Symptoms become apparent 4–10 days after the bite of an infected mosquito and usually last for 2–7 days. In up to 20% of cases decrease in fever is accompanied by sudden onset of complications due to a vascular leak syndrome consisting of plasma leakage, severe bleeding, respiratory distress or organ impairment. Medical care and maintenance of blood fluid volume during the 24–48 h critical phase can decrease mortality from more than 20% to less than 1%. Warning signs that predict severe dengue are useful for deciding which patients need hospital admission and more intensive monitoring[8]; however, they may not be recognised and

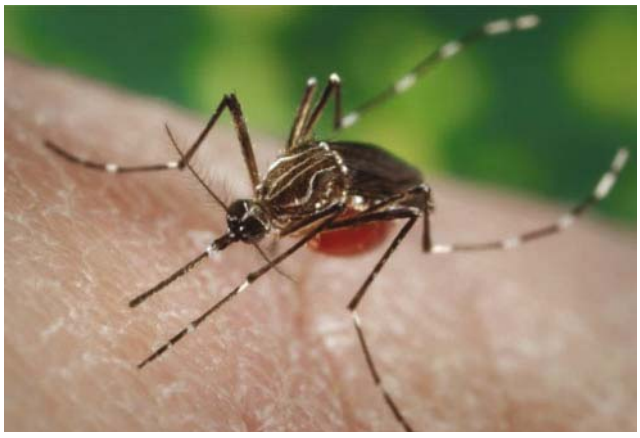

Figure 1. The principal dengue vector, *Aedes aegypti*. Centers for Disease Control and Prevention. https://blogs.cdc.gov/publichealthmatters/2014/01/coming-to-america/aedes-aegypti/.

10.1071/MA18019

case management may not be optimal, exposing patients to unnecessary risk[9].

Dengue is an acute viral infection and virus can be detected in blood by RT-PCR or NS1 protein ELISA from disease onset for up to 9 days. During the viremic phase infected people may transmit DENV to a susceptible host via the bite of the appropriate mosquito vector, after an extrinsic incubation period of 8–12 days following the blood meal during which the virus replicates within the mosquito and is amplified in the salivary glands.

Immune responses

Immunity to DENV infection is thought to be lifelong, and DENV-specific neutralising antibody responses have been detected more than 60 years after infection[9,10]. Infection with one serotype does not protect against infection with the other three serotypes, and indeed epidemiological observations indicate secondary infection with heterologous DENV serotypes is associated with increased probability of severe dengue[11]; 80–90% of severe dengue cases occur in individuals experiencing secondary infection with heterologous DENV.

DENV genomes and correlates of epidemic virulence

The dengue viruses are RNA viruses which form their own antigenic complex within the Flaviviridae family. Like many other RNA viruses DENV undergo high rates of mutation, and comparison of DENV sequence data allows analysis of evolutionary relationships and epidemiological linkages. The four serotypes, DENV-1–DENV-4, share amino acid sequence homology of approximately 70% and are further classified into genetically distinct genotypes, and lineages within the genotypes, based on phylogenetic analysis of the E gene or whole genomes. DENV-1 and DENV-2 can be divided into five and six genotypes, respectively, and DENV-3 and DENV-4 into four genotypes, including the sylvatic lineages found in non-human primates. DENV epidemic virulence has been linked to introduction and transmission of specific serotypes, genotypes and lineages. Introduction of a Southeast Asian genotype of DENV-2 into the Caribbean resulted in the first epidemic of severe dengue in the region, with over 10 000 cases of dengue haemorrhagic fever (DHF) and 158 deaths[12]. The Southeast Asian genotype replaced the American genotype which had circulated for some time and was not associated with severe disease. Similarly, introduction of a lineage of DENV-3 genotype III, a variant associated with DHF in India and East Africa, into Sri Lanka coincided with emergence of DHF in 1989[13]. In contrast, an outbreak of severe dengue in the Solomon Islands in 2013 that resulted in hospitalisations and deaths was caused by a lineage of DENV-3 circulating in Madang on the northern coast of Papua New Guinea (PNG) in 2007–2008[14]. Unlike the previous instances in Cuba and Sri Lanka where the introduced virus was associated with dengue haemorrhagic fever, severe disease was not identified among patients in Madang. Dengue virulence has been associated with viral and host factors.

Dengue in Australia

Dengue was first reported in Australia in 1873, with a report of 8 cases in Melbourne in May 1873 on board the Charles Auguste, a ship originating in Mauritius[15]. Local cases were prevalent in northern Queensland in Townsville in 1879 and Rockhampton in 1885, and epidemics in Queensland, northern NSW, Northern Territory and Western Australia occurred up until the 1940s[16]. Dengue activity in Australia since the first reports at the end of the 19th century has been linked to distribution of its major vector *Ae. aegypti*, a highly efficient vector that preferentially feeds on human blood and is capable of biting several people in a short period for one blood meal. It is highly adapted to urban environments, living in intimate association with humans. The species was widely distributed in the north-east coastal areas of Australia at the end of the 19th century and spread along road and rail links with the movement of people[17]. Disappearance of *Ae. aegypti* from New South Wales, Western Australia and the Northern Territory in the 1960s, and a decline in distribution in Queensland in the 1960s and 1970s followed changes in water storage practices and the introduction of scheme water supply[18]. Established populations of *Ae. albopictus*, the other major dengue vector, were first recognised in Australia when they were discovered in the Torres Strait in 2005[19], and may have served as a vector in a DENV-2 outbreaks in Torres Strait in 2016[20]. *Ae. Albopictus* is a less efficient DENV vector than *Ae. aegypti*[21] and under most conditions would be unlikely to be responsible for large-scale dengue outbreaks. This species is not known to be present in mainland Australia.

Introduction of DENV in travellers

Dengue reappeared in northern Queensland in 1981 after an absence of 26 years with an outbreak of DENV-1[22]. A significant increase in the number and frequency of outbreaks was observed after the international airport was opened in Cairns in 1984[23], linked to DENV importation by international visitors and returning residents. Major epidemics occurred, for example introduction of DENV-2 in 1992 resulted in an outbreak that lasted for 64 weeks, with >1000 cases of DENV-2[24].

Travellers from countries where dengue is not transmitted and who do not have pre-existing immunity are at significant risk of DENV infection in endemic countries. The frequency of dengue diagnoses

in febrile returned travellers increased from 2% in the early 1990s to 16% by 2005[25]. Many countries in the Asia-Pacific region are popular tourist destinations and national notification data show that dengue is the most significant introduced mosquito-borne disease in Australia[26] (Figure 2). Severe dengue has been identified in Australia, however a systematic analysis of incidence among imported and local cases has not been undertaken.

Between 2002–2010 DENV infections notified in Queensland were most commonly acquired in Southeast Asia – predominantly Indonesia, Thailand and the Philippines – followed by Papua New Guinea and other Pacific Island nations[27]. Imported DENV was identified as the source of outbreaks of DENV-2 in 2003–2004[28] and DENV-3 in 2008–2009[29]. The DENV-2 outbreaks that began in late February 2003 in Cairns were likely imported by a PNG national who developed a febrile illness in late January soon after arriving from PNG. DENV E gene sequencing showed the Cairns DENV-2 was similar to a strain isolated from an outbreak that began in Townsville in May 2003, as was DENV-2, isolated from a second outbreak that began in Townsville in October 2003. The Townsville and Cairns viruses were very similar to an imported strain isolated from a traveller from PNG into Townsville in April 2003, and all three viruses represented the same lineage within the Cosmopolitan genotype of DENV-2, indicating that PNG was the source of the February, May and October outbreaks in Cairns and Townsville. PNG was also the likely source of a distinct lineage of DENV-2, Cosmopolitan genotype, that was isolated from a resident of Yam Island in the Torres Strait during an outbreak that began in early November 2003. PNG nationals had attended a funeral ceremony on the island in mid-September. The Yam Island virus showed 100% homology with DENV-2 isolated during an outbreak that began in Cairns in February 2004, suggesting that the Cairns virus belonged to a lineage that originated in PNG. A different strain of DENV-2, belonging to the Asian I genotype, was isolated from a small outbreak that began in Cairns in later October 2003; this virus was very similar to one isolated from an outbreak in Kuranda, near Cairns, in 2002 and both strains were very similar to DENV imported from Thailand in 2001. Genetic analysis thus illustrated separate introductions into northern Queensland of distinct lineages of DENV-2 that circulated in different countries in the region between 2002 and 2003. A clear linkage between DENV importation by travellers and local transmission was also shown for DENV-3: an explosive outbreak in Cairns from November 2008 to May 2009 was likely initiated by a traveller infected in Kalimantan, Indonesia. The epidemic virus was distinct from DENV-3 isolated earlier in Townsville in 2006 and in Cairns in 1998.

Although dengue has not been present in Western Australia since the 1940s, the state notifies the most dengue cases among the 8 Australian States and territories despite having only 10% of the Australian population and no sustained endogenous transmission, due to the absence of the mosquito vectors[26]. In recent years new budget airlines began offering affordable package holidays and travel increased 300-fold between 2006 and 2010, coinciding with a sharp increase in dengue cases notified to the Communicable Diseases Division of the WA Department of Heath[30]. Analysis of

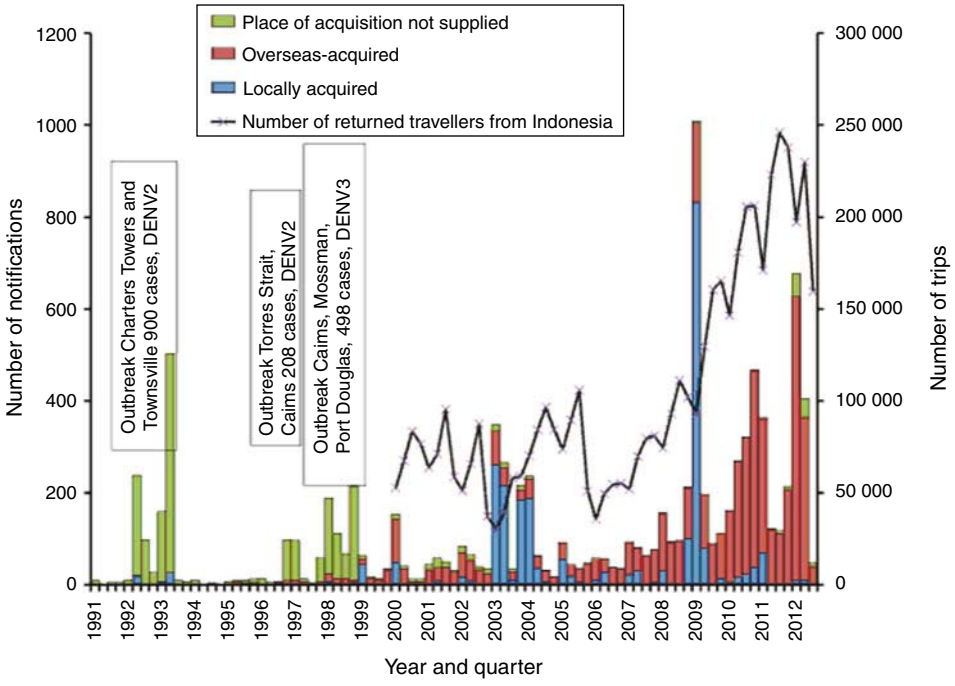

Figure 2. Increasing notifications of dengue in Australia related to overseas travel, 1991 to 2012. http://www.health.gov.au/internet/main/publishing.nsf/content/cda-cdi3701f.htm.

DENV derived from febrile travellers entering WA after visiting seven countries throughout Asia between 2010–2012 identified a diverse range of DENV genotypes and lineages within all four DENV serotypes[31] (Figure 3). Most of the travellers had entered from Bali, a popular holiday destination for residents of WA. Many of the imported DENV were local regional variants with strong epidemic potential known to have circulated in the region, particularly in Indonesia and Singapore, for some time. Other DENV were more recently introduced into Bali from other countries in the region. A new lineage of DENV-2 that appeared to be associated with a major outbreak in Bali in 2012 was also identified. Importation of this lineage has continued up to 2017. The data from this study suggest that Bali is a melting pot of substantial DENV diversity and serves as a hub for DENV transmission and mixing.

The dengue vectors were present in WA up until the end of the 1960s and *Ae. aegypti* was recorded as far south as Harvey, approximately 150 km south of Perth[18]. Changes in water storage and supply practices led to complete elimination of *Ae. aegypti*, and no native mosquito species are known to have the potential to transmit DENV. In October 2013 the first case of locally acquired dengue fever occurred in a male with no history of travel outside WA for many years and who was likely exposed in the Pilbara region in north-west WA[32]. The case may have been exposed to infected mosquitoes imported on international cargo vessels docked at local iron ore export ports or via direct international flights into Port Hedland or Perth airports. Passive transportation of mosquitoes by both air and sea has been demonstrated in previous studies in Southeast Asia and has been suggested as a mechanism for global spread of DENV[33,34]. Between February 2014 and March 2016 *Ae. aegypti* was frequently detected at Australian international airports including Perth, Melbourne, Adelaide, Brisbane, Sydney and Darwin[35,36]. The most regular detections occurred at Perth International Airport[32] but there is no evidence that *Ae. aegypti* has become established.

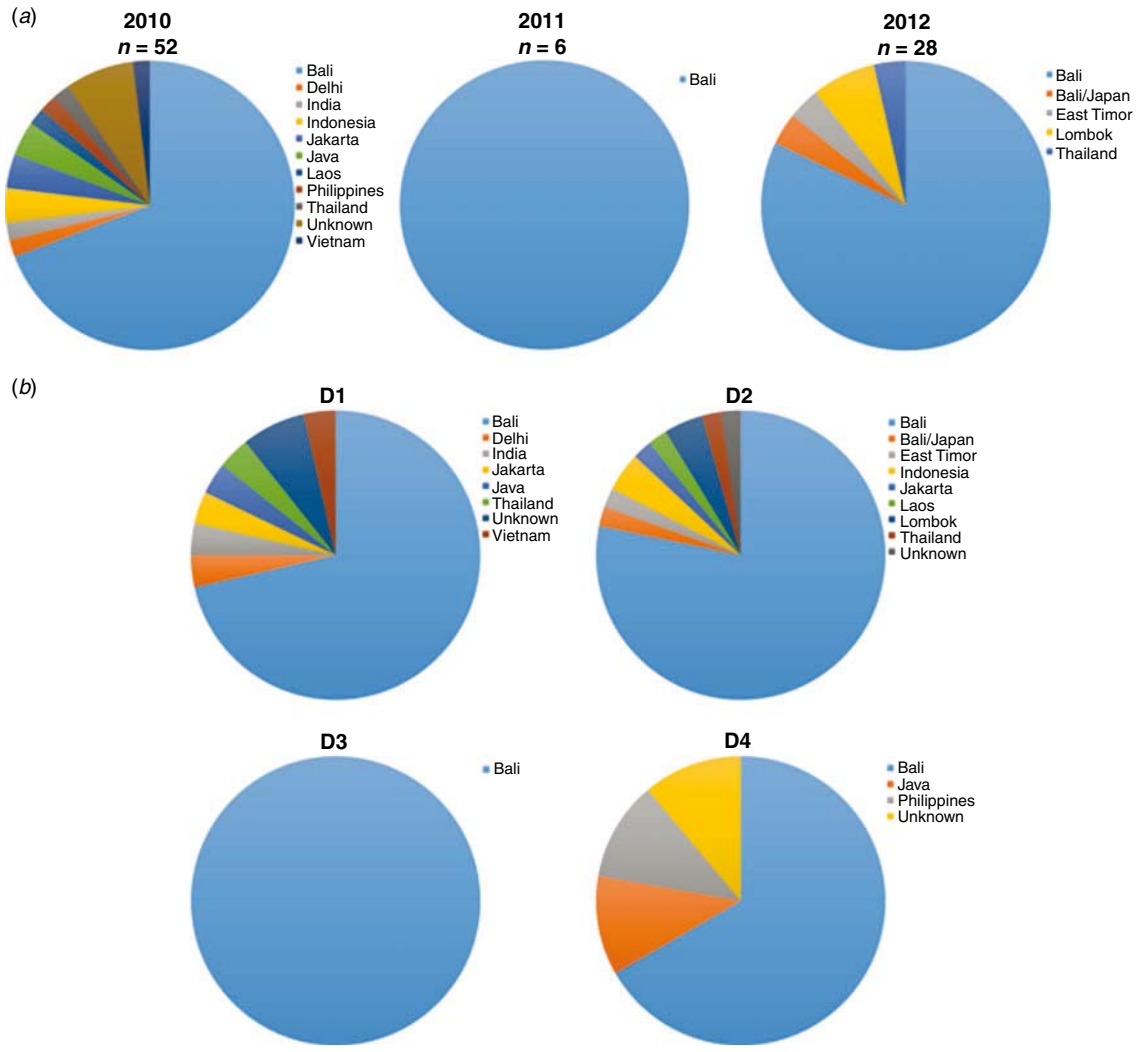

Figure 3. Origin and diversity of dengue viruses isolated from travellers entering Western Australia during 2010–2012. http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003442.

Conclusion

Surveillance of DENV imported by travellers provides information on origin and movement of DENV in the region and in countries where locally generated detailed genetic data may not be available. Ongoing public health surveillance and rapid response measures are necessary to detect incursion of *Ae. aegypti* and *Ae. Albopictus* and to prevent re-introduction of dengue into states where the disease has not been present for many decades.

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Biography

Associate Professor Allison Imrie is a teaching and research academic in the School of Biomedical Sciences within the Faculty of Health and Medical Sciences at the University of Western Australia, and a Research Scientist at PathWest Laboratory Medicine WA. Her current research interests focus on arboviruses, anti-viral immunity and viral genetic epidemiology, influenza and other respiratory viruses, discovery, and laboratory-based surveillance of infectious diseases. She has previously worked with HIV and AIDS.

