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Newly characterized arboviruses of northern Australia

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ABSTRACT

Arboviruses circulate in the biota of northern Australia, sometimes causing disease in animals and humans. Five different arboviruses, isolated and stored for over 30 years, were re-cultured and sequenced using high-throughput sequencing technology (Ion Torrent PGM) for identification and genetic characterization. Phylogenetic analysis, primarily of sequence fragments of the large segment, indicated classification as members of the Family *Bunyaviridae* (Belmont, Little Sussex, Parker's Farm, and Thimiri viruses). Another was identified as a member of the Family *Rhabdoviridae* (Beatrice Hill virus) based on phylogenetic analysis of the RNA polymerase region.
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1. Introduction

Arboviruses, or arthopod-borne viruses cause large disease outbreaks in animal and human populations which place a strain on veterinary and medical services and have a huge disease burden globally (Weaver and Reisen, 2010). Hence, surveillance is widely conducted to determine seasonal risk and to facilitate targeted control strategies (Geoghegan et al., 2014). Arboviruses mainly belong to 6 families: *Togaviridae*, *Flaviviridae*, *Bunyaviridae*, *Rhabdoviridae*, *Reoviridae* and *Orthomyxoviridae* (Weaver, 2006). Mosquitoes, ticks, biting midges and sandflies are the main vectors depending on the virus and host. Within Australia, there have been 75 isolations of different arboviruses (Russell and Dwyer, 2000), a proportion of which are associated with animal and human disease.

Globally, insect-borne infections with rhabdoviruses cause diseases of veterinary importance, but occasionally result in pathology in humans. For example vesicular stomatitis virus (VSV), a mosquito and sandfly-transmitted arbovirus (Genus *Vesiculovirus*), is found in the Americas and can cause fever and vesicular lesions in cattle, horses and pigs, and acute fever in humans (Rodriguez, 2002). Bovine ephemeral fever virus (BEFV) is a mosquito and biting midge-transmitted arbovirus (Genus *Ephemerovirus*) responsible for fever and mucosal discharge in cattle and water buffalo, which causes significant economic impact in affected regions due to reduced milk production (Walker, 2005). In Australia, the virus is enzootic, and outbreaks in cattle occur in summer and autumn (Walker, 2005). There are many other rhabdoviruses that circulate widely in the biota worldwide and have been detected in a wide diversity of hosts including insects, bats, fish, macropods, lizards, birds, ungulates and rodents, but have not been shown to be associated with disease (Walker et al., 2015).

Numerous bunyaviruses cause disease in animals and humans worldwide. For example, the bunyaviruses California encephalitis virus and La Crosse virus are both mosquito-transmitted arboviruses (Genus *Orthobunyavirus*) known to cause encephalitis in

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humans in the United States (Rust et al., 1999; Zeller and Bouloy, 2000). Crimean–Congo haemorrhagic fever virus is a tick-transmitted arbovirus (Genus *Nairovirus*) causing a potentially fatal arboviral hemorrhagic disease also in humans (Zeller and Bouloy, 2000). A number of other viruses such as Rift Valley fever (Genus *Phlebovirus*), the Bunyamwera group (Genus *Orthobunyavirus*), Nairobi sheep disease and Dera Ghazi Khan virus group (both Genus *Nairovirus*) cause disease in both humans and livestock (Schmaljohn and Nichol, 2007). Finally, the tick-borne Uukuniemi (Genus *Phlebovirus*), Hughes and Qalyub (both Genus *Nairovirus*) virus groups cause disease in seabirds (Schmaljohn and Nichol, 2007). However, in Australia only Akabane virus (Genus *Orthobunyavirus*, Simbu group) causes disease of any significance, with infection resulting in abortion and deformities in gestating cattle (Geoghegan et al., 2014).

Since the 1950s, arthropod collection and subsequent virus isolation have been conducted across northern Australia to survey for known and novel arboviruses. This has resulted in the collection of numerous virus isolates which have been archived in reference collections. When surveillance for these viruses was initially conducted, and up until the 1980s, characterization was generally limited to experimental pathology in animals and serology-based classification. More recently, virus taxonomy has been simultaneously assisted and challenged by the advent of nucleic acid sequencing. The development of high-throughput sequencing (HTS), also known as next-generation sequencing, has accelerated this process. This sequencing technology is the method of choice to characterize many archived isolates as multiple virus samples can be sequenced in a single experiment using this technology.

In this study, we utilized HTS to obtain genome sequences from 5 virus samples stored in archival collections, for which no information or only serological information was available. To facilitate this, a high density chip was used with multiple samples on the Personal Genome Machine (PGM, Life Technologies) platform. The sequences obtained confirmed previous serological classification for two bunyaviruses. Additional isolates were identified as two bunyaviruses and a rhabdovirus. This work confirms the power of HTS to rapidly identify viruses without prior knowledge of their identities or genome sequences.

2. Methods

2.1. Virus collection, isolation and culture

Viruses were isolated from collections of mosquito and biting midges as previously described (Doherty et al., 1979, 1972; Standfast et al., 1984). Virus samples were thawed and re-cultured in Vero cells for one passage, and then subsequently in C6/36 cells for another passage. Tissue culture supernatant was harvested and then stored at −80 °C prior to use.

2.2. Viral genome sequencing

The sequencing of viral RNA genomes was conducted as described previously (Warrilow et al., 2014). In brief, virus was purified from tissue culture supernatant using preferential nuclease digestion and ultra-centrifugation (RNase ONE and RQ1 DNase, Promega), followed by sequence-independent amplification. A library was constructed from the products which were then sequenced on a Personal Genome Machine (Ion Torrent PGM™, Life Technologies) using a 318 chip.

2.3. Virus sequence assembly and phylogenetic analysis

The virus genome sequence was assembled *de novo* using Geneious R8 (Kearse et al., 2012). The generated contiguous sequences (contigs) were then mapped using related rhabdovirus or bunyavirus segments from GenBank (large, L; medium, M; and small, S) as scaffolds. The combined contigs were then used as a reference sequence with the complete sequence data to generate the final contigs to each of the segments. The open reading frames (ORFs) of each segment were used for phylogenetic analysis. Best match by Basic Local Alignment Sequence Tool for protein (BLASTP) was performed using the National Center for Biotechnology Information online tool (http://www.ncbi.nlm.nih.gov). Multiple sequence alignments were generated using the MAFFT plugin of Geneious R8 (Kearse et al., 2012). The alignment was then used as input for determining phylogenetic relationships by the maximum likelihood model using MEGA5 software (Tamura et al., 2011) with an appropriate outgroup. Robustness of the generated trees was determined using 1000 bootstrap replicates.

3. Results

3.1. Sequencing