Could Australian ticks harbour emerging viral pathogens?



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Tick-borne viruses contribute significantly to the disease burden in Europe, Asia and the US. Historically, some of the most well-known viruses from this group include the human pathogens, tick-borne encephalitis virus and Crimean-Congo haemorrhagic fever virus. More recently multiple emerging tick-borne viruses have been associated with severe disease in humans with Bourbon virus and Heartland virus isolated from patients in the US and severe fever with thrombocytopenia syndrome virus reported from China, Japan, and South Korea. Such examples highlight the need for broader approaches to survey arthropod pathogens, to encompass not only known but novel pathogens circulating in Australian tick populations.

There are currently 70 recognised species of ticks in Australia, with 22 reported infesting on humans[1,2]. *Ixodes holocyclus* is the most significant tick in terms of human and animal health in Australia, causing a myriad of problems from toxin-induced paralysis, mostly in domestic animals[3], to allergic reactions in humans[4]. The feeding behaviour of *I. holocyclus* as a three-host tick, makes it a successful vector for Rickettsial pathogens that cause Queensland tick typhus, Flinders Island spotted fever, and Australian spotted fever[5]. Research into 'Debilitating Symptom Complexes Attributed to Ticks' has led to the discovery of several new bacterial species in *I. holocyclus* and other native Australian ticks from families containing known human pathogens[6,7]. Despite this, little is known of the viruses carried by Australian terrestrial ticks, with most isolations from seabird ticks collected on offshore islands[8–10].

Viruses isolated from Australian seabird ticks show similarities with tick-borne pathogens in other parts of the world (Table 1). The flavivirus Gadgets Gully virus (GGYV), first isolated from *Ixodes uriae* ticks in 1985, clusters with the mammalian tick-borne flavivirus group including human pathogens Powassan virus and Tick-borne encephalitis virus[9]. While GGYV has not been associated with disease in humans, a serological survey demonstrated evidence for infection in inhabitants of a research station on Macquarie Island[11]. Similarly, a second flavivirus, Saumarez Reef virus (SREV), was isolated from *Ixodes eudyptidis* and *Ornithodoros capensis* ticks taken from nests of sooty terns (*Onychoprion fuscatus*) and silver gulls (*Chroicocephalus novaehollandiae*) after reports of illness and tick bites by technicians servicing weather stations on Saumarez reef (~330 km off the Central Queensland coast)[10]. Although no serological evidence of SREV infection was gathered, it was noted that the closely

Table 1. Virus isolates from ticks collected in Australia and New Zealand.