Newly discovered mosquito viruses help control vector-borne viral diseases

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Many well-known mosquito-borne viruses such as dengue, Zika, West Nile, chikungunya and Ross River viruses can be transmitted to vertebrates and are associated with disease in man or animals. However, the use of deep sequencing and other open-minded approaches to detect viruses in mosquitoes have uncovered many new RNA viruses, most of which do not infect vertebrates. The discovery of these 'insect-specific' viruses (ISVs) has redefined the mosquito virome and prompted the lines of viral taxonomic classification to be redrawn[1,2]. Despite their benign phenotype, ISVs have become a hot topic of research, with recent studies indicating they have significant application for biotechnology.

The main focus of our lab is the study of new and emerging mosquito-borne viruses. For most of the past decade we and our collaborators have developed a comprehensive system for high throughput virus detection and isolation from mosquito and vertebrate samples. This has enabled the discovery of many new viruses and detection of known viruses occurring in new ecological or pathological contexts. We have also focussed on the development of novel research tools and reagents to characterise these viruses both *in vitro* and *in vivo*.

To conduct investigations into the biodiversity of viruses in Australian mosquito populations, we have had access to extensive archival collections of mosquito pools collected from different parts of Australia over several decades. These collections were part of previous targeted research projects or routine surveillance operations and were pivotal to the success of our virus discovery program. Another key to our success was the development of a sequence-independent system to detect and isolate new and known viruses in a high throughput manner. This was based on a novel set of monoclonal antibodies we generated specific to double-stranded RNA (dsRNA), which have the crucial ability to recognise the replicative dsRNA intermediates produced by most RNA viruses during growth in cell culture. These antibodies, also known as 'MAVRIC' (monoclonal antibodies to viral replicative intermediates in cells), are used in ELISA to detect viral replication in C6/36 mosquito cells in 96-well plates inoculated with mosquito samples[3]. This allows us to target the MAVRIC-positive cultures for viral isolation and amplification by generic viral RT-PCRs or deep sequencing to identify the viral agent.

To date, the work of several postdocs as well as PhD and honours students in the lab has resulted in the detection, isolation and characterisation of more than 20 new arthropod-borne viruses. These new viruses represent at least nine viral taxa, including flaviviruses, bunyaviruses, mesoniviruses, negeviruses, reoviruses, iflaviruses, nodaviruses, birnaviruses and totiviruses[4–12]. It is interesting to note that only one of the newly discovered viruses was able to infect vertebrate cells, albeit in a highly restricted fashion[4]. The high yield of these new insect-specific viruses in our studies likely reflects the fact that previous approaches for virus discovery and surveillance have relied on the use of vertebrate systems (mice or cell lines) for the detection and isolation of mosquito-borne viruses. Whilst this was effective in the discovery of many true arboviruses that cycle between mosquitoes and vertebrates, such methods preclude the detection of insect-specific viruses.

Most of our efforts to characterise these new viruses have focussed on the insect-specific flaviviruses (ISFs). While ISFs share the same genome structure and basic replication strategy as flavivirus pathogens such as West Nile (WNV), Zika (ZIKV) and dengue (DENV)

viruses, they do not infect or replicate in vertebrates (Figure 1*a*, *b*). Phylogenetic analysis of ISFs also group them into two distinct genetic clusters – referred to as Lineage I ISFs and Lineage II ISFs (reviewed in[1] – see Figure 1*c*). The Lineage I ISFs are the most genetically divergent and are thought to represent the ancestors of all flaviviruses. This supports the hypothesis that all arboviruses originally evolved in arthropods[2]. Lineage II ISFs on the other hand are genetically much more closely related to the pathogenic flaviviruses and are hypothesised to have recently evolved from a vertebrate-infecting ancestor. This also provides support for convergent evolution amongst ISFs.

The inability to replicate in a vertebrate host indicates that ISFs utilise a form of vertical transmission, a process that has been demonstrated in the laboratory for a number of ISF species[13,14] (Figure 1*c*). Our own studies with Parramatta River virus (PaRV), a Lineage I ISF isolated from *Aedes vigilax* in Sydney, revealed that a high proportion of both male and female progeny of wild-caught female mosquitoes that were hatched and reared in the laboratory were infected with PaRV (unpublished data). Just how the virus infects progeny mosquitoes via the egg has not been determined.

Efficient vertical transmission by ISFs can result in a very high frequency of infected mosquitoes in some populations. This has been shown to reach 80–100% in some studies[6,14]. Curiously, a high prevalence of persistent ISF infection in mosquito populations may have a significant effect on the transmission of flavivirus pathogens such as WNV or dengue. Indeed, laboratory studies by us and others have shown that female *Culex* mosquitoes previously infected (naturally or artificially) with Lineage I or Lineage II ISFs reduced their susceptibility to infection by WNV and their ability to transmit this virus, likely due to the apparent localisation of ISF replication to the cells of the mosquito mid-gut[15,16] (Figure 2). This suggests that ISFs may naturally regulate the transmission of pathogens in some mosquito populations and may present an opportunity to develop novel strategies to reduce the transmission of mosquito-borne viral disease.

To understand why ISFs do not replicate in vertebrate cells, we developed a series of research tools to identify the stages of the cellular replication cycle at which restriction occurs. These included monoclonal antibodies to detect the viral proteins of ISFs produced during replication, and infectious DNA-clones of these viruses to identify viral factors associated with host restriction[6,9,17,18]. These infectious DNAs have enabled us to replace different parts of the ISF genome with the corresponding region of West Nile virus, a flavivirus that successfully replicates in most vertebrate cell types. These chimeric viruses have revealed that the structural genes that code for the virion envelope proteins of ISFs are unable to facilitate entry of the virus to vertebrate cells, while

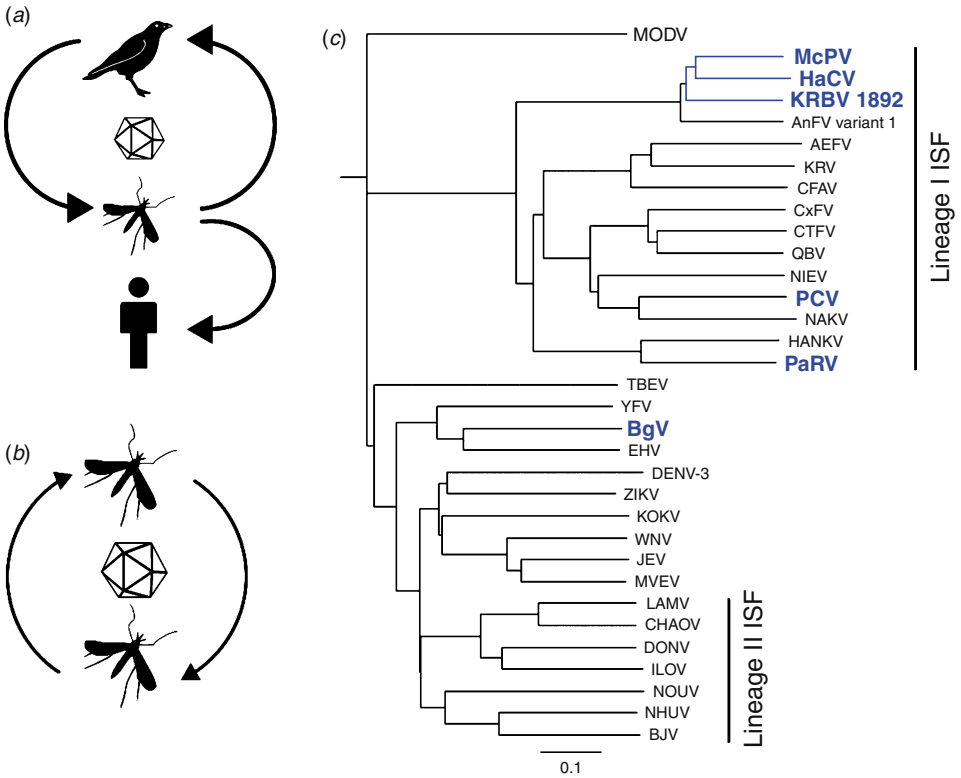

Figure 1. (*a*) Typical arbovirus transmission cycle. (*b*) Proposed transmission cycle of insect-specific viruses. (*c*) Phylogenetic tree showing the different genetic lineages of insect-specific flaviviruses within the genus. Viruses discovered by our lab are highlighted in blue.

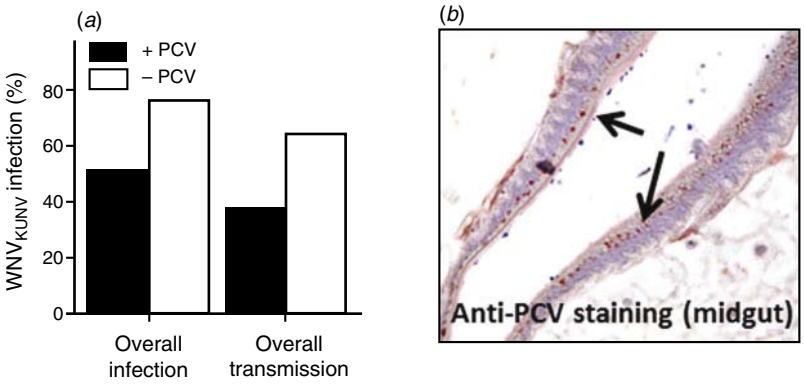

Figure 2. (*a*) Transmission of West Nile virus by *Culex annulirostris* previously infected with the insect-specific flavivirus Palm Creek virus (PCV) was reduced from 64% (29/45) in PCV-free controls to 37% (14/41) in PCV-infected mosquitoes. (*b*) An IHC image showing PCV-infected cells in the mosquito midgut. Figure modified from Hall-Mendelin *et al*.[16].

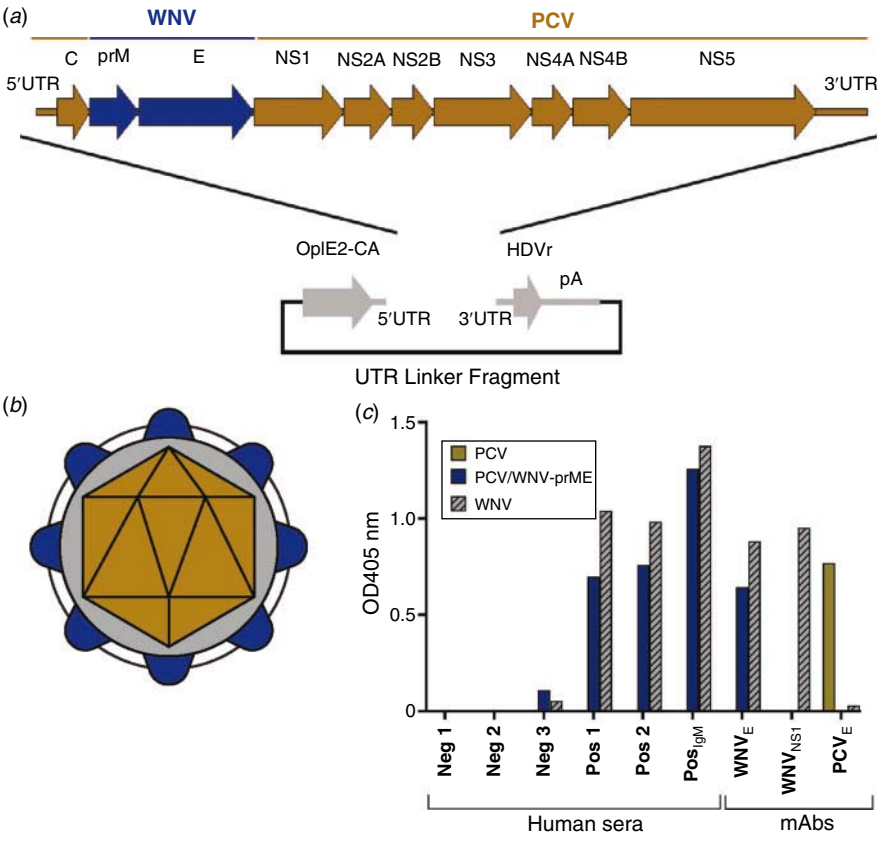

Figure 3. (*a*) Schematic of the CPER strategy to generate infectious DNA of PCV/WNV-prME. (*b*) Stylised schematic of PCV/WNV-prME particles displaying WNV prM and E structural proteins on the virion surface (blue) and capsid protein of PCV (gold). (*c*) Evidence for the utility of the PCV/WNV-prME chimera in diagnostic assays as demonstrated by the recognition of WNV-immune human sera to WNV-chimeras in fixed cell ELISA using virus-infected C6/36 mosquito cell monolayers. Figure modified from Piyasena *et al*.[18].

components in the remainder of the genome (non-structural proteins and untranslated regions of the positive strand RNA genome) render ISFs incapable of initiating replication in the cytoplasm of vertebrate cells.

Our success in producing chimeric viruses between an ISF (PCV) and a pathogenic flavivirus (WNV) led us to express the immunogenic antigens of WNV and other pathogenic flaviviruses to develop a new platform for the safe and simple production of diagnostic antigens and vaccine candidates. Using PCV as the genetic backbone, we were able to construct viable chimeric viruses that expressed the prM and E virion proteins of WNV, ZIKV or DENV 2[18] (Figure 3*a*, *b*; unpublished data) that are antigenically authentic and suitable as diagnostic antigens (Figure 3*c*). Subsequently, we have identified other ISF species that can be also used for this purpose. Importantly, the chimeric viruses exhibit the host restriction phenotype of the parental ISF and do not replicate in vertebrate cells. They can also be grown to high titre in mosquito

cell culture, often to orders of magnitude greater than the parental pathogenic virus.

Future directions

We are currently elucidating the precise mechanisms involved in the transmission of ISFs and the ecological context in which this occurs. Elucidating the molecular basis of their host restriction and how this relates to the evolution of different ISF lineages is also another interesting facet of our research. While the application of ISFs to develop recombinant platforms for safe diagnostics and vaccines provides exciting potential for biotechnology, we are also intrigued by the apparent regulation of pathogen transmission in mosquito populations that carry ISFs and the possibilities of exploiting this phenomenon to control the transmission of flavivirus diseases.

Acknowledgements

We acknowledge the many staff and students in the School of Chemistry and Molecular Biosciences at UQ and in Public Health Virology at Queensland Health who have contributed to this research and to the many collaborators who have provided arthropod and clinical samples that have enabled us to undertake the work. Special thanks to Agathe Colmant for preparing the phylogenetic tree, Jessica Harrison and Greg Sullivan for editing the manuscript and Tobias Hall for the schematic artwork.

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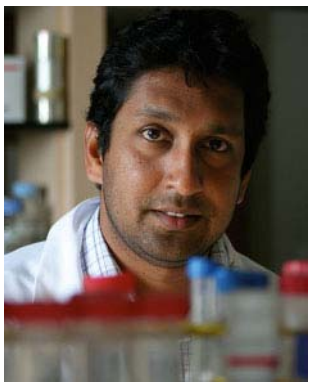
Biographies

Professor Roy Hall is a specialist in vector-borne virology at the University of Queensland. His research explores emerging mosquito-borne viruses with a focus on their pathogenesis and the development of novel vaccine and diagnostic platforms. His work has led to the design and development of novel diagnostic assays and vaccine candidates and the discovery of several new mosquito-borne viruses.

Dr Jody Hobson-Peters is a virologist at the University of Queensland specialising in mosquito-borne virus discovery and the development of novel diagnostic assays. Following almost a decade working in industry in the development and commercialisation of rapid point-of-care assays, her most recent research interests have culminated in a greater understanding of the mosquito virome, producing an extensive suite of monoclonal antibodies to novel mosquito-borne viruses and the optimisation of safe and authentic viral protein production for next-generation mosquito-borne virus vaccines and diagnostics.

Chikungunya: treatments, opportunities and possibilities





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The natural progression of chikungunya virus (CHIKV) disease can consist of three stages – acute, post-acute and chronic, each having different clinical features. The acute phase (up to 3 weeks) is characterised by high viremia, fever, rash, polyarthralgia, synovitis and intense inflammation. Complete recovery is achieved in most symptomatic cases after this phase. However, in a large proportion of patients symptoms persist into a post-acute phase and in some may even continue to become chronic. In the post-acute phase, which can last up to 4 months, there is clinical persistence of joint inflammation or relapse after transient improvement. These can lead to musculoskeletal disorders and eventually chronicity of disease. The main symptoms being chronic inflammatory rheumatism that can last for several years in some cases. With the near global reach, debilitating nature and recent outbreaks of CHIKV there has been much research effort put towards combatting it. New antivirals and medications to counteract inflammation are being developed. Development of CHIKV vaccines is also an area with intense research focus.

Chikungunya virus is a member of the *Togaviridae* family, and belongs to the Alphavirus genus. Its name is translated from the African dialect of Makonde which means, 'that which bends up'[1] and refers to the effect of the incapacitating arthralgia experienced by the majority of patients with CHIKV fever. This arthralgia affects the small joints of the hands, wrists, ankles, and feet. The arthritis-like symptoms are accompanied by other symptoms such as maculopapular rash, myalgia, nausea, headaches, nasal discharge, conjunctivitis, retrobulbar pain, photophobia, and lymphadenopathy[1]. In the majority of cases recovery is achieved within 10 days; however, in some people the joint pain may persist for months (even years)[1].

CHIKV is transmitted to people from its natural reservoirs, which include monkeys, rodents, bats, and birds, and subsequently between people by the bite of two types of mosquitos: *Aedes aegypti* and *Aedes albopictus*[1]. Historically, CHIKV cases have been associated with developing countries in Africa. However, it has begun to show signs of re-emergence following decades of low activity[2]. Several outbreaks have occurred infecting millions of people in the Indian Ocean islands, Asia, Caribbean Islands, Pacific Islands and the Americas[2]. The disease has an enormous economic burden due to medical costs and decreased productivity. As there are no vaccines or antivirals for prevention and treatment of CHIKV infections, there is critical need for the development of such treatments.

Antiviral and anti-inflammatory compounds

Currently, there are no specific licensed antiviral medications for CHIKV infections and treatments are limited to the use of non-steroidal anti-inflammatory drugs (NSAID) or corticosteroid drugs to relieve fever and joint swelling[2]. However, with the re-emerging

threat of CHIKV, recent years have seen interest in the discovery and testing of novel antiviral and anti-inflammatory medications to combat CHIKV.

Chloroquine, an antimalarial drug was found to be effective against alphaviruses *in vitro*[3]. However, in a double-blinded clinical trial with infected patients during the La reunion epidemic chloroquine was found to be ineffective[4]. Ribavirin, an antiviral agent, in combination with interferon-alpha was found to be beneficial in inhibition of SFV and CHIKV replication *in vitro*[5]. Arbidol, licensed for the treatment of influenza and other respiratory infections, was also found to be effective in inhibiting CHIKV replication *in vitro* (IC50 <10 μg/mL)[6].

High throughput screening is a common approach to identify novel antiviral compounds. Screening of compounds resulted in identification of coumarin 30 as an effective antiviral agent in inhibiting the replication of CHIKV[7]. Harringtonine, a cephlotoxoid alkaloid, was identified by screening a natural compound library for inhibition of CHIKV replication[8].

Varghese *et al*.[9] have also adapted similar screening strategies to identify several compounds that inhibited CHIKV replication in a dose-dependent manner with some showing broader antiviral activity against other alphaviruses. Compounds, such as niclosamide and nitazoxanide, were found to restrict the entry of virus, restrain both viral discharge and cell-to-cell virus transfer, and exhibit expansive anti-alphavirus function against CHIKV and other alphaviruses[10]. Alternate modes of action for antivirals include the inhibition of viral fusion by neutralising the acidic environment of endosomal vesicles. The small-molecule antagonist of the Bcl2 family of proteins, Obatoclax, demonstrated such efficacy against CHIKV as well as SINV and influenza A[11]. Such discoveries of antiviral activity might provide a basis for the development of new human drug therapies against CHIKV and other alphavirus infections.

Other significant research discoveries include several breakthroughs in understanding the mechanism of alphaviral infections and their interactions with host defense systems. A recent study revealed the role of inflammasomes in causing severe inflammatory disease in arboviral infection. Activation of the NLRP3 inflammasome in humans and mice following CHIKV infection was shown. Inhibition of NLRP3 activation *in vivo* using the inhibitor MCC950 resulted in reduced inflammation and bone loss in mice. Furthermore, this *in vivo* inhibition showed reduced inflammation in the closely related Ross River virus (RRV), but not for mice infected with the flavivirus West Nile virus[12].

Similar approaches for the identification of anti-inflammatories of specific viral-host interactions are ongoing and offer hope for the effective treatment of CHIKV. For example, the glycan derivative drug Pentosan polysulfate (PPS), that has been used to treat cystitis in the US, was shown to decrease the level of joint swelling, cartilage damage and inflammatory proteins in both CHIKV and RRV mouse models[13]. PPS is currently in a phase 2 clinical trial for the treatment of RRV at 4 different locations across Australia.

Antibodies

Neutralising antibodies have been found to be effective in animal models of CHIKV infection[14,15]. In rhesus monkeys, administration of monoclonal antibody (mAb), SVIR001 that mimics the human anti-CHIKV mAb 4N12, resulted in rapid clearance of CHIKV infection[15]. A combination therapy with CTLA4-Ig (abatacept) and 4N12 antibody decreased the periarticular swelling and joint inflammation even after administration several days after infection[16]. Neutralising antibodies could be used prophylactically for individuals with high risk of infection such as pregnant women and persons with underlying disabilities such as diabetes and cardiovascular diseases.

Vaccines

At present there is no licensed vaccine for prevention of CHIKV infections. Much research effort is being directed in the development of an effective vaccine with studies ranging from the preclinical stage through to phase 2 trials. A common approach to developing vaccines is often through the use of an attenuated variant of the virus. A phase 2 clinical trial utilising a live, attenuated virus showed very promising results for short-term immunity with 98% of participants being immune after 28 days. However, this fell to 85% at the one-year follow up stage[17]. Side-effects were reported in a small percentage of patients, with some people reporting joint pain for a short period of time.

Mutation of the nucleolar localisation sequence (NoLS) in the N-terminal region of CHIKV capsid protein (C protein) is another example demonstrating potential as a live attenuated vaccine candidate. Mice infected with CHIKV-NoLS did not exhibit any signs of disease, had reduced viremia and proinflammatory cytokines when compared to wild type CHIKV. When the mice were challenged with CHIKV-WT at 30 days post immunisation, no disease signs and no detectable viremia were observed[18].

One particular concern with the use of live attenuated viral vaccines is the potential of the virus to revert back to its native wild-type phenotype through random mutation. The vaccine tested in the phase 2 trial was attenuated due to two point mutations in the E2 glycoprotein region[19]. Such small changes in the viral genome, although targeted, also make the possibility of reversion high. This would not be a concern for the NoLS vaccine candidate because a substantial number of changes have been made within C protein

that affects a number of its functions. This thereby makes the likelihood of reversion extremely low. Indeed, *in vitro* data shows a high level of stability even after multiple passages.

Alternative attenuation strategies have also been examined for the development of a CHIKV vaccine. Replacement of the subgenomic promoter of CHIKV with the internal ribosome entry site of encephalomyocarditis virus resulted in a highly attenuated, immunogenic vaccine candidate with good efficacy in mouse models. In addition, the virus was unable to replicate within mosquito cells *in vivo*[20]. Deletion of a large segment of the nsP3 gene or the entire 6K gene and subsequent administration of viral particles or infectious DNA genomes proved efficacious in animal models[21].

The external envelope proteins, E1 and E2, which form heterodimers on the virion's surface and are involved in CHIKV attachment and entry are also prime targets for treatment strategies. In a study involving both cell-based and murine models the N218 of CHIKV E2 protein was found to be a potent neutralising epitope. CHIKV attachment to cells was completely blocked when the IgM 3E7b antibody was used in a pre-binding neutralisation assay by binding to the surface-accessible E2-N218 residue. Prophylactic administration of 3E7b to neonate mice markedly reduced viremia and protected against CHIKV pathogenesis in various tissues. Given therapeutically at 4 h post-infection, 3E7b conferred a 100% survival rate and similarly reduced CHIKV load in many tissues. These findings demonstrate the importance of the E proteins in the possible future development of an epitope-based vaccine[22].

The use of virus-like particles (VLPs) is another strategy that has managed to pass phase 1 clinical trials[23]. VLPs are multi-protein structures that mimic native viral proteins often found on the outside of the virus. However, they lack the viral genome and are thus non-infectious and unable to replicate within the human recipient. This approach holds the promise of potentially yielding safer and cheaper vaccine candidates. In the phase 1 trial, neutralising antibodies were detected in all 25 adult volunteers after the second vaccination. The antibodies remained detectable even 6 months after the third vaccination. The vaccination proved to be safe and well tolerated, without serious adverse reactions[23]. This trial represents an important milestone in the development of a vaccine to combat this pathogen, although further studies are needed in larger and more diverse cohorts and a phase 2 trial has been planned[24].

Non-replicating viral vectors are another approach to developing vaccine candidates for CHIKV. Modified Vaccinia Ankara (MVA) is a highly attenuated, avirulent poxvirus vector engineered to be unable to productively grow in human cells. MVA demonstrates a high level of antigen gene expression, even in cells that are not susceptible to infection. Investigations using MVA vaccines in animal models, has proven that it is immunogenic and protective against various infectious agents[25]. Several pre-clinical studies have evaluated the efficacy of MVA expressing various CHIKV structural proteins such as C, E3, E2, 6K, and E1, either alone or in combination. Positive results were obtained, with the candidates eliciting neutralising antibodies in animal models and some providing protection against challenge with CHIKV[26,27].

Nucleic acid based vaccines have been tested as vaccine candidates with varying success. Although proven to be safe in humans, achieving long lasting immunity has proven challenging for DNA vaccines, often requiring multiple doses. As a way to improve immunogenicity, the use of DNA-launched alphavirus replicon vectors shows promise. Replacement of the alphaviral structural genes with the antigen gene of interest elicited strong immune responses[28]. The use of this approach in combination with protein antigen doses resulted in antigen-specific immune responses against CHIKV, particularly when used in a prime-boost strategy[29].

Many pre-clinical studies investigating vaccine candidates often report efficacy in terms of neutralising antibodies; however, these may not be accepted as a correlate of protection by some regulatory agencies. The requirement of a Phase 3 trial for proof of vaccine efficacy and licensure means that the time and cost of development expand rapidly. This is compounded further by the fact that epidemics/outbreaks are sporadic and hard to predict. Significant numbers of trial participants in endemic countries would also need to be screened since a large proportion of people would have pre-existing CHIKV antibodies. In the absence of a Phase 3 trial it may be possible to perform a comprehensive analysis of a vaccine using animal models instead, e.g. macaques, to accelerate the vaccine to market. Alternatively, a strictly controlled Phase 3 'challenge' trial model could be employed whereby vaccinated individuals are challenged at a set time point post vaccination. This could be a controversial approach but concerns could be allayed through the use of viral strain in the challenge that is not linked to causing any form of arthralgia.

CHIKV has a major economic impact on affected nations. The huge populations of India (1.3 billion) and South American countries (400 million) means well over a billion people are vulnerable to future CHIKV outbreaks. Add to this the continuing changes in global climates that could further expand the habitat of *A. albopictus* across the globe. This creates opportunities for future outbreaks to occur in countries not usually associated with CHIKV. It is clear that development of new therapeutics and solutions to regulatory challenges will depend on innovative thinking and collaborations between developers, laboratory personnel, regulators, funders and governments.

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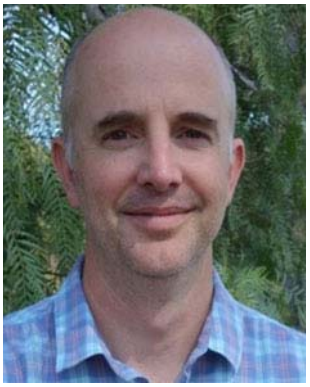
The detection and significance of emerging insecticide resistance in mosquitoes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Mosquito-borne arboviruses are increasing in incidence around the world. Australia enjoys some protection from pests and diseases afforded by its geographic isolation coupled with strict biosecurity control at its borders. However, as the volume of global trade, travel and transport expands, risk of exotic incursions to Australia is increasing. Detection of foreign mosquitoes at airports and seaports around Australia is becoming commonplace. The Asian tiger mosquito, *Aedes albopictus*, which has expanded its range throughout Europe and the Americas[1], has not become established in mainland Australia, but is encountered as an exotic incursion[2]. The yellow fever mosquito and dengue vector, *Aedes. aegypti*, occurs naturally in northern Queensland, but is also captured at Australia's ports on a recurrent basis as an incursion from overseas[3]. Although *Ae. aegypti* is established in Australia, its detection as an incursion is still cause for concern. Apart from the possibility that invasive mosquitoes will carry exotic arboviruses, genetic characteristics of a foreign insect population can be very different from those observed in local mosquitoes, particularly in terms of insecticide resistance. Our recent research has shown that invading mosquitoes from overseas carry insecticide resistance alleles not found in Australia[4] and our development of a global genomic database is helping us to pinpoint their source.

Disease transmission is a major human health consequence of mosquito activity and, in many cases, management of mosquito-borne diseases is addressed by interfering with the mosquito vector. Interference may take the form of physical methods such as source reduction, for example, by removing water containers around dwellings, thereby destroying the aquatic stages of the mosquito and their oviposition sites[5]. More recently, mosquito population replacement or suppression programs have been developed based on virus-blocking endosymbionts, particularly *Wolbachia pipientis*[6]. Variations on the sterile insect release technique using genetic modification are also being employed to reduce abundance of pest mosquitoes[7]. The World Health Organization (WHO) is promoting integrated vector management (IVM) to reduce the reliance on chemical insecticides for vector control[8]. Despite these developments, insecticide application is still the main method of mosquito control[9] in tropical urban areas where the focus species are *Ae. aegypti* and *Ae. albopictus*. As vectors of dengue, Zika, chikungunya and yellow fever viruses, *Ae. aegypti* and *Ae. albopictus* are regular targets of insecticide treatment.

Pyrethroid insecticides are valuable tools for mosquito control because of their rapid action, high efficacy, low mammalian toxicity relative to some other insecticide groups and low cost[10]. Pyrethroids can be applied in a variety of ways such as thermal fogging of neighbourhoods[11], internal domestic space and residual spraying[12,13], as spatial repellents[14], in lethal ovitraps[15], as treatment of bed nets[16] and for impregnation of clothing[17]. These diverse applications have led to a dependency on pyrethroids around the world, but have also resulted in their overuse[18]. As a consequence, multiple instances of pyrethroid resistance have developed in

populations of *Ae. aegypti* throughout much of its range[18], although some gaps in testing exist[19], and there is evidence for similar developments of resistance within *Ae. albopictus* populations, though not to the same extent[20].

Pyrethroid resistance levels and mechanisms vary by region[18,20], which can help identify the significance of mosquito incursions to Australia. Resistance levels in adult mosquitoes from Central and South America are generally higher than those from Asia, Africa and the USA[20]. The two major pyrethroid resistance mechanisms encountered in *Ae. aegypti* and *Ae. albopictus* within these regions are detoxification by cytochrome P450 monoxygenase enzymes and modification of the pyrethroid target site which is the insect's voltage sensitive sodium channel (*Vssc* gene)[19,20]. Combinations of different *Vssc* mutations also show geographic patterns[20], which, together with other methods, can assist in determining the geographic origin of mosquitoes detected as incursions in Australia. Recently we discovered that Australian *Ae. aegypti* mosquitoes (Figure 1) show none of the common mutations in the *Vssc* gene that are associated with resistance to pyrethroid insecticides[4]. In contrast, most incursions of this species to Australia do carry pyrethroid resistance mutations which we have determined by screening *Ae. aegypti* with TaqMan® assays developed from DNA sequence data acquired during a study of insecticide resistance in Indonesia[21].

Several problems are posed by the presence of resistance in exotic incursions. Firstly, insecticide resistance in mosquitoes entering the country represents a risk that the resistance alleles will become established in the Australian mosquito population where they will have an advantage on occasions when pyrethroid insecticides are applied. Secondly, there is the problem of the immediate or preventative control of mosquitoes as they arrive in the country, particularly at airports. Aircraft disinsection on international flights into Australia depends entirely on the use of pyrethroid insecticides because of their rapid knockdown of susceptible insects and low mammalian toxicity. They are the only class of insecticide recommended for disinsection by the WHO[22] and are applied as a space spray (knockdown) or residual treatment. Insects that are resistant to pyrethroids generally will survive disinsection and may or may not be detected by biosecurity officers at airports, depending on the monitoring regime. Without a safe and effective disinsection method, the risk of insecticide-resistant mosquitoes becoming established in Australia is high and the risk of 'airport cases'[23] of mosquito-borne diseases becomes a possibility. It, therefore, becomes necessary to track the sources of insecticide-resistant, invasive mosquitoes in the hope that alternative pest management tactics can be employed in source locations overseas to reduce mosquito populations around airports and minimise their likelihood of dispersal by aircraft. There are alternate pathways of introduction of exotics, specifically through eggs laid on personal belongings/souvenirs. In these cases, insecticide use in aircraft and around airports will not be effective barriers to entry. If an incursion, away from an airport occurs, the insecticide resistance will hamper eradication efforts.

Figure 1. Female (left) and male (right) *Aedes aegypti* mosquitoes (photograph by Perran Ross).

We are using genome-wide single nucleotide polymorphism (SNP) analysis to construct a comprehensive population genomic database for *Ae. aegypti* and *Ae. albopictus* from around the world. We pioneered the use of this technique for investigating broad and fine scale patterns of genetic relatedness in *Aedes aegypti*[24] and have been building on this initial study to include mosquitoes from geographic regions relevant to risk of incursion. Genomic profiles of mosquitoes captured as exotic incursions to Australia at international airports and seaports are compared with those in the database to determine their likely origin. Using thousands of SNP loci and genotyping of the *Vssc* resistance loci, we established recently that most Australian incursion samples of *Ae. aegypti* at international airports during 2015–2016 had originated in south east Asia, with only a single incursion specimen appearing to have come from South America (Figure 2). We were also able to identify that one incursion sample originated in north Queensland, highlighting that incursions can occur through both domestic and international routes at airports.