Virus designation	Associated tick species	Region isolated	Virus genus	Closest relative (% amino acid[A])	Available sequence
Family: *Orthomyxoviridae*					
Upolu virus	*O. capensis*	Upolu cay (GBR)	Thogotovirus	Aransas bay virus (93% PB1)	Complete KC506156 – 61
Johnston Atoll virus	*O. capensis*	Johnson Atoll (NZ), Qld	Quarjavirus	Tjuloc virus (84% PB1)	Partial FJ861696 – 7
Family: *Phenuiviridae*					
Albatross Island virus	*I. eudyptidis*	Albatross Island (Tas.)	Phlebovirus	Heartland virus (67% RdRP)	Complete KM198925 – 7
Hunter Island group virus (HIGV)	*I. eudyptidis*	Albatross Island (Tas.)	Phlebovirus	Albatross Island virus (99% RdRP)	Complete KF848980 – 2
Precarious Point virus (PPV)	*I. uriae*	Macquarie Island	Phlebovirus	Murre virus (81% RdRP)	Complete HM566179 – 81
Catch-me-Cave virus (CMCV)	*I. uriae*	Macquarie Island	Phlebovirus	Precarious point virus (98% nucleoprotein, partial)	Partial EU274384
Family: *Nairoviridae*					
Finch Creek virus (FCV)	*I. uriae*	Macquarie Island	Orthonairovirus	Taggert virus (99% RdRP)	Partial EU267169
Taggert virus (TAGV)	*I. uriae*	Macquarie Island	Orthonairovirus	Avalon virus (80% RdRP)	Complete KU925491 – 3
Vinegar Hill virus (VINHV, CSIRO1499)	*A. robertsi*	Gatton (Qld)	Orthonairovirus	Dera Ghazi Khan orthonairovirus (97% RdRP)	Complete MF17881 – 3
Family: *Reoviridae*					
Nugget virus (NUGV)	*I. uriae*	Macquarie Island	Orbivirus	Great Island virus (serological data only)	None
Sandy Bay virus (SBV)	*I. uriae*	Macquarie Island	Orbivirus	Great Island virus (71% VP5)	Partial EU685329 – 33
Family: *Flaviviridae*					
Gadgets Gully virus (GGYV)	*I. uriae*	Macquarie Island	Flavivirus	Powassan virus (72% polyprotein)	Complete DQ235145
Samaurez Reef virus (SREV)	*I. eudyptidis, O. capensis*	Coral Sea Islands, Tasmania, Macquarie Island	Flavivirus	Tyuleniy virus (73% polyprotein)	Complete DQ235150
Unknown					
Lake Clarendon virus (CS704)	*A. robertsi*	Gatton (Qld)	Unknown	–	None
Little Diamond Island virus group (CSIRO 1759-1762)	*I. kohlsi*	Diamond Island (Tas.)	Unknown	–	None

[A]Amino acid similarity to closest relative by Blastx analysis. RdRP, RNA-dependent RNA polymerase; TAS, Tasmania; QLD, Queensland; GBR, Great Barrier Reef; PB1, polymerase basic subunit 1.

related Tyuleniy virus showed a 6% seroconversion rate in inhabitants of the Commodore Islands[12].

In 2002, a disease outbreak in shy albatross (*Thalassarche cauta*) on Albatross Island (129 km north-west of Burnie, Tasmania) led to the isolation of Hunter Island Group virus. Next-generation sequencing identified the virus as a phlebovirus related to human pathogens severe fever with thrombocytopenia syndrome virus and Heartland virus[13]. Following this, sequencing of Albatross Island virus (ABIV), an isolate from *I. eudyptidis* ticks from the same location in 1983, showed that the two viruses were the same species[14].

More recently, a novel orbivirus and two bunyaviruses were reported from *I. uriae* collected during a survey of ticks on penguins at Macquarie Island[15]. The two bunyaviruses, tentatively named Catch-me-Cave virus and Finch Creek virus, show similarities to previously isolated Precarious Point and Taggert viruses[9,16]. However, full genome sequencing is required to confirm whether these viruses are contemporary strains of known viruses or new species. During this study, GGYV was also re-isolated from *I. uriae* suggesting that it is probable that the viruses first described between 1975 and 1985 are still circulating in the tick and bird populations on Macquarie Island[15].