Information about place of origin and pathway to Australia enables authorities to focus on mosquito incursions from south east Asia, at least in the short term, and potentially form relevant collaborations to address the problem. *Vssc* mutations detected in the group of mosquito incursions from south east Asia were mostly all the same and consisted of a combined haplotype of three mutations which is

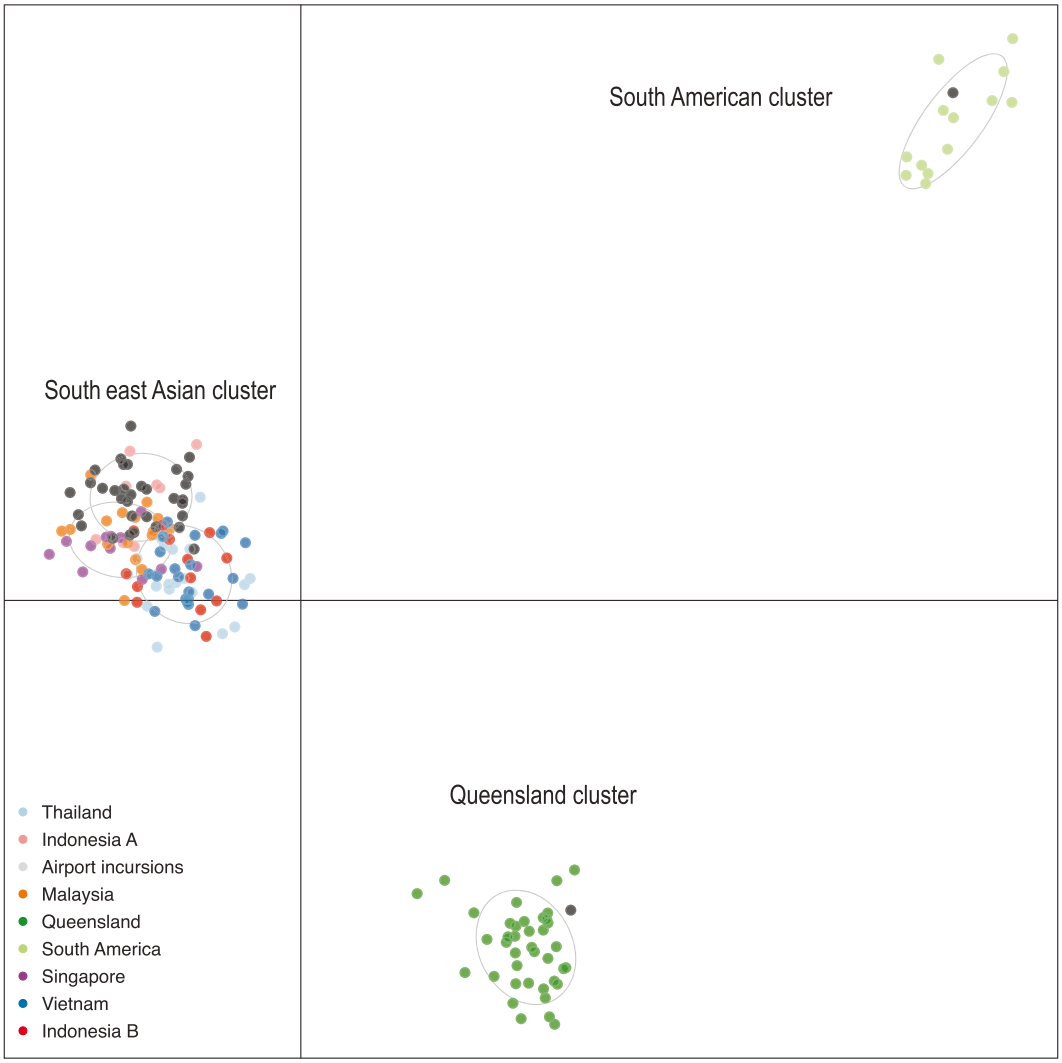

Figure 2. Discriminant analysis of principal components (DAPC) plot using ~5000 genome-wide SNPs in *Ae. aegypti* samples from various geographic locations with Australian airport incursion samples (black dots) sitting in relevant geographic groups. Most incursion samples fall within the south east Asian cluster.

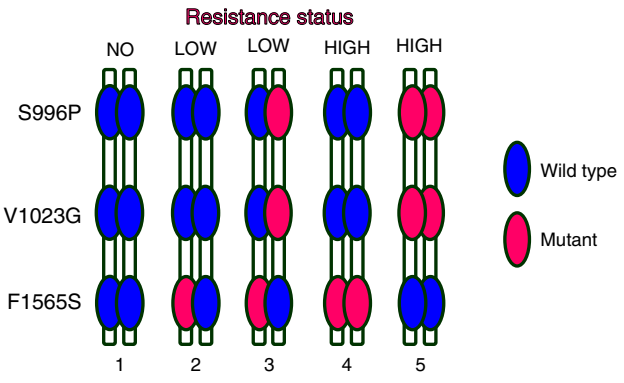

Figure 3. *Vssc* mutations in *Aedes aegypti* and associated pyrethroid resistance status. Haplotype 1 is the only one found in Australian mosquitoes. Haplotype 5 is the most common haplotype found in incursion mosquitoes thought to be from south east Asia. Haplotypes 2 to 4 have been found at a lower frequency in mosquito incursions thought to originate from south east Asia.

known to confer resistance to Type I and Type II pyrethroids (1023G mutant homozygote, 1565F (wild type) and 996P mutant homozygote) (Figure 3) and is consistent with bioassay data showing high levels of resistance to permethrin (Type I) in *Ae. aegypti* from this region[25]. Addressing management practices in the country of origin will be the most effective way to reduce the influx of pyrethroid-resistant mosquitoes to Australia and further research should be conducted into the full resistance profile including multiple mechanisms and current efficacy of insecticide groups other than pyrethroids which may be used on other life stages of *Ae. aegypti*. Under some circumstances, alternative insecticides to pyrethroids can be used, for example, carbamates, that could kill pyrethroid-resistant *Ae. aegypti*[26] and non-insecticidal methods may also be considered. Careful stewardship of pyrethroid insecticides has been practised in Queensland since the 1990s using strategic applications combined with a variety of delivery methods that include indoor residual spraying, lethal ovitraps and use of insect growth regulators as larvicides[4]. Under these conditions, resistance mutations in the *Vssc* have not been selected for in *Ae. aegypti* in Queensland[4]. Our continued research into identification of mosquito origin and monitoring of *Vssc*

resistance mutations in incursion specimens will keep our management programs relevant, even in the event of temporal changes in mosquito distributions and levels of insecticide resistance. Continued progress should result in maintenance of insecticide susceptibility in *Ae. aegypti* in Australia and reduced risk of arbovirus transmission.

Acknowledgements

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Biographies

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The risks to Australia from emerging and exotic arboviruses

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The recent pandemic spread of mosquito-borne arboviruses across multiple continents, as exemplified by West Nile (WNV)[1], chikungunya (CHIKV)[2], and Zika (ZIKV)[3] viruses, together with the continuing disease burden of epidemic dengue viruses (DENVs)[1], multiple importations of yellow fever virus (YFV) into populous areas of Asia[4], and the potential threat of some other, possibly unknown, emerging arboviral threat, constitute a wake-up call for governments to strengthen surveillance programmes and enhance research into mosquito-transmitted diseases[5–7]. Rift Valley fever[8] (RVFV) and Japanese encephalitis[1,9] (JEV) viruses are also important examples of threats to human and/or livestock health. Australia is vulnerable to these arboviral diseases, with risk of importation and outbreak potential varying between viruses[10]. The risk of exotic arboviral diseases establishing transmission cycles in Australia is dependent on the availability of competent vectors and suitable vertebrate hosts. Therefore, knowledge of the vector competence of Australian mosquito species for exotic arboviruses, potential for the introduction and establishment of exotic vector species, and suitability of vertebrate hosts, are essential components of understanding and mitigating these arboviral threats.

Mechanisms of emergence and spread

The factors involved in the emergence and spread of these viruses are complex and multi-factorial, but are clearly associated with human influences, such as unchecked urbanisation and changes in land use, inadequate water and waste management that leads to a proliferation of larval habitats, as well as increased global movement of humans and trade through air and sea travel[1,11]. Importation of arboviruses can occur via viraemic travellers, or by the introduction of infected mosquitoes on aircraft or other vessels[1]. Virus dissemination can also occur through the movement of vertebrate hosts, such as birds and bats, or infected mosquitoes transported by wind currents.

Vectors and vertebrate hosts of endemic arboviruses, and introduction of exotic vector species

Over 75 arboviruses occur in Australia[12], although relatively few are human or animal pathogens. Transmission cycles of endemic pathogenic viruses inform the likelihood that their vertebrate hosts and vector species may participate in transmission cycles of exotic viruses. This is the case of WNV[13] and probably JEV[14], viruses related to Murray Valley encephalitis virus, for which wading birds and *Culex annulirostris* are important hosts and vectors, respectively.

YFV, DENV, ZIKV and CHIKV have developed human-to-human transmission cycles predominately involving urban *Aedes aegypti* and, in some cases, *A. albopictus* transmission. *A. aegypti* is currently restricted to northern Queensland, but was historically widely distributed in Western Australia and New South Wales[15]. *A. albopictus* is currently absent from mainland Australia, but was recognised in the Torres Strait (TS) in 2005 and, due to a very effective control strategy, has been prevented from spreading to the mainland[16]. In addition to exotic viruses, *A. albopictus* may also become involved in transmission of endemic Australian alphaviruses, Ross River and Barmah Forest viruses[17]. There is

an ongoing risk of these two species expanding their range in Australia or being introduced from overseas by air or sea transport. To help mitigate the risk, active surveillance is in place at international seaports and airports to detect introductions (see Sly and Mack, this issue).

Exotic arboviruses: specific examples

The exotic viruses believed to present a potential threat to Australia are described briefly below. YFV, DENV, JEV, WNV and ZIKV are all flaviviruses; CHIKV is an alphavirus; and RVFV is a bunyavirus.

Yellow fever virus

Recent epidemic activity of YFV in Africa and South America, and the importation of cases of YFV into China from Angola[4], have highlighted the vulnerability of SE Asia and Oceania to the introduction of YFV[18]. Vector competence studies with Australian mosquitoes have confirmed that Australian *A. aegypti* are efficient vectors of YFV, but of greater concern, have shown that *A. notoscriptus*, a relatively common species found widely across Australia, including urban areas, may also be a potential vector for both African and South American strains of YFV[19]. Why YFV has not emerged in Asia previously remains an enigma, but with a susceptible population of two billion people and extremely limited infrastructure to respond effectively[18], the risks of emergence are enormous, presenting an increased threat to Australia. It is essential that surveillance of incoming travellers from endemic areas and the requirement for current YFV vaccination is maintained to reduce the risk of a viraemic traveller introducing the virus into receptive areas, particularly north Queensland.

West Nile virus

The risk of an exotic and pathogenic strain of WNV entering Australia are believed to be low[20,21]. The nearest land mass with a pathogenic strain of WNV is the United States, so the most likely route of introduction would be via an infected mosquito carried on aircraft. Current disinsection procedures for aircraft make this unlikely. Nevertheless, endemic Australian mosquito species are competent WNV vectors[22], and Australian avifauna would almost certainly be able to participate in transmission cycles.

Japanese encephalitis virus

JEV is widely dispersed across southern and eastern Asia, including Indonesia, and PNG[9]. Outbreaks of JEV have occurred in Australia, with human cases and widespread swine infection in the TS, and on Cape York Peninsula[9,14]. Sentinel pig and mosquito surveillance conducted between 1995 and 2005 suggested that the virus had become endemic in the TS, but not on mainland Australia[14]. Its inability to become established on the mainland may be due to the presence of different lineages of *C. annulirostris*, which vary in vector competence or limited mosquito feeding on pigs, which are major JEV hosts[14]. However, this may not reflect the potential for establishment elsewhere in northern Australia. There is little doubt that JEV remains a threat to human and animal health in northern Australia.

Dengue viruses

There are four distinct, but closely related, serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4). It is estimated that there are 390 million DENV infections annually around the world, of which 100 million are symptomatic[5]. The highest burden of disease is in Asia, which accounts for 70% of infections[23]. The disease is now endemic in more than 100 countries in tropical and subtropical regions of the world, and is the most common arbovirus disease of humans. Australia has had regular outbreaks of DENVs in north Queensland over the past three decades, each initiated by an infected traveller to the region. This is discussed in more detail in an accompanying article (see paper by Pyke, this issue). Dengue is the most common arboviral disease imported into Australia by travellers.

Zika virus

ZIKV emerged from obscurity in 2007 with an outbreak on Yap in the Federated States of Micronesia[3,24] (see paper by Jamal I-ching Sam, this issue). Previously described as a mild self-limiting fever, ZIKV has become associated with major complications, including foetal developmental defects and Guillain-Barré syndrome in adults. ZIKV then appeared in French Polynesia in 2013–14 where severe complications were first reported. In 2015, the virus jumped from the Pacific to Brazil causing a widespread epidemic which involved large numbers of microcephaly cases. The epidemic spread to other countries and peaked in 2016, when it was declared a public health emergency of international concern (PHEIC) by the World Health Organization (WHO)[24,25]. In April 2017, WHO reported 84 countries or territories with current or previous ZIKV transmission[24]. Of Australian mosquitoes, *A. aegypti* is the primary potential vector, so the receptive zone is restricted to north Queensland[26]. However, multiple non-vector routes of transmission have been reported for ZIKV[27]. Of these, sexual transmission is of particular concern with respect to assessing risk of entry of ZIKV, particularly because live virus can persist in semen for over 60 days[27].

Chikungunya virus

CHIKV causes a rapid-onset febrile illness characterised by moderate to severe joint pain, and is often mistaken for dengue.

Three related lineages occur: the East, Central and South Africa lineage (ECSAL), the Asian lineage (AL), and most recently, the Indian Ocean lineage (IOL)[2]. Since 2004, all lineages have shown a propensity to spread and establish in new areas[2,25]. The epidemic vector is *A. aegypti*. However, a mutation in the E1 envelope glycoprotein gene in a circulating ECSAL strain in East Africa around 2005 resulted in the ability of the virus to replicate efficiently in *A. albopictus*, giving rise to the IOL. Significant CHIKV outbreaks caused by this new lineage then occurred on Indian Ocean islands and southern and SE Asia, resulting in millions of infected persons, with infected travellers spreading the virus to many regions of the world, including Italy in 2007 and southern France in 2011. The AL spread from SE Asia or Oceania to the Caribbean in 2013, followed by much of Central and South America. CHIKV-infected travellers have frequently imported the virus into Australia, although there has been no evidence of local transmission[28]. Despite native Australian species, particularly *A. vigilax*, *A. procax*, and *Coquillettidia linealis*, being highly competent vectors[29], the blood feeding behaviour of *A.aegypti* and *A. albopictus*, incriminates these two species as the primary CHIKV vectors[30].

Rift Valley fever virus

RVFV infection of sheep and cattle causes severe and often fatal illness, which can occasionally result in spill over infection of other domestic animals and humans. In 1–2% of infected humans, severe disease manifestations occur, including hepatitis, encephalitis, retinitis, blindness, and/or a haemorrhagic fever; the case fatality rate is approximately 10–20%[8]. The combination of competent vectors in many countries, high level viraemia in domestic animals, and globalisation of travel and trade, make RVFV a considerable worldwide threat to both human and animal health. This was exemplified in 2000-01, when RVFV spread out of Africa for the first time to cause a major epidemic in the Arabian Peninsula. While the risk of RVFV introduction to Australia is low, importation via an infected human could occur, and several mosquito species could play a role in epidemic transmisson[31].

Other exotic viruses

There are several additional exotic arboviruses of which we need to be vigilant for, although they may not represent an immediate threat to either humans or animals. These include Tembusu[32] and related flaviviruses, which cause widespread disease in poultry in Asia, especially ducks, in China, Thailand and Malaysia; and Mayaro virus, an alphavirus from South America which is closely related to CHIKV, and appears poised for urban spread[6].

Will a novel or unexpected arbovirus emerge to surprise the world, a little like ZIKV has done? New arboviruses continue to emerge, but so far none of them have any indication of pathogenic potential. One virus which could yet cause a surprise is the flavivirus Sepik from PNG[33], the closest known virus to YFV. Only time will tell.

Conclusions

There is little doubt that exotic arboviruses constitute a significant risk to human and/or animal health in Australia. To help prevent or mitigate the consequences of their importation, it is critical that ongoing surveillance be maintained and strengthened at all levels, from border protection to human and animal health. Continued strategies to prevent the entry of *A. albopictus* and *A. aegypti* are essential, both across the TS and through border entry points. Finally, research into exotic arboviruses and their vectors needs to be supported and enhanced.

For brevity, this short review has not included the possible emergence of a hitherto unrecognised mosquito-borne arboviral disease, although novel arboviruses are regularly described in the literature. Nor have we examined additional problems associated with the arrival of infected travellers, where there is not only the risk of local virus transmission, but the also the threat to blood safety[28,34]. Similarly, establishment of a veterinary arbovirus could have implications for Australia's livestock disease-free status.

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Endemic Australian arboviruses of human health significance

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Each year many thousands of cases of human arbovirus infection are notified within Australia, acquired either within Australia or when travelling overseas[1]. These cause diseases varying from fever and aches, to debilitating joint disease, to encephalitis and death. The arboviruses endemic to Australia are all maintained in a cycle between mosquitoes (and rarely midges) and a bird or mammalian host[2]. As such, the virus activity is dependent on rainfall and temperature conditions that are conducive to mosquito breeding, and to virus replication and amplification (Figure 1). Those conditions being met, there have to be suitable amplifying animal hosts nearby, and their absence is one of the factors that protects most of the larger urban populations in Australia. Then, of course, humans have to be exposed to the infected mosquitoes to get disease.

The most common arbovirus infections in Australia are due to the alphaviruses, almost entirely Ross River virus (RRV) and Barmah Forest virus (BFV). Serologically proven RRV disease was first described in 1928 in south-eastern Australia[3], but has been postulated to have been responsible for an earlier outbreak in Victoria in 1868[4]. It is transmitted by a range of mosquito species (especially *Culex annulirostris, Aedes vigilax* and *Aedes camptorhynchus*) and the primary amplifying hosts are the macropods (kangaroos, wallabies and euros)[2,5,6]. While the virus has been found in all Australian states and territories, the largest numbers of cases occur in south-eastern Queensland, coastal NSW and in the south-west of WA. However, the highest individual risk of infection is in the less populous area of northern Queensland, northern WA and the NT. Infection is asymptomatic in 25–99% of infected individuals. The acute clinical illness is unpleasant and typically lasts up to 4 weeks but persists much longer in some. Joint pains, muscle pains, and lethargy may continue for months or years, especially if the illness is superimposed on pre-existing joint disease[5,7,8]. This is a common feature of the arthritogenic alphavirus infections worldwide including chikungunya and the Sindbis viruses[5,9], the clinical illness resulting from a complex interaction between the virus and host responses that we are just beginning to understand[5,7,9]. Treatment remains symptomatic[5].

BFV ecology and geographic distribution are similar to those of RRV, but human disease is less common[2,6]. Localised epidemics may occur as it enters new areas, after which it settles down to a pattern of low level endemic activity.

Sindbis virus in Africa and Europe causes an RRV-like illness, but the lineage found within Australia rarely causes illness[2,5,6]. Chikungunya virus has never been detected within Australia despite having circulated within our region over many decades and the presence of a vector, *Ae. aegypti*, in north Queensland.

The diagnosis of the alphavirus infections is mainly serological as any detectable viraemia is generally gone by the time patients see a doctor. For both RRV and BFV[10,11], detection of IgM has proven unreliable as an indicator of recent infection in itself, due to false positive results and the long-term persistence of IgM. Therefore, wherever possible, confirmation of recent infection should be sought by testing of paired acute and convalescent serum samples to show either IgG seroconversion (usually using the enzyme immunoassay tests) or a significant rise in IgG levels (usually using the haemagglutination inhibition titres)[12].

While less common as a cause of illness in Australia, the flaviviruses have the potential to cause much more serious disease[6,13]. Most of the serious disease is due to Murray Valley encephalitis virus (MVEV), with occasional cases due to the Kunjin strain of West Nile virus (KUNV).

MVEV is closely related to Japanese encephalitis virus and is maintained primarily in a mosquito (*Culex annulirostris*)-water bird cycle[2,6,13]. Encephalitis develops in only 1 : 200–1 : 1000 infected people, carrying with it a mortality of 20–25% and persisting neurological deficits in approximately 50% of survivors. Epidemics occurred on the eastern coast of Australia in the early 20th century, then in 1951 and 1974 in the Murray Valley region of the southeast.

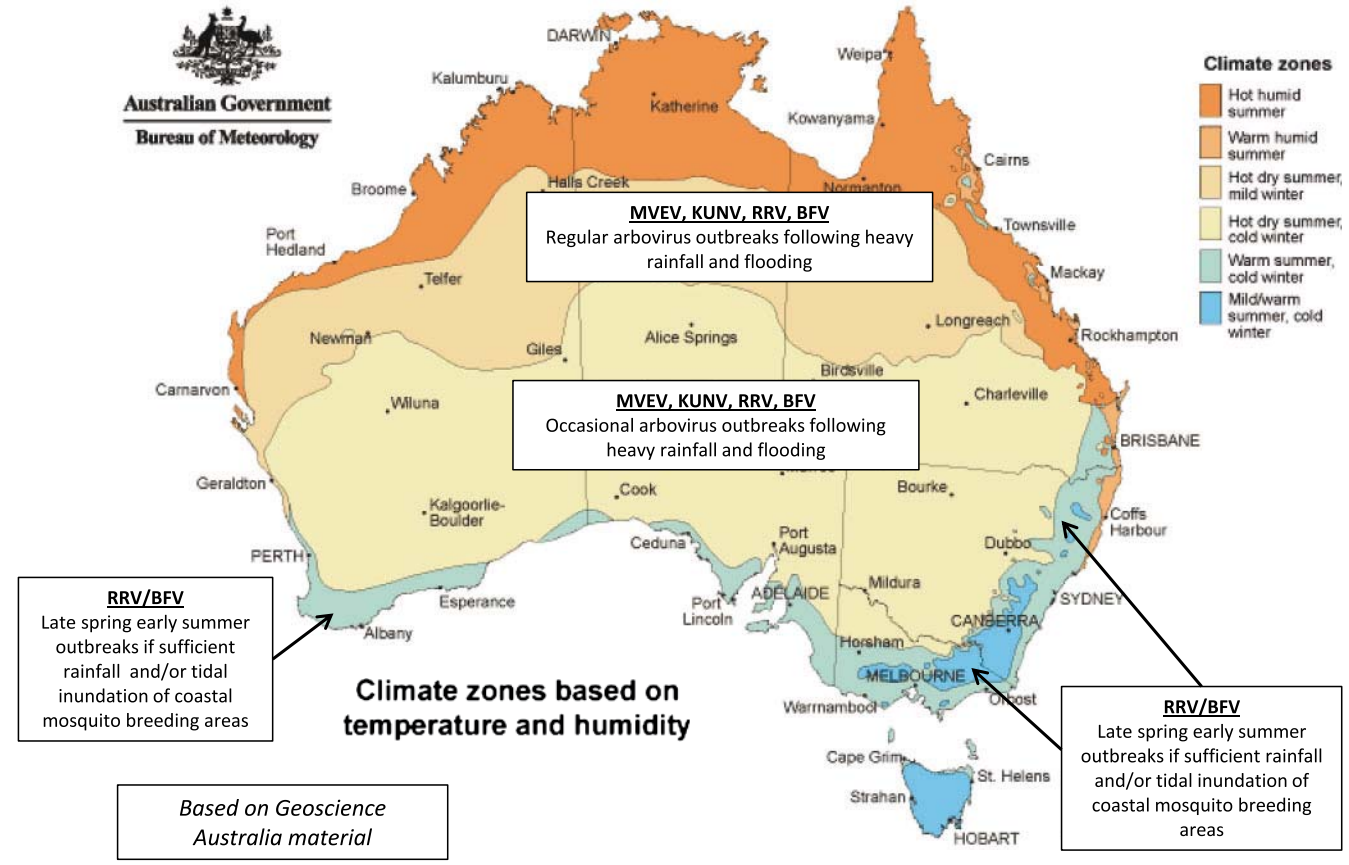

Figure 1. Australian climatic zones, the predominant endemic arboviruses of human health significance that occur in those regions. http://www.bom.gov.au/climate/averages/maps.shtml (accessed 9 February 2018).

Since then activity has been almost completely confined to north western and central Australia[14]. This enzootic focus results in a small number of human cases each year in north western Australia. It was also believed to be the source of all of the occasional larger outbreaks that spread beyond these areas, resulting from carriage of virus by infected migratory waterbirds into areas of flooding. The last major outbreak in 2011 changed that perception and suggested more than one enzootic focus in Australia. It included 16 confirmed human cases and many equine cases, in two separate but simultaneous outbreaks (one in south-eastern Australia and the other in north-western Australia) between March and May[13]. The former was the first substantial activity in the south-east since 1974, while the latter represented a severe but not exceptional season for the north-west. It further demonstrated the significant changes in the epidemiology of MVEV disease since 2000. While the burden of disease and death continues in Aboriginal communities, especially in young children, there has been a relative increase in cases and deaths in non-Aboriginal adults. This change is believed to be due to the spread of the virus into wider areas of WA, increased work and tourist travel in the risk areas, and growth in the resident populations in those areas[13].

Japanese encephalitis virus (JEV) is found widely throughout Asia, including our immediate northern neighbours[2]. It is maintained in a cycle between pigs and mosquitoes, principally *Ae. aegypti* and *Ae. albopictus*. In 1995, JEV entered and established in the Torres Strait Islands (TSI) and has transiently entered the Cape York Peninsula. No human cases have occurred within mainland or territorial Australia since 1998. However, the virus itself is still present in TSI and the threat of introduction into northern Queensland remains, as both *Ae aegypti* and large populations of feral pigs are present.

Dengue virus (DENV) was endemic across northern Australia and some more southerly areas up until the 1940s[15]. Control of its major vector species (*Ae. aegypti)* led to DENV elimination from all of Australia, though the mosquito has remained in parts of northern Queensland, and occasional limited outbreaks occur following introduction of the virus into local mosquito populations by viraemic travellers[16]. Control of the outbreaks relies on control of the vector mosquitoes and avoidance of exposure, including novel biological control programs[17].

The other endemic flaviviruses of Australia that have been shown to infect humans include Kokobera (KOKV), Stratford (STRV), Edge Hill and Alfuy viruses[6]. Human infection is uncommon and diagnosed clinical illness is rare; KOKV and STRV having been described as causing fever and polyarthralgia.

However, it is clear that there are many other arboviruses present within our mosquito populations[18]. Some of these have been shown to infect humans and to cause disease in animal models[19], but further work is needed to properly assess the significance of these arboviruses and those yet to be discovered.

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Biography

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The Asia-Pacific origins of the current outbreaks of Zika virus

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Zika virus (ZIKV) is a mosquito-borne arbovirus from the *Flaviviridae* family, first isolated in 1947 from a monkey in Uganda. In the ensuing decades up to the 2000s, there have been sporadic reports of infections and seropositivity in humans in Africa and Asia[1,2]. The first isolation of ZIKV outside Africa was from *Aedes aegypti* mosquitoes in Malaysia in 1966[3]. Seropositivity has also been reported in wild monkeys in Malaysia[3], although the relevance of this in sylvatic transmission of ZIKV is unknown. These studies suggest that there was endemic and mostly undetected transmission in Asia during this period. Re-emergence from Asia has now brought this relatively neglected virus into the focus of global attention.

Following the first ever reported outbreak in Yap Island, in the Western Pacific in 2007[4], epidemics occurred in several countries in the Pacific between 2013 and 2016, starting with French Polynesia[5], and spreading to 20 other Pacific countries, including New Caledonia, the Cook Islands, Easter Island (Chile), the Solomon Islands, Tonga and American Samoa[1,6]. ZIKV was probably introduced in Brazil in early 2014[7], although cases were only first diagnosed there in 2015, before rapidly spreading to 48 other countries in the Americas and Caribbean[6]. The extent of the epidemics, and their occurrence in continents where ZIKV had never previously been reported was unprecedented. ZIKV usually causes either no symptoms or a mild febrile illness accompanied by rash, myalgia, arthralgia and conjunctivitis. However, the sheer number of affected patients also revealed startling new evidence of the neurotropism of the virus, as increased incidence of neurological diseases such as Guillain-Barré syndrome and congenital Zika syndrome (including microcephaly, and abnormalities of the brain, eye and musculoskeletal system) was seen[6].

There are two genotypes of ZIKV, African and Asian, with the 1966 Malaysian isolate representative of the Asian ancestral strain[8]. Phylogenetic analysis shows that ZIKV sequences of the Asian genotype obtained from Southeast Asian countries between 2010 and 2014 are situated basally to viruses from the recent 2013-2016 outbreaks in the Pacific and Americas[8,9]. This suggests that the outbreaks in the Pacific and subsequently the Americas likely originated from Southeast Asia, where the virus continues to circulate endemically. Interestingly, when chikungunya virus (another mosquito-borne virus) re-emerged between 2004 and 2016, it reached the Americas by a similar route, originating in Southeast Asia before spreading to the Pacific and then to the Americas[5].

The main mosquito vector of ZIKV in the recent outbreaks is *Ae. aegypti*[10], which also transmits dengue and chikungunya viruses. *Ae. albopictus*, which played the key role in the worldwide epidemics of chikungunya virus, was implicated in a ZIKV outbreak in Gabon[11] and has shown high competence for ZIKV in several laboratories[12]. *Ae. albopictus* may therefore potentially be an important vector in future outbreaks. These two *Aedes* species are distributed throughout tropical Asia. However, the full extent of vectors competent for ZIKV is not known, in particular the roles of more locally-relevant species in areas with little or no *Ae. aegypti*. For example, *Ae. hensilli* is by far the most predominant mosquito species on Yap Island and has been shown to be susceptible to ZIKV[13].

Despite the extensive epidemics in the Pacific and the Americas in recent years, there has only been one outbreak reported in Asia, which occurred in Singapore in August 2016 and affected 455 people[14]. Sporadic, autochthonous (locally-acquired) cases - some identified retrospectively - have been reported in Southeast Asian countries including Cambodia, Indonesia, Malaysia, Philippines, Vietnam and Thailand (reviewed by Lim *et al.*[2]). It is unclear why there have not been large outbreaks reported in Asia, apart from Singapore. In the past, this may have been due to lack of ZIKV-specific diagnostics and surveillance, and difficulties in distinguishing ZIKV disease from other tropical illnesses with similar symptoms, such as dengue. However, even Southeast Asian countries with recent focused surveillance have found very low levels (0.02–1.3%) of ZIKV RNA in patients with dengue-like symptoms[2,15], supporting the clinical reports. A possible explanation

for this is pre-existing population immunity against ZIKV in these ZIKV-endemic countries, based on limited historic studies showing 4–44% seropositive rates[2], whereas populations in the Pacific and the Americas were ZIKV-naive. To assess this possibility, contemporary population serosurveys are required in Asia using specific assays to minimise confounding by other flavivius infections.

What is the risk of ZIKV in Australia? Vector competence studies of local mosquito species show that *Ae. aegypti* is the most likely vector, although it is currently confined to northern Queensland[16,17]. *Ae. albopictus*, which is present in the Torres Strait islands but not in mainland Australia, is a potential invasive threat[16]. No or low rates of dissemination and transmission of ZIKV was reported for other common *Aedes* species, including *Ae. notoscriptus*, *Ae. vigilax* and *Ae. camptorhynchus*[16,17]. Between 2013 and 2017, there were 133 imported cases of ZIKV in Australia (although mostly in areas where *Ae. aegypti* does not occur), comprising 63 from Pacific countries, 56 from the Americas or the Caribbean, and 13 from Southeast Asia[18]. This shows that while the main risk is from travellers coming from countries experiencing outbreaks, there is a background risk from travellers from Southeast Asia. Thus, as Australia has competent mosquito vectors and imported cases, and continuing extensive traffic with other countries in the Asia-Pacific region, there remains an ongoing threat of a ZIKV outbreak in Australia[19]. This can be mitigated by continued surveillance for human cases, infected mosquitoes and potential mosquito vectors, and effective vector control programs[20].

Numerous important questions about ZIKV in Asia remain. What is the true burden of ZIKV in Asia? Why has there not been more clinically-apparent disease, such as outbreaks of illness and microcephaly? With the apparent low level of circulation in humans, how is the virus maintained in nature, and what is the role of non-human primates? What were the underlying reasons for its recent global emergence from Asia, and can these be predicted for prevention of future outbreaks? Even as the WHO declared an end to ZIKV as a Public Health Emergency of International Concern in November 2016, there is still much urgent work to be done.

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Biography

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The origins of dengue outbreaks in northern Queensland, Australia, 1990–2017

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Dengue is one of the world's major infectious mosquito-borne diseases and although not endemic in Australia, is a significant public health concern. Queensland is vulnerable to outbreaks of dengue viruses (DENVs) and indeed, due to endemic populations of the mosquito vector *Aedes aeypti*, has been the only state since the 1950s to record local transmission. Determining DENV outbreak origins, and monitoring strain movement and diversity greatly assists outbreak management. It also confirms epidemiological links and potentially identifies incursions of rare or highly pathogenic viruses. There have been 73 DENV outbreaks recorded in northern Queensland within the past three decades and it has been the role of Public Health Virology, Department of Health, Queensland Government, to provide DENV genotyping and characterisation to facilitate this essential surveillance. This review summarises the likely origins of the recent northern Queensland outbreaks and describes the complex dynamics of DENV genotypic diversity that have characterised local transmission events.

Boasting tropical and subtropical climates, Queensland attracts more than two million overseas visitors annually[1]. The increased frequency and affordability of travel and expanded business and trade have contributed to the influx of travellers to Queensland, including visitors and returning Queensland residents coming from Southeast Asia, the western Pacific and other dengue virus (DENV) endemic regions. Populations of the primary DENV mosquito vector *Aedes aegypti* are present on mainland Queensland and the Torres Strait islands[2]. Another DENV vector, *Ae. albopictus*, is currently restricted to the Torres Strait islands[3]. Thus, Queensland is prone to DENV outbreaks and other arthropod-borne virus disease threats, such as chikungunya and Zika viruses[4,5].

At least 73 DENV outbreaks, each involving one or more locally acquired case(s), have been recorded in northern Queensland between 1990 and 2017[6–14] (*Dianne Brookes, Tropical Public Health Unit (TPHU) Cairns, and Jan Humphreys, TPHU Townsville, unpublished data*) (Figure 1*a*). Whilst all four DENV serotypes (DENV 1–4) have caused outbreaks (DENV-1, 36%; DENV-2, 42%; DENV-3, 12%; DENV-4, 10%), DENV-1 and DENV-2 account for almost 80% of outbreaks. Major outbreak epicentres included the Torres Strait islands (northernmost outbreak location), Cairns, Townsville, Port Douglas, Mossman, Kuranda, Mareeba, Innisfail, Tully, Ingham and Charters Towers (furthest southern/western outbreak location). Recent outbreak trends for the period between 1990 and 2016, showed a steady increase in the recorded number of outbreaks, despite variable numbers of locally acquired cases during the same period and a recent decline in cases between 2011 and 2016[8]. Determining the origin and genetic relatedness of DENV strains plays a key role in disease mitigation strategies, by affirming epidemiological links and providing early warning of sustained transmission or, importantly, endemicity, if it was to occur.

For most DENV outbreaks, index cases are not known and local transmission is normally identified after laboratory confirmation of DENV positive patient(s) who have no history of travel abroad. Therefore, the determination of outbreak origins has become increasingly reliant on viral nucleotide sequencing and phylogenetic molecular techniques. Nucleotide sequencing and phylogenetic analysis of complete DENV envelope (E) genes was used to establish the possible geographical origins of Australian outbreak strains and compare their genetic diversity with other imported and globally circulating DENVs. Figure 1*b* summarises the number of outbreaks between 1990 and 2017 and their most likely overseas geographical sources based on phylogenetic analyses. The data include primary outbreaks initiated by viraemic international travellers and secondary outbreaks likely resulting from further spread of the same virus strain(s) to new locations (for example, Cairns to Townsville). For the current report, secondary outbreaks were also assigned the likely overseas source to highlight the impact of respective overseas incursions on transmission and disease.