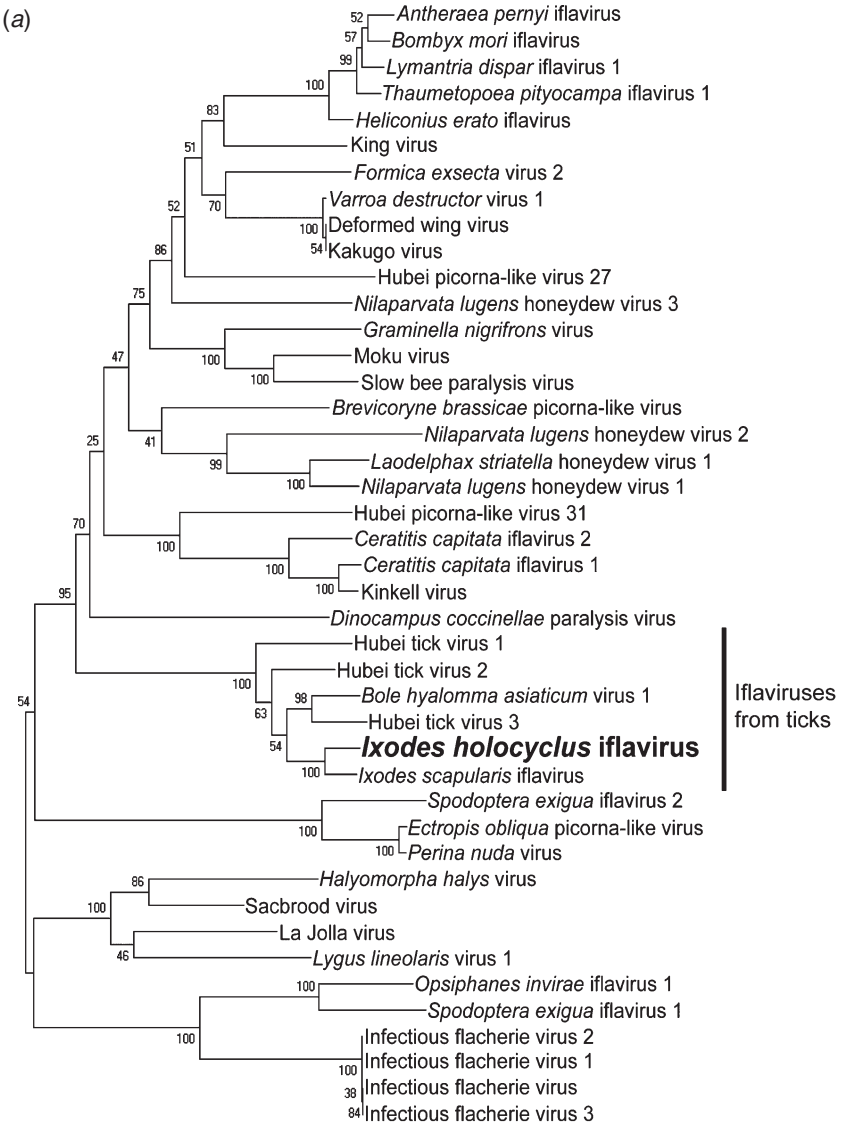
In contrast to the panel of viruses isolated from Australian seabird ticks, attempts to screen terrestrial tick populations have yielded only three viruses, all from *Argas robertsi*, a tick also found in Asia. Lake Clarendon virus (CSIRO704) was isolated from *A. robertsi* ticks collected at the Lake Clarendon cattle egret colony in Gatton, Queensland in 1980[17]. The virus was not identified and showed no relatedness to known arboviruses by serum-neutralisation and complement-fixation tests. Neutralising antibodies in cattle egret sera suggested the virus was able to infect the birds with no apparent disease[17]. To our knowledge, this isolate remains unidentified. A second virus was isolated from *A. robertsi* ticks at the same colony following reports of death in nestling chicks. This virus, originally designated CSIRO1499, was shown to have no serological similarity to Lake Clarendon virus. Experimental infections subsequently showed that the isolate was able to infect and cause mortality in birds[11]. More recently, the full genome sequence of this virus revealed it to be closely related to Dera Ghazi Khan virus (family *Nairoviridae*) and the name Vinegar Hill virus (VINHV) was proposed[8]. Serological surveys found antibodies to VINHV in 3.4% of avian samples and 1% of human serum samples tested[11]. Finally, a virus isolated from *A. robertsi* in the Northern Territory (NT15470) was suggested to be a member of the Dera Ghazi Khan genogroup, thought to be either a strain of Kao Shuan virus (KSV) or a close relative[18]. As no genome sequence is available for this isolate, it remains to be seen whether it is in fact KSV or another isolate of VINHV.