Likely origins of outbreaks were determined following sequence alignment and phylogenetic tree analysis as previously described[8,9]. Outbreak strains were considered to have been sourced from a particular region after demonstrating very high percentage sequence identities (≥99%) and evolutionary relatedness with the strain identified and sequenced from the index case (where available) or clusters of strains (two or more) which were themselves

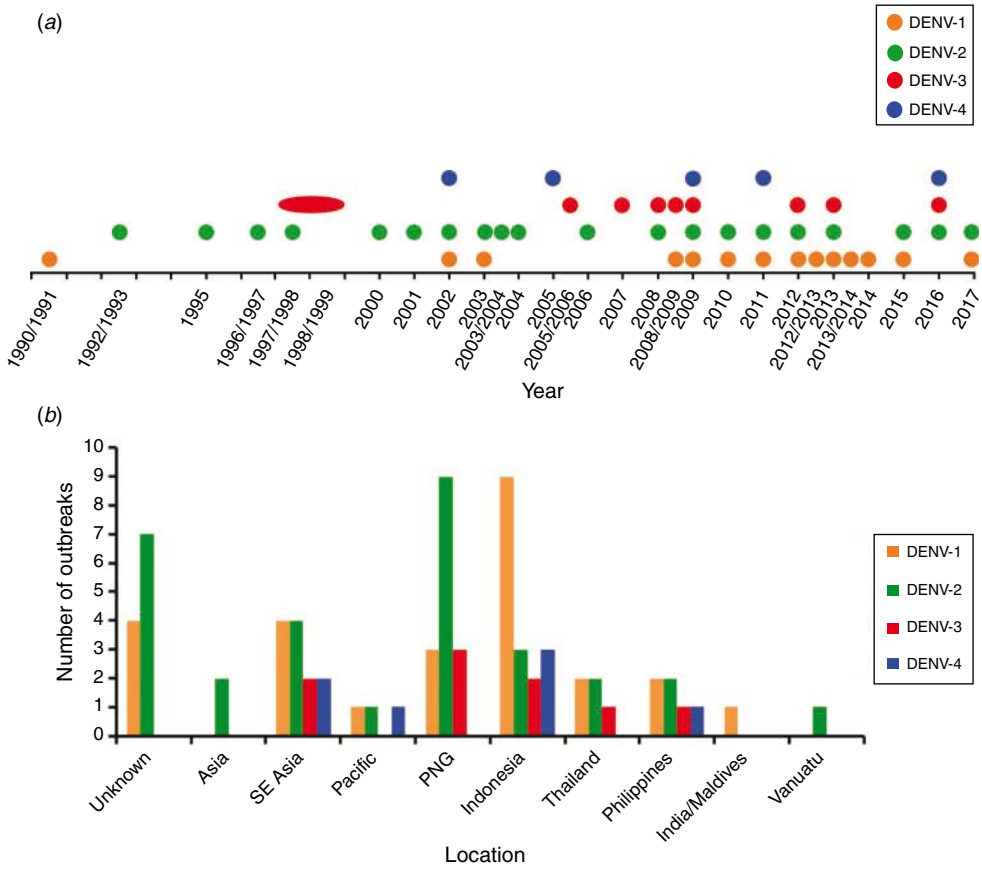

Figure 1. Northern Queensland dengue virus (DENV) outbreaks, 1990–2017. (*a*) Timeline summarising the occurrence of northern Queensland outbreaks between 1990 and 2017 based on circulating DENV 1–4 serotypes. The sustained DENV-3 outbreak in 1997–1999 is represented by the elongated ellipse symbol. (*b*) The number of northern Queensland outbreaks occurring between 1990 and 2017 are plotted against their most likely geographical source. The data include secondary outbreaks that resulted from further spread of imported DENV strains into new Queensland locations.

closely related and recently circulating in that region. Specific country categories included Papua New Guinea (PNG), Indonesia, Thailand, the Philippines, Vanuatu and India/Maldives. For some outbreaks, a broader regional classification (Asia, Southeast Asia and the Pacific) was assigned if related phylogenetic clusters displayed an ambiguous geographical source (contained sequences from several countries of that region) or the outbreak strain was only closely related to one other DENV strain. Unfortunately, the origin of 11 outbreaks (4 DENV-1 and 7 DENV-2) could not be determined due to absence of isolates or DENV PCR positive patient samples for sequencing.

The largest number of outbreaks by country involved imported strains from Indonesia ($n = 17$; 23%). Indonesia has been shown previously to be a major source of imported DENVs into Queensland, Australia[9], and likely reflects the high frequency of travellers from this country, in particular, Bali. Within the last seven years, the annual number of visitors who travelled from Indonesia to Queensland was ≈20 000[1]. PNG accounted for the second highest number of outbreaks ($n = 15$; 21%) and remains a major source of imported DENVs into northern Queensland[8]. Collectively, Southeast Asia (including Indonesia, the Philippines and Thailand) was responsible for 40 outbreaks (55%) and continues to be the highest contributor of outbreaks, due to all four DENV serotypes[9].

In addition to the co-circulation of multiple serotypes, some concurrent or closely successive outbreaks were caused by a single DENV serotype, and it was only after performing phylogenetic analysis, that involvement of one or more viral strain(s) could be determined. Occasionally, this further assisted the identification of secondary outbreaks in the absence of clear epidemiological links. In 2003–2004, three unrelated strains of DENV-2 (two from PNG and one from Thailand) were responsible for outbreaks in north Queensland resulting in ≈890 cases, including two haemorrhagic cases and one death[7]. Phylogenetic analysis revealed that three separate DENV-2 strains were responsible for outbreaks in (1) Cairns, early 2003, and two later subsequent outbreaks in Townsville, 2003, (2) Cairns, late 2003, and (3) the Torres Strait islands, late 2003, followed by an outbreak in Cairns, early 2004[7]. Similarly, in 2012–2013, four different DENV-1 strains from Southeast Asia (including one from Thailand and two from Bali) were responsible for separate outbreaks in (1) Cairns/Townsville/Ingham, (2) Townsville, (3) Innisfail and (4) Cairns.

Sustained transmission of DENV strains over long, uninterrupted periods increases the risk of endemicity. Although northern Queensland outbreaks have not resulted in endemicity to date, several prolonged outbreak events, extending from summer through winter have occurred, including DENV-3 in 1997–1999 (70 weeks duration, 498 cases) originating from Thailand[11], DENV-2 in 2003–2004 (69 weeks duration, ≈500 cases) originating from PNG[7] and DENV-1 in 2012–2013 (29 weeks duration, ≈170 cases) originating from Thailand *(Dianne Brookes, TPHU Cairns, and Jan Humphreys, TPHU Townsville, unpublished data)*.

In the absence of a suitable vaccine, concerted mosquito control aimed at supressing *Ae. aegypti* and *Ae. albopictus* populations is the primary strategy for counteracting the DENV threat in northern Queensland. Biological approaches such as the release of *Wolbachia* infected *Ae. aegypti* are also being trialled in several high risk areas to potentially reduce transmission[15]. Importantly, constant vigilance and surveillance to detect DENV introduction and identify potential geographical sources of strains is crucial for early case identification, accurate monitoring of strain dissemination and disease epidemiology. These factors also ensure that limited mosquito control resources are deployed where they can have the greatest impact, thereby further confining DENV outbreaks in northern Australia.

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Biography

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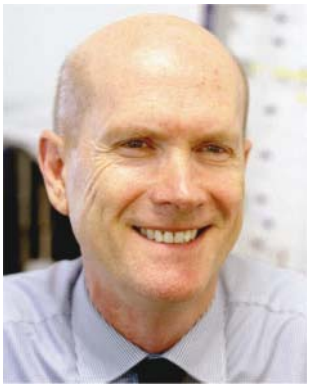
Arboviruses in pregnancy: consequences of maternal and fetal infection



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Epidemics and localised outbreaks of infections due to arthropod borne (arbo) viruses, have been described for hundreds of years. Few viruses to date are known to transmit from mother to fetus, causing either teratogenic effects or fetal demise (see recent reviews Charlier *et al.*[1] and Marinho *et al.*[2]). Many arboviruses are zoonotic but there appear to be few parallels between the effect of these viruses following human or animal infection during pregnancy. Higher rates of MTCT (mother to child transmission) may be seen (1) where herd immunity is reduced, either because virus is newly introduced into a population (as occurred in Brazil with ZIKV), or where the virus has only recently become endemic (as occurred with West Nile virus (WNV) in the USA in the 1990s), (2) where the arthropod vector is present, (3) where the vector transmits virus efficiently, and (4) in groups of pregnant women exposed, allowing transmission[3].

Transmission

There are ~200 million pregnancies annually worldwide, with 90% in regions where arboviruses are endemic: often these are regions with reduced diagnostic capacity[4]. Arbovirus epidemics have the potential for significant outbreaks of disease in pregnant women and their unborn infants, as evidenced by ZIKV in Brazil where ~17 000 pregnant women had been infected by April 2017[5]. Except for DENV and ZIKV, much is unknown about MTCT of arboviruses, the antenatal and perinatal effects of infection are not well described, and mechanisms of viral teratogenicity are only recognised for some arboviruses[1]. This means links between adverse pregnancy outcomes and arboviral infections are problematic, particularly where the clinical outcomes are mild, develop over time or are subtle, such as failure to thrive, and mild neurodevelopmental delay.

Effects on the mother

Pregnant women suffer similar symptoms and signs as non-pregnant women, although in general terms, they have a higher risk of more severe effects of viral infection due to the immunosuppression of pregnancy[6]. The typical syndromes include short incubation time (<1 week), fever, malaise, rash, encephalitis/meningoencephalitis, or haemorrhagic fever (Table 1). Maternal and other infections are asymptomatic in 70–80% of infected individuals, excepting CHIKV (>95% symptomatic often with severe arthritis)[8], Yellow fever virus (YFV, ~50% symptomatic)[9], and ZIKV Asian lineage (~50% symptomatic with prominent pruritic rash and arthritis)[10]. Infection in pregnancy with DENV results in increased maternal mortality and severe illness (OR 3.38, 95% CI 2.1–5.42), increased caesarean section and increased postpartum haemorrhages[11].

Effects on the fetus and newborn

It remains uncertain whether arboviral infection causes fetal damage through direct fetal infection when it occurs, and/or as a result of placental dysfunction causing fetal damage through malnutrition and reduced function, as occurs in other congenital infections[12,13]. The fetal and neonatal disease caused by arbovirus infection includes fetal demise, premature birth, and neurodevelopmental defects from viral teratogenicity. The latter has been recognised particularly with ZIKV and Venezuelan equine encephalitis (VEE) virus infections, as summarised in Table 1, and in recent reviews[1,2]. Ross River Virus infection may cause fetal infection, although it is uncertain if fetal disease results[14–16].

DENV is the most common arboviral infection globally, and is associated with severe illness *in utero*, including premature birth (21% versus background rate 11.5%), and miscarriage particularly during the first trimester (T1), and fetal death (13% versus background *in utero* death rate of 1.8%)[11]. Maternal symptomatic presentation correlates with rates of fetal demise, with no clear association with serotype. The immune enhancement syndrome seen with second infection from a serotype discordant with a prior DENV serotype infection is also seen in pregnant women, and the fetus[17]. Other arboviruses appear to less frequently affect the fetus[18,19].

Table 1. Some arboviruses of significance in pregnancy.

Virus	Virus family	Distribution	Clinical presentation acute disease	Major fetal effects
Chikungunya (CHIKV)	Alpha (Toga)	• 1952 Initially Tanzania • 1958 Asia • 2013 Americas • 2014 Brazil	IP 2–10 days (1–12 days) Duration 7–10 days • Fever • Arthralgia • Rash	VT 27–48% • Newborn fever, irritability • Postnatal global neurodevelopmental delay • Fetal demise • TG
Dengue (DENV types 1, 2, 3, 4)	Flavi	• DENV – like for centuries • 1943 isolated Japan (DENV1) • 1945 Hawaii (DENV2)	IP 3–10 days • Fever • Rash • Myalgia • 3 phases • 2015 Live attenuated vaccine	VT • MC • Fetal demise • Prematurity • TG not reported
Japanese Encephalitis (JEV)	Flavi	• Asia for centuries	IP 5–15 days • Fever • Myalgia • Headache • Vaccine	VT rare • Fetal demise • Neurological infection
West Nile Virus (WNV)	Flavi	• Africa • Asia • Middle East • 1999 USA • 2000 Mediterranean France	IP 3–14 days • Asymptomatic 80% • Meningoencephalitis	VT rare • TG with brain malformation • Retinal scarring, neurological disease • Miscarriage T1 • T3 transmission with perinatal neurological disease
Yellow fever (YFV)	Flavi	• Africa for centuries • 1700s Americas • 2015 Angola urban • 2017 Brazil	IP 3–6 days • Fever • Myalgia • Headache • 1930 live attenuated vaccine	PT • Newborn fever, hepatitis, death
Zika (ZIKV)	Flavi African Asian lineages	• 1947 Initially Uganda • 1954 Nigeria • 1969 Outside Africa • 2007 Micronesia (Yap) • 2015 Brazil	IP 4–5 days 80% asymptomatic Clinically as for non-pregnant • Rash • Fever • Arthralgia, myalgia • Conjunctivitis	VT Fetal disease worst T1 • Neurological syndromes • Microcephaly • Fetal demise

VT, vertical transmission; MC, miscarriage; PT, perinatal transmission; IP, incubation period range from systematic review Rudolph *et al.*[7]; TG, teratogenicity; T, trimester of pregnancy.

The most prominent recent arbovirus-induced fetal malformation has been ZIKV infection, particularly with the Asian lineage in the Americas. This outbreak, initially most severe in Brazil, has been discussed in this journal[20], and elsewhere by those at the forefront of epidemic response[21–23], and more recently in the United States of America[24]. It has become clear that regarding ZIKV: (1) infection in pregnant women may result in fetal demise, intrauterine growth restriction, microcephaly, and neurological abnormalities[25]; (2) disease includes abnormalities of the eye, hearing, craniofacial area, musculoskeletal system; (3) a range of pathological changes occur, with brain abnormalities present in 1–13% of infants of mothers with ZIKV infection; (4) the highest, but not only, risk period is T1, T2; (5) apparently healthy neonates born following maternal ZIKV infection may have longterm neurodevelopmental adverse outcomes; (6) not all outbreaks produce identical phenotypes of fetal and neonatal disease, although infections during pregnancy predominantly result in neurological adverse outcomes of varying severity; (7) screening for ZIKV in pregnant women has

been recommended for at-risk populations[5]; and (8) ZIKV is spread sexually, unusually for an arthropod borne virus, and such spread may result from seminal ZIKV present for up to 120 days in semen, albeit at low titre[26].

The diagnosis of arbovirus infections in pregnancy utilises standard molecular methods (predominantly arbovirus specific RT-PCR of blood, cerebrospinal fluid, urine, saliva) and serology (predominantly EIA for detecting virus-specific IgG, IgM), with evidence of seroconversion where specimens are available. There are a range of commercial diagnostic assays available but they vary significantly in their sensitivity and specificity and require care when interpreting results[27].

Control of arbovirus infections

Many editorials discuss that recent ZIKV outbreaks highlight the effects of arboviruses on pregnant women, rather than ZIKV being the only cause of virus-induced congenital malformation[28]. Of the arboviruses known to pose a risk to pregnancy (Table 1), there are vaccines against YFV, JEV and WNV, with vaccines against DENV, CHIKV and ZIKV in various stages of development and testing. Sexual transmission of these viruses (particularly ZIKV) including from asymptomatic hosts, poses a significant challenge. Guidelines are available to inform efforts to minimise this risk. As public health and vaccine efforts are enhanced, it is hoped control of MTCT and neonatal disease from arboviruses will improve.

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Biography

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Neurological disease caused by flavivirus infections

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The *Flavivirus* genus contains dozens of species with varying geographical distributions. Most flavivirus infections in humans are asymptomatic or manifest as a non-specific febrile illness, sometimes accompanied by rash or arthralgia. Certain species are more commonly associated with neurological disease and may be termed neurotropic flaviviruses. Several flaviviruses endemic to Australia and our near northern neighbours are neurotropic, such as Murray Valley encephalitis virus, West Nile (Kunjin) virus and Japanese encephalitis virus. Flavivirus neurological disease ranges from self-limiting meningitis to fulminant encephalitis causing permanent debilitating neurological sequelae or death. The recent Zika virus outbreak in South America has highlighted the dramatic effects of flavivirus neurotropism on the developing brain. This article focuses on the neurotropic flaviviruses endemic to Australia and those of international significance.

Neurotropic flaviviruses of Australia

Murray Valley encephalitis virus (MVEV) and Kunjin virus (WNV_{KUN}), a clade of West Nile virus (WNV), are endemic to Australia, causing sporadic neurological disease and occasionally outbreaks associated with increased mosquito activity during the wet season. Both viruses are maintained in mosquito-waterbird cycles primarily in northern Western Australia, the top end of the Northern Territory and possibly northern Queensland[1,2]. However, heavy rainfall with flooding may lead to spread of these viruses into normally arid areas, carried by waterbirds[3]. There were several outbreaks of MVEV on the east coast of Australia in 1951 and 1974 along the Murray-Darling River basin that gave the virus its name. The last widespread outbreak of MVEV occurred in 2011, with 17 cases across WA, NT, SA and NSW.

It is estimated that between 1 : 150 and 1 : 1000 of those infected with MVEV will develop encephalitis, which may manifest as seizures, altered mental state, focal neurological abnormalities, coma, or flaccid paralysis[1]. Characteristic thalamic involvement may be seen on brain MRI (Figure 1) but the changes may take several days to develop. The case fatality rate is 15–30% with long-term neurological sequelae occurring in 30–50%[4]. WNV_{KUN} follows a similar

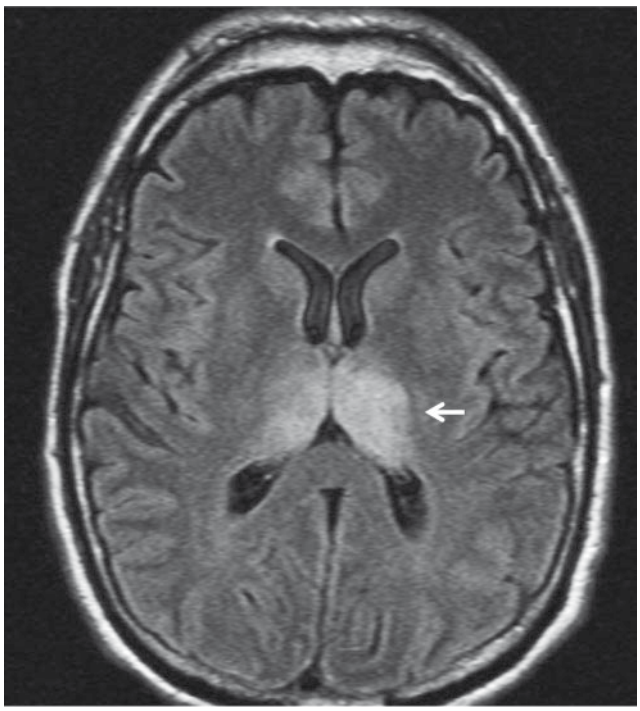

Figure 1. MRI brain scan of Murray Valley encephalitis 8 days after illness onset. FLAIR MRI scan demonstrating increased signal intensity in the thalami (arrow).

10.1071/MA18029

epidemiological and clinical pattern to MVEV, although neurological disease tends to be milder, with no recorded cases of fatal infection[3].

The public health management of MVEV and WNV_{KUN} includes surveillance using antibody testing in sentinel chicken flocks and, more recently, detection of viral RNA in trapped mosquitoes. These data serve as an early warning system for increased flavivirus activity and potential increased risk of human cases to prompt timely public health advice to the public for mosquito avoidance measures and to medical services for appropriate diagnostic test ordering[5].

Neurotropic flaviviruses outside Australia

The most important neurotropic flaviviruses for human health worldwide are Japanese encephalitis virus (JEV), WNV and Zika

Table 1. Distribution, clinical features, vectors and vertebrate hosts of selected neurotropic flaviviruses.

Flavivirus	Geographical distribution	Incubation period	Neurological manifestations	Arthropod vector	Vertebrate host(s)
Murray Valley encephalitis virus	North-western Australia Occasional outbreaks in other parts of Australia Papua New Guinea	5–15 days	Seizures, altered mental state, focal neurological abnormalities, coma, acute flaccid paralysis	*Culex annulirostris*[19,20]	Waterbirds
West Nile virus	Asia Europe Africa Middle East North and South America	2–14 days	Meningitis, encephalitis, altered mental state, extrapyramidal symptoms, focal neurological abnormalities, acute flaccid paralysis	*Culex pipiens*, other *Culex* spp.	Birds
WNV-Kunjin virus clade	North-western Australia Occasional outbreaks in other parts of Australia Papua New Guinea	5–15 days	Similar to Murray Valley encephalitis but usually milder	*Culex annulirostris*	Waterbirds
Japanese encephalitis virus	Asia Western Pacific islands	5–15 days	Meningitis, encephalitis, focal neurological abnormalities, seizures	*Culex tritaeniorhyncus*, other *Culex* spp.	Waterbirds, pigs
Zika virus	Asia Africa Central and South America Caribbean	3–14 days	Congenital Zika syndrome, Guillain–Barré syndrome, meningoencephalitis, transverse myelitis	*Aedes aegypti*, *Ae. albopictus*	Humans
St Louis encephalitis virus	North and central America	7–14 days	Meningitis, meningoencephalitis, cranial nerve dysfunction	*Culex* spp.	Birds
Tick-borne encephalitis virus	Northern and eastern Europe Northern Asia	7–14 days	Meningitis, encephalitis, transverse myelitis, acute flaccid paralysis	*Ixodes persulcatis*, *Ix. ricinus*	Small mammals, birds
Powassan virus	Canada USA Russia	7–14 days	Encephalitis, paralysis, altered mental state, seizures	*Ixodes cookei*, *Ix. persulcatis*, *Haemaphysalis longicornis*	Small mammals, birds

virus (ZIKV). Infection with these viruses should be suspected in travellers returning from areas where these viruses are endemic who develop compatible symptoms within the relevant incubation period. Depending on the travel and exposure history, the differential diagnosis may include other neurotropic arboviruses, such as alphaviruses and bunyaviruses.

JEV is widespread in Asia with over 50 000 cases of JEV encephalitis reported every year, mostly in children, with a case-fatality rate of 20–30% and neurological sequelae in 70% of survivors[6]. JEV vaccination programs have been implemented in several countries including China, India, Japan and Thailand, though data on the effectiveness of these programs is still emerging.

WNV was endemic only to Africa and Eurasia until it was introduced to New York in 1999, then spread across North and South America[7]. Since 2007, a yearly average of over 1000 cases of neurological disease has been reported in the USA[8]. Between 1 : 150 and 1 : 240 of those infected develop neurological disease[9,10], with the risk increasing with age[11].

ZIKV was restricted to Africa and Southeast Asia until outbreaks occurred in Micronesia in 2007, French Polynesia in 2013 and Brazil in early 2015. By July 2016, more than 150 000 cases were reported in Brazil and the virus had spread to central and South America and the Caribbean[12,13]. It is estimated that 80% of Zika infections are asymptomatic. An increase in cases of Guillain-Barré syndrome (GBS) was reported following the outbreak in French Polynesia and subsequently also reported in Brazil, Colombia, and El Salvador[13,14]. Less commonly, ZIKV may cause meningoencephalitis and transverse myelitis in adults.

During the 2015 outbreak in Brazil, an increase in congenital microcephaly was noted and suspected to be linked to maternal Zika virus infection. Retrospective investigation of the outbreak in French Polynesia outbreak also found an increase in microcephaly notifications. Further data support a causal link between maternal ZIKV infection and congenital Zika syndrome, manifestations of which include microcephaly, obstructive hydrocephalus, cerebral calcifications, congenital contractures, and hypertonia[13,15–17]. The full neurological spectrum of congenital Zika syndrome will become clearer with longer term follow-up studies of the ZIKV infected infants.

Dengue virus has the most extensive geographical distribution of all the flaviviruses known to infect humans and is the most frequently diagnosed flavivirus infection in travellers returning to Australia. While severe dengue infection usually manifests as shock from plasma leakage or haemorrhage, rare neurological complications of dengue such as encephalitis, meningitis, transverse myelitis and Guillain–Barré syndrome have also been described[18].

See Table 1 for further information on selected neurotropic flaviviruses.

Laboratory diagnosis of flavivirus neurological disease

IgM antibody may be the earliest serological marker in flavivirus encephalitis[21] and, in patients who develop an encephalitic illness, detection of flavivirus IgM antibody in serum confirms the diagnosis[22]. Recent flavivirus infection is usually confirmed by IgG or haemagglutination-inhibiting antibody seroconversion or a significant titre increase, or detection of flavivirus by cell culture or RT-PCR. Interpretation of flavivirus antibody titres is made more difficult in those with immunological memory from previous flavivirus exposure due to ‘original antigenic sin’. Given the high proportion of mild or asymptomatic cases, confirmation of infection with a neurotropic flavivirus does not necessarily indicate neurological disease. Where invasive specimens are available, definitive diagnosis can be made by detection of flavivirus in cerebrospinal fluid (CSF) or brain biopsy by cell culture or RT-PCR, or by detection of specific flavivirus IgM antibody in CSF.

When requesting diagnostic testing for flavivirus, it is important to consider the range of likely infecting flaviviruses, which is seasonally and geographically dependent. Relevant clinical and travel history should be provided to the testing laboratory to assist with test selection and interpretation.

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Biographies

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Results of ASM Members' survey

Recently we asked members to participate in a brief survey. Five hundred members took the time out, not only to answer the questions, but also to write comments. This was a fantastic result and we are most grateful to those of you who answered the questions. This kind of information and insight is most useful and helps guide our decision-making.

Although survey participants have already been given this information I thought other members might be interested in seeing the outcomes.

Regarding *Microbiology Australia*

Q1. 75% of respondents received a printed copy of *Microbiology Australia* (MA).
Q2. 66% of respondents thought we should maintain the print version of MA.
Q3. 70% of respondents found it easier and more convenient to read a printed copy of MA than to read it online.
Q4. 35% of respondents always read MA from cover to cover.
Q5. 87% of respondents considered receiving MA, either as a hard copy or online, as an important membership benefit.

Our response?

We have just re-signed our contract with Team Macreadie who put the four editions of MA together, and are about to re-sign with CSIRO who are responsible for both the print and electronic versions. The survey results give us confidence that this is something members want and consider a worthwhile expenditure item.

Regarding our annual scientific meeting

Q6. 30% of respondents regularly attended the ASM scientific meeting.
Q7. 25% of respondents did not attend because they did not find it was relevant or helpful to them in their work.
Q8. 57% of respondents did not attend because of the total cost.
Q9. 21% of respondents did not attend because their workplace manager would not support them taking time off work.
Q10. 40% of respondents did not attend because their workplace manager would not support them financially to attend.

Our response?

We have introduced a very generous incentive to come to the Brisbane meeting this year in response to the response about cost but regret that we cannot do much about travel and accommodation costs.

Regarding membership

Q11. 77% of respondents thought that ASM membership represented good value for money.

Our response?

A sigh of relief that our hard work has not been in vain but a desire to make the figure 100 not 77.

We are still working through the two hundred comments and finding some excellent suggestions and ideas to make ASM more relevant and useful!

Congratulations to Alice McCarthy, MASM, who works with the Forensic and Analytical Science Service and who won a year's free membership to ASM for participating.

And make sure you apply for one of the 100 reduced price registrations for the Brisbane conference!

Executive standing committee

Arbovirus infections of animals: congenital deformities, encephalitis, sudden death and blindness

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Viruses from five different taxonomic families have been shown to be the cause of disease outbreaks in either domesticated or wild animals. These include viruses spread by both mosquitoes and biting midges from the genus *Culicoides*, especially *C. brevitarsis*. A number of arboviruses also present significant impediments to the international movement of live animals, semen and embryos.

Alphaviruses

Ross River virus is intermittently incriminated as a cause of fever, lethargy and arthralgia in horses. However, there are few cases supported by convincing laboratory confirmation.

Flaviviruses

Infection of dogs, chickens and horses with Murray Valley encephalitis virus (MVEV) and West Nile Virus (WNV) occurs intermittently in regions where there are large mosquito populations. However, disease is rare and has only been reported in horses. In 2011 there was an outbreak of neurological disease in horses in southern Australia[1], with MVE identified in five horses that died in Victoria[2]. A few MVE cases were also confirmed in NSW and SA. Otherwise there have only been a few sporadic cases confirmed[3,4] with anecdotal reports of others[5].

Concurrent with the MVE cases in 2011 in NSW there was an extensive outbreak of encephalitis in horses due to WNV, with approximately 1100 cases observed and a case fatality rate of 11% (A.J. Read *et al*., unpubl. data). A number of cases were also observed in SA and Victoria. This large outbreak had a number of unusual features. This was the first epidemic of equine neurological disease due to WNV in Australia. Unlike the 1999 outbreak in the USA, no cases were observed in birds. The NSW 2011 outbreak was due to a variant strain of WNV of Australian origin[6]. The geographic distribution was also unusual. While there were cases throughout the Riverina Region (where the first outbreak of MVE had occurred in people), cases were distributed throughout the Central and Northern Tablelands (extending from Forbes to Narrabri), with a large number of cases in the Hunter Valley, the Sydney Basin and Illawarra region. WNV had never been detected on the eastern side of the Great Divide previously.

Orbiviruses

The *Orbivirus* genus of the family *Reoviridae* contains a large spectrum of viruses that cause disease in livestock and wildlife. Most are transmitted by biting midges but some are spread by mosquitoes and ticks. Viruses belonging to the bluetongue serogroup (BTV) have an extremely high profile globally, either as a cause of disease, mainly in sheep, or through international trade restrictions. Of the 29 serotypes, 12 have been detected in Australia. Most are detected intermittently in the far north of Australia, although serotypes 1 and 21 are widespread along the east coast from Cape York south to the Hunter Valley region, with occasional movement onto the NSW south coast as the distribution of their vector, *Culicoides brevitarsis*, fluctuates.

In northern Australia incursions of novel genotypes of BTV have been detected, with nucleic acid sequencing indicating that these have been introduced by long distance dispersal of vectors from South-East Asia.

Australia has not experienced the severe outbreaks with large scale mortalities as have occurred in Africa, the Mediterranean basin and the USA. Experimental infection studies in sheep have shown that some of the serotypes of BTV found in Australia are asymptomatic while others can produce moderately severe disease. The absence of disease in Australia is largely due to the lack of overlap between the distribution of *C. brevitarsis* and sheep flocks. The presence of BTVs is the greatest impediment to the export of live animals, semen and embryos from ruminant and camelid species. Consequently, because the principal vector has well defined geographical limits determined by seasonal influences,

animal health authorities in Australia have established a 'bluetongue free zone' to support the export of ruminants and germplasm. The National Arbovirus Monitoring Program[7] (NAMP), based on systematic sampling of sentinel cattle and vectors, underpins this zoning strategy.

Viruses belonging to the epizootic haemorrhagic disease of deer (EHDV) group, which are closely related to BTVs, are also widespread in Australia and mainly infect cattle and deer. Disease has never been observed in Australia.

Between 1994 and 1996 an unusual epidemic was observed in kangaroos, characterised by varying degrees of blindness. The epidemic commenced in western NSW in summer 1994, spread south and west into Vic and SA, and, after interruptions to transmission during winter and spring, eventually reached southern WA in 1996. Disease affected mostly western grey kangaroos, but eastern grey and red kangaroos and even euros were involved. Blindness was shown to be due to chorioretinitis and mild encephalitis[8]. The disease was successfully reproduced after the inoculation of eastern and western grey kangaroos with isolates of Wallal virus[9]. The vector was not proven but this virus has been isolated from several *Culicoides* species.

A syndrome of sudden death almost always without any prior signs, has occurred on several occasions in captive populations of Tammar wallabies, held mainly in research institutions in eastern NSW and southern Qld. The first outbreak in 1998 decimated research populations in the Sydney region with more than 230 animals affected[10]. The gross pathology was very similar to severe acute bluetongue where vascular damage results in extensive congestion and haemorrhage. Eubenangee virus, a close relative of BTV, was isolated from these animals. However, the vector involved remains unclear.

In the Northern Territory between 1999 and 2004, a similar syndrome of acute death in northern black wallaroos was associated with infection with a Wallal group virus, blindness in agile wallabies with a Eubenangee group virus and death in red kangaroos with a Wongorr group virus (L. Melville *et al*., unpubl. obs.).

Finally, the equine population is not spared from infections with orbiviruses. In Australia, Elsey virus is a mosquito-borne orbivirus that has been associated with encephalitis in several horses in the NT and Qld[11].

Bunyaviruses

The Bunyaviruses (Family *Peribunyaviridae*, genus *Orthobunyavirus*) comprise the largest group of vector-borne viruses with members transmitted by mosquitoes, biting midges, sandflies and ticks. Akabane virus is the most prominent to infect animals in Australia and was the cause of a large outbreak of congenital deformities in calves in 1974, with approximately 8000 cases in NSW. Outbreaks occur at intervals of about 15–20 years. Climatic variations have a profound impact on the distribution of the principal midge vector, *C. brevitarsis*, either as an outcome of higher rainfall and mild winters resulting in greater dispersal of midges and virus, or temporary reductions in population immunity in times of drought as a result of restricted midge and virus distribution[12]. The major epidemics have been mostly confirmed in regions of NSW that adjoin the endemic North and Central Coast regions[13]. The impact of the virus is greatest in cattle due to their long (9 month) gestation but sheep and goats can also suffer losses. The type of defect depends on the stage of gestation at which the dam was infected[14]. In cattle infected late in gestation calves can be born with encephalitis. Infection in the fifth and sixth months of gestation results in arthrogryposis and scoliosis, with the severity and incidence of abnormalities greater in earlier stages of pregnancy. The most severe defects follow infection in the third and fourth months of gestation and affected calves are born with hydranencephaly. The most severe cases have almost complete destruction of the cerebral hemispheres although the brain stem and cerebellum are generally unaffected. When cattle are infected at the most susceptible stages of gestation, up to 50% of calves can be born with defects[15]. In small ruminants, with a much shorter pregnancy, severe hydranencephaly and arthrogryposis occur concurrently and animals are usually still born[16].

Aino virus has been associated with an outbreak of congenital defects reported about 40 years ago[17], but no further outbreaks have occurred.

Rhabdoviruses

Also known as 'Three Day Sickness' by farmers due to the spectacular acute but transient illness, bovine ephemeral fever (BEF) virus (Family *Rhabdoviridae*, genus *Ephemerovirus*) had attracted attention because of the large scale epidemics that had spread from Northern Australia through most of NSW and sometimes into Vic. and SA between 1930–1970[18]. BEFV only infects cattle and buffalo, causing a severe but short febrile disease, often associated with recumbency, locomotor difficulties, shifting lameness and difficulties in swallowing[19] – signs similar to rabies in cattle. There is a high morbidity but generally low mortality and the majority of animals recover rapidly (typically in about three days, hence the colloquial name). In the endemic coastal regions of NSW north of Sydney and in Qld and the NT, infection is restricted to younger animals born since the last occasion that the virus was spread[20]. Beyond the endemic areas, cattle of all ages are affected[21].

The disease can have a severe impact in dairy herds because there is almost complete cessation of milk production in infected cows[22]. In beef herds, the decline in milk availability can severely impact on calf health and growth. The severe fever often induces a temporary infertility in males that lasts for several months but can be permanent. Although the epidemiological patterns and association of outbreaks with high rainfall suggest a mosquito vector, there remains a dearth of supporting evidence. There is some evidence to incriminate *Culex annulirostris* as the most likely vector[23,24].

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Biography

Dr Peter Kirkland is the Head of the Virology Laboratory at the state government Elizabeth Macarthur Agriculture Institute at Menangle NSW. Dr Kirkland has had a long career in diagnostic and research projects in animal health. He has been instrumental in the identification of several new viruses, including Menangle virus that was transmitted from flying foxes to pigs, a novel pestivirus that was responsible for a major disease outbreak in pigs and viruses that have caused blindness and sudden deaths in macropods. In 2007 he led the EMAI team during the diagnosis and response to the equine influenza outbreak and in 2011 the investigation of the large West Nile virus outbreak in horses in NSW. His research interests include vector borne viruses and the development and evaluation of rapid diagnostic assays for viral diseases of animals.

The molecular epidemiology of Murray Valley encephalitis virus in Australasia

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Of the viruses transmitted by mosquitoes in the Australasian region, Murray Valley encephalitis (MVE) virus is the major cause of brain disease in humans. There is no vaccine to prevent MVE, nor are there effective antiviral drugs available to treat infections. Therefore, surveillance of MVE is essential to control efforts. A key element to this is understanding the virus at a genetic level, which allows the tracking and identification of known or novel genetic types and can tell us about their circulation patterns.

In the last century, the major epidemics of MVE occurred in southeastern Australia, centred on the Murray Valley region[1]. However, since 1978 the majority of cases have occurred in northern Western Australia and the Top End of the Northern Territory, where the virus is now endemic. Nowadays, MVE only occasionally re-emerges in south-eastern Australia following periods of heavy and prolonged rainfall. The last such event occurred in 2011, resulting in 17 cases of MVE nationwide and 3 deaths, including 2 cases (1 death) in South Australia and 1 case in New South Wales[2]. Approximately 100 equine cases were also reported in South Australia and the Eastern states in 2011[3]. To the north of Australia, the virus is found in Papua New Guinea and Irian Jaya, where cases have been reported and virus isolated from mosquitoes[1,4–6]. MVE virus cycles between *Culex* species mosquitoes and water birds, such as herons and egrets[1]. It is not well understood how it reappears in places where occasional cases or activity is found, but it may be re-introduced by infected migratory water birds and/or wind-blown mosquitoes.

Four distinct genetic lineages or genotypes (G1-G4) of MVE virus have been identified (Figure 1), based on RNase oligonucleotide mapping[5,7] or sequencing regions of the virus genome, such as the 5' untranslated region or the structural genes pre-membrane and envelope[8–10]. Studies that analysed genes encoding the structural surface proteins of the virus have been the most comprehensive to date. As for other viruses, the surface proteins of MVE virus offer an attractive choice for phylogenetic analyses, since they are the primary target of the host immune response and subject to relatively strong selection pressures. This means that the genes encoding these proteins have high levels of sequence variation that allows sensitive levels of phylogenetic discrimination.

G1 is the major type of MVE virus on mainland Australia and the most recent to evolve from ancestral strains[9,10]. The last viruses identified from Papua New Guinea, isolated from mosquitoes caught in 1998, also belong to this type. Molecular clock analysis revealed that G1 evolved between 1930 and 1949[10]. Within G1, two distinct sub-lineages (1A and 1B) have co-evolved and exhibit different patterns of transmission in Australia. G1A viruses were estimated to emerge in the 1970s and 80s from a common ancestor of early virus isolates from the 1950–51 and 1974 epidemics in southeast Australia. Paradoxically, Australian G1A viruses have only been found in the northwest of the country. The emergence of G1B in the 1980s overlapped that of G1A but, in contrast to the latter, G1B viruses have been found across mainland Australia (Figure 1). Both types have been associated with disease. We don't yet know why they have different circulation patterns, but further studies may reveal subtle differences in the genome of G1B viruses that enables them to infect mosquitoes and waterbirds more efficiently, allowing them to outcompete their G1A counterparts.

Interestingly, the progenitor of G1B viruses emerged between 1968 and 1973[10], coinciding with construction of Stage 2 of the Ord River Irrigation scheme in the northeast Kimberley region of Western Australia. This involved damning the Ord River to create Lake Argyle, a vast freshwater reservoir, and resulted in profound changes to the local ecosystem. It has been hypothesised that this led to MVE virus becoming established in this region[11]. G1B subsequently became the dominant and most widespread lineage of MVE virus in Australia. It has been proposed that the changes to the ecosystem in the northeast Kimberley facilitated the emergence of this lineage, via increased populations of vector mosquitoes and waterbirds, and the creation of a permanent ecosystem suitable for maintaining mosquito-borne viruses[10].

A second minor genotype (G2) is also found in Australia and consists of only a handful of mosquito isolates (~5% of all viruses propagated from trap-caught mosquitoes)[9,12] and a single human isolate (J. Druce, *personal communication*), all from

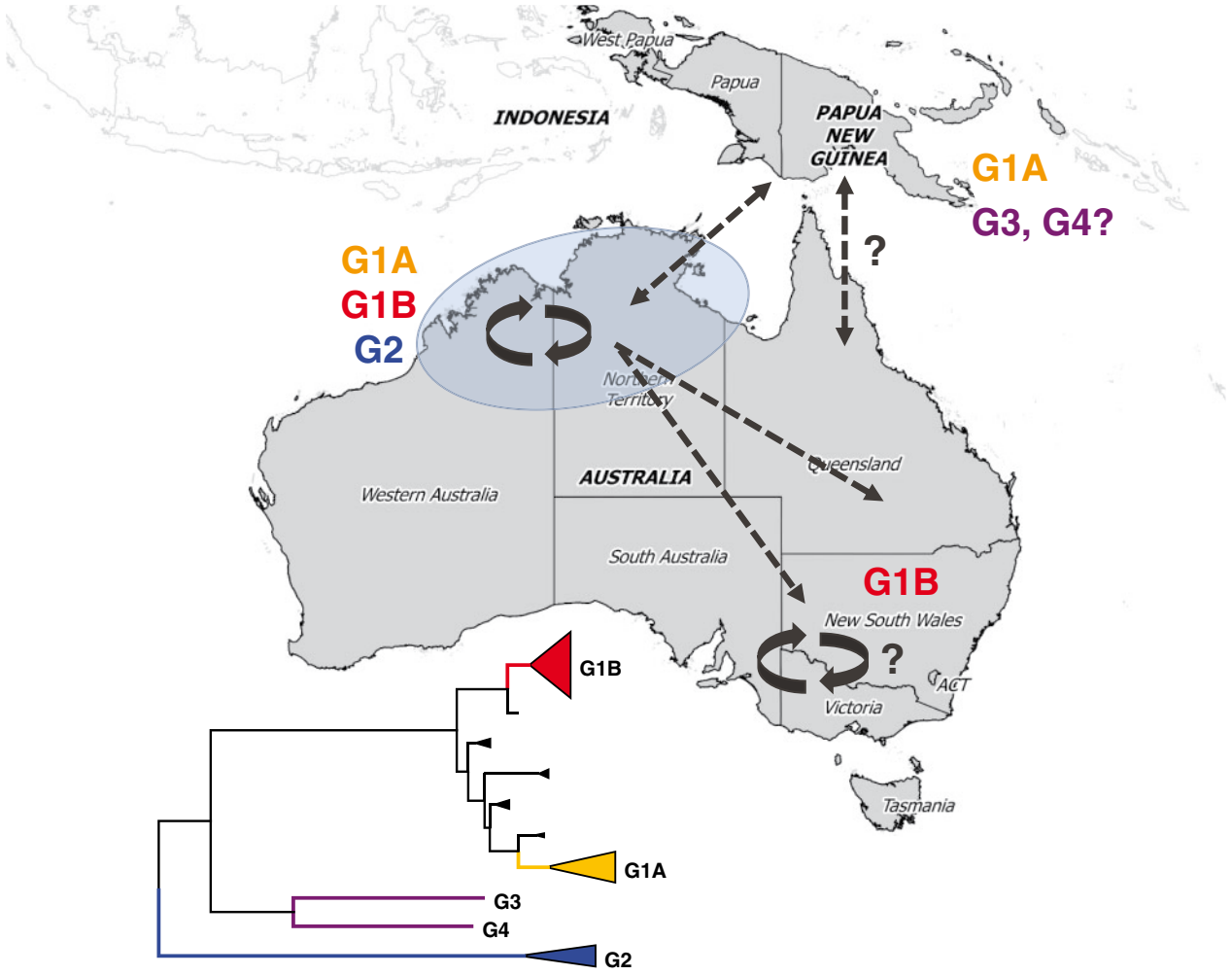

Figure 1. Geographic distribution of genotypes and subgenotypes of Murray Valley encephalitis virus and their circulation pattern in Australia and Papua New Guinea. The enzootic focus of MVEV in northwestern Australia is indicated by blue shading. A phylogenetic tree of complete prM and Env genes of MVEV is shown below the map. The tree was drawn using an alignment of 67 sequences and the neighbour-joining method (MCL substitution model) in MEGA7[17].

northwestern Australia. G2 is the oldest lineage of MVE virus, emerging directly from the ancestral virus of this species around 200 years ago[10]. Up until recently, it was thought that G2 viruses were attenuated, based on experiments using a mouse model of MVE[10,13] and the absence of human cases linked to G2 viruses. However, the first isolation of a G2 virus from a case of MVE in the Northern Territory in 2015 indicates that G2 viruses have the same potential to cause severe disease as G1 viruses. It is not understood why G2 viruses are a minority type restricted to northwestern Australia. G2 strains may occupy a rarely-sampled ecological niche, or their genetic differences (to G1 viruses) may underlie altered biological properties that affect their replication and virulence, as for G1B viruses, thereby restricting their transmission[9,10,12]. In support of the latter, several unique amino acids are encoded in the major envelope surface glycoprotein that have potential biological significance.

The remaining two genotypes of MVE virus are 'one hit wonders' – single isolates from Papua New Guinea derived from a human case in 1956 (G3)[4] and mosquitoes collected in 1966 (G4)[5]. No other strains of these genotypes have been found; however, surveillance activities in PNG and Irian Jaya have been limited and this may reflect under-sampling.

The co-circulation of all contemporary genotypes of MVE virus in northwestern Australia provides further evidence that this region is the enzootic focus for this virus. Continued surveillance throughout mainland Australia through existing mosquito surveillance programs, passive surveillance of cases and enhanced surveillance strategies will remain important to gather environmental and clinical samples to test for the presence of MVE viruses and gain a greater understanding of the molecular epidemiology of this potentially devastating virus. Although surveillance activities in PNG present challenges, the contribution to our knowledge of the epidemiology of MVE and other mosquito-borne diseases may be invaluable. Another gap for MVE virus molecular epidemiology is the lack of full length genomes. Currently, the complete genomes or ORFs of only seven different strains have been published[14–16]. Additional full length genomes will enable more comprehensive genetic studies that may uncover new insights into factors that

determine transmission and pathogenicity. As next generation sequencing technologies become mainstream, this should be achievable in the near future.

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Biography

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Protecting Australia from disease vectors: exotic mosquito management at the border

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Mosquitoes, through the diseases they transmit, are considered the deadliest animals in the world[1]. While Australia is relatively free of many of the mosquito species capable of transmitting diseases such as dengue, yellow fever, chikungunya, and Zika virus, Australia is not immune to the risk of these arboviruses becoming endemic through the introduction of exotic mosquito vectors. In 150 separate instances there were 525 individual exotic mosquitoes

detected at the Australian border between 2014 and 2017 (Department of Agriculture and Water Resources, unpublished data). Accordingly, there is a strong focus on surveillance and control activities to prevent exotic mosquito incursions and possible local establishment.

Aedes aegypti (Linnaeus) and *Aedes albopictus* (Skuse) are highly invasive mosquito species responsible for the transmission of diseases significant to public health across many parts of the world. *Aedes albopictus* is not yet established on mainland Australia and *Ae. aegypti* is confined to a limited distribution through eastern Queensland[2]. As a result, diseases such as dengue, chikungunya, Zika virus, and yellow fever are relatively rare or non-existent in Australia. While Queensland continues to experience localised dengue outbreaks stemming from imported cases, the rest of Australia remains free from disease transmission.

The lack of a competent vector dramatically reduces the public health risk posed by arboviral diseases. For example, there were 1740 cases of dengue reported in Western Australia between 2014 and 2017[3], predominantly associated with overseas travel. This is the highest recorded of any state or territory during this period, but in the absence of a competent vector no dengue outbreaks have occurred. The establishment of either *Ae. aegypti* or *Ae. albopictus* in this region of Australia would have significant public health consequences.

Australia's island geography aids in preventing the introduction of mosquito vectors, but a reliance on imports and increasing international passenger movements has provided a pathway for invasive mosquitoes via international conveyances and imported cargo. To prevent the establishment of vectors through import pathways and maintain a risk free environment with respect to the previously mentioned arboviruses, the Australian Government employs specific measures at the border, primarily: disinsection of all aircraft entering Australia, targeted inspections of international vessels and cargo, and vector surveillance at first points of entry (air and sea ports).

Exotic mosquito surveillance is legislated under the *Biosecurity Act 2015* and undertaken by the Australian Government Department of Agriculture and Water Resources. In line with the World Health Organization's *International Health Regulations 2005* surveillance is carried out to a minimum distance of 400 metres from facilities that are used for operations involving travellers, conveyances, cargo, and postal articles[4].

The Department of Agriculture and Water Resources deploys a range of mosquito surveillance traps at first points of entry as part of its mosquito surveillance program. These traps are targeted at collecting different life stages of mosquitoes including Biogents Sentinel traps and Encephalitis Vector Surveillance CO_2 traps for collecting adults, Sentinel tyre traps for collecting larvae, and ovitraps for collecting mosquito eggs (Figure 1). The vast majority of mosquitoes collected through this program are local species with 117 exotic mosquitoes (37 separate instances) identified from more than half a million mosquitoes collected at Australian first points of entry in 2016 (Department of Agriculture and Water Resources, unpublished data). The type and number of traps deployed at a first point of entry varies according to the size, the volume of arriving international conveyances and cargo, and local environmental conditions at the port. The larger the port or the higher the volume of international traffic arriving at a port, a greater number of traps and trap types are used. Surveillance activities are also escalated at times of heightened international traffic. For example, additional surveillance traps are deployed to cover increased international military arrivals during Australian Defence Force exercises.

The detection of a single exotic mosquito will trigger a rapid response involving Federal, State and, in some jurisdictions, Local Government. A typical response to an exotic mosquito detection will involve knockdown adulticide treatments (Figure 2), residual insecticide harbourage spraying, and treatments of potential mosquito breeding sites. Enhanced surveillance is undertaken to monitor the effectiveness of the treatments applied and to ensure no exotic mosquitoes remain. Site surveys around the detection point are undertaken to ensure that there is no localised breeding of exotic mosquitoes. To assist this cross government response, the Australian Government Department of Health has developed a set of guidelines to support decision making and describe the roles and responsibilities of the key stakeholders involved in the response activities[5].

Pathway analysis is another important part of the response process. Determining the origin of an exotic mosquito and how it arrived

Figure 1. Cigar shaped *Aedes* mosquito eggs collected from an oviposition trap.

Figure 2. Thermal insecticide fogging treatment being conducted within a baggage unpack area at an International Airport. Image courtesy of Brisbane City Council.

allows for targeted control measures to be implemented. Genome-wide single nucleotide polymorphism (SNP) analysis is used as a population genetics tool to compare genetic similarities between exotic mosquitoes detected at the border and an established reference database in order to determine their origins[6]. This, as well as investigations into insecticide resistance profiles of exotic mosquitoes using genetic markers and biological assays, is helping to better understand new and emerging risk pathways.

To date, pathway controls, early detections of exotic mosquitoes at the border, and rapid response activities have helped prevent exotic mosquito vectors from establishing in Australia, however, exotic mosquitoes continue to probe our borders. Through continued efforts at the border, researchers are provided with more time to develop new and innovative ways to prevent and control these mosquito vectors, and the diseases they transmit ahead of their possible introduction to Australia.

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Biographies

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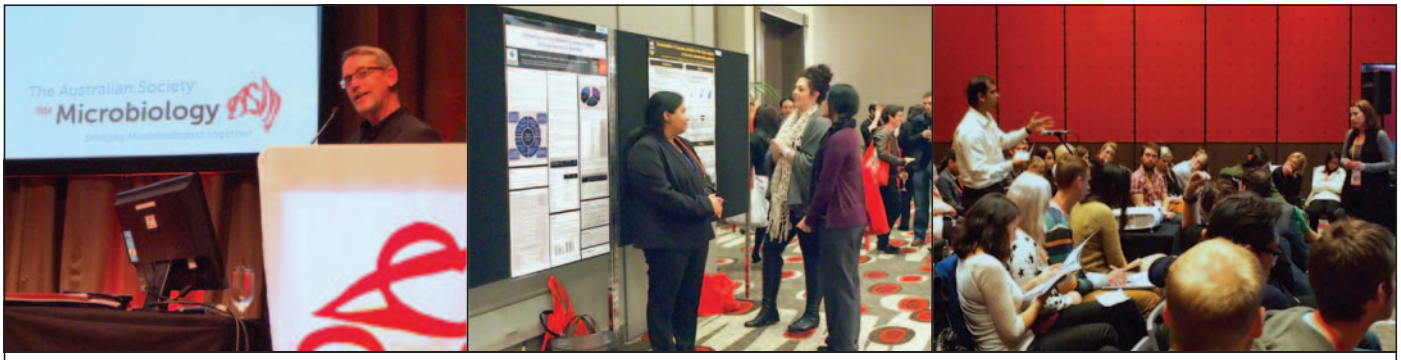

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