To our knowledge, there have been no virus isolations from an Australian terrestrial hard tick species. Next generation sequencing performed on *I. holocyclus* ticks collected in New South Wales and Queensland have allowed some insights into their potential virome[19]. Recently we published the genome of a novel iflavirus assembled from the transcriptome of *I. holocyclus* salivary glands (Figure 1*a*)[20]. Interestingly, related iflaviruses have also been identified in the *Ixodes scapularis* cell line (ISE6) and in ticks collected from China[21,22]. Several bunyavirus nucleocapsid sequences have also been identified in *I. holocyclus* transcriptome sequence data but whether these sequences belong to a virus or an integration in the host genome is yet to be elucidated (Figure 1*b*). This data indicates that the virome of Australian terrestrial ticks may mirror that of terrestrial ticks found in the northern hemisphere.

We have developed a broad-spectrum screening system that detects viral isolates in cell cultures inoculated with mosquito homogenates. The MAVRIC system (Monoclonal antibodies against viral RNA intermediates in cells) targets long (>30 bp) double-stranded RNA molecules, produced during replication of viruses, in a sequence-independent manner[23]. MAVRIC led to the isolation of at least 9 new viral species from 7 different families allowing the identification of numerous viruses previously not known to exist in Australian mosquito populations[24]. Based on the success of MAVRIC in mosquito screening, we aim to apply this system to screen Australian terrestrial ticks. One barrier is the lack of suitable cell lines derived from Australian tick species and their hosts. While the use of vertebrate cell cultures (generally BHK-21 and Vero cell lines) has proven successful for the isolation of mosquito and seabird tick viruses in Australia[9,25], alternative cell lines reflecting the common hosts of terrestrial ticks (i.e. marsupials), may need to be considered for tick virus isolation on the mainland. Furthermore, while mosquito cell culture has been well established, tick cell culture has proven more difficult requiring a complex mix of vitamins and minerals which must be formulated in-house[26].

Ixodes holocyclus iflavirus was unable to replicate in the *I. scapularis* cell line (ISE6), but appeared to replicate in the host tick raising the question of the suitability of cell lines derived from ticks of the northern hemisphere to isolate Australian tick-borne viruses[20,27]. Phylogenetic analyses have demonstrated that *I. holocyclus* and *I. uriae* are divergent from 'other' *Ixodes*

(*a*)



(*b*)

0.50

Sequence name	Length (nt)	Protein identity	Closest relative (% amino acid)	Sequencing technology
***Ixodes holocyclus* iflavirus (IhIV)**	9260	Complete polyprotein	*Ixodes scapularis* iflavirus (73%)	Illumina, 454
Phlebovirus-like 1	711	Nucleoprotein	Sandfly fever Sicilian virus (31%)	Illumina
Phlebovirus-like 1	1003	Non-structural protein	Saint Floris virus (32%)	Illumina
Phlebovirus-like 3	269	Nucleoprotein	Mukawa virus (36%)	Illumina
Rhabdovirus-like	337	RNA-dependent RNA polymerase	Chandipura virus (39%)	Illumina

Figure 1. (*a*) Evolutionary relationship of *Ixodes holocyclus* iflavirus within the family *Iflaviridae.* Mid-point rooted maximum likelihood phylogenetic tree was constructed based on an alignment of peptidase and RdRP proteins corresponding to position 2036–2944 of the IhIV polyprotein. (*b*) Summary of virus sequences identified in *I. holocyclus* by transcriptome sequencing[19].

species[28]. Preliminary analysis from our group has suggested that some of the vertebrate-infecting viruses of *I. uriae* and *I. eudyptidis* are able to replicate in the ISE6 cell line (Figure 2), however this remains to be demonstrated for viruses of terrestrial ticks. Bell-Sakyi and Attoui recently discussed the role of tick cell culture in virus discovery, particularly in relation to tick-specific viruses[29]. In this instance, the development of cell lines from Australian native ticks may be necessary.

Finally, the risk that Australian seabird-associated tick viruses pose to human health should be considered. A study undertaken to investigate the health risk posed to residents and tourists on the islands in the Great Barrier Reef and Coral Sea by seabird-

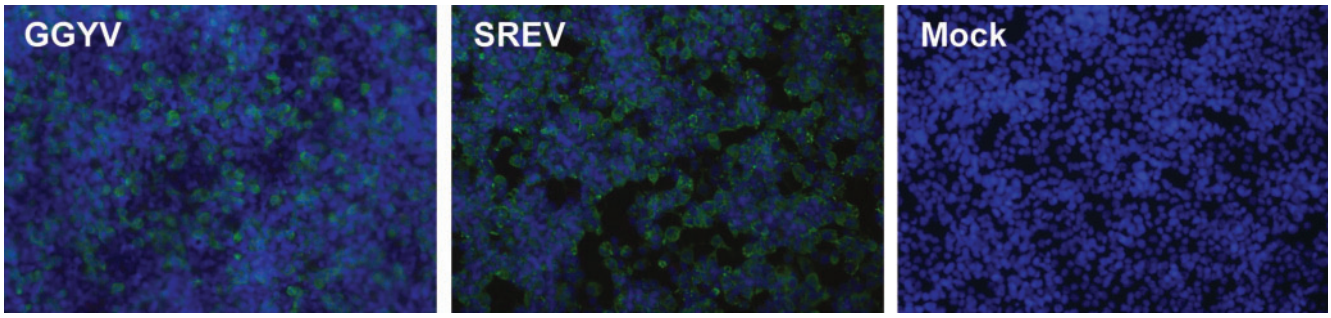


Figure 2. Anti-flavivirus E protein (green) staining in *Ixodes scapularis* (ISE6) cells infected with Australian tick-borne flaviviruses Gadgets Gully virus (GGYV) and Saumarez Reef virus (SREV).

associated arboviruses identified two isolates in *O. capensis* collected on Masthead Island, which appeared closely related to VINHV[30]. This may demonstrate the potential for incursion of seabird-associated viruses to the mainland and vice versa. Serological surveys undertaken after the initial isolation of ABIV identified a black noddy (*Anous minutus*) from Heron Island off the coast of Queensland with neutralising antibodies to the virus[30]. Finally, SREV was isolated from *O. capensis* ticks found on Saumarez reef off the coast of Queensland and 2000 km away in Tasmania[10].

While our knowledge of the viruses harboured by Australian ticks is still limited, the data thus far suggests that our tick viromes may mirror those seen in the northern hemisphere. Next generation sequencing of terrestrial ticks performed by our group and others will greatly contribute to the characterisation of tick-viruses in Australia. In this context, a recent deep sequencing study of Australian ticks by Eddie Holmes' group at the University of Sydney, has identified a plethora of novel viral sequences that will provide a useful reference for further studies (unpublished data available online: https://www.biorxiv.org/content/early/2018/08/07/386573). Complementary to next generation sequencing, a system for efficient isolation of newly discovered viruses will allow for complete characterisation. Finally, comprehensive characterisation of the current tick-borne virus isolates held in archive is required to avoid re-discovery of these viruses in future studies.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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Biographies

Caitlin O'Brien is a final year PhD student focusing on the optimisation of novel culture-based methods for virus discovery in Australian arthropods. Her most recent work includes the identification and characterisation of novel virus species in Australian mosquitoes and ticks. Her six years in research have led to the discovery of novel biological control mechanisms for pathogenic arboviruses, development of monoclonal antibodies to flaviviruses and insect specific viruses and the establishment of the ISE6 cell line for use in Australian tick virus work.

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

Professor Ala Lew-Tabor is a molecular biologist and 'research focused' academic at the University of Queensland. Research highlights include the developing novel vaccines and molecular assays for ticks and tick-borne diseases, respectively. Her group produces translational outputs for cattle and pets including patented vaccines for commercial uptake (cattle tick and paralysis tick), and laboratory assays for government-based diagnostic facilities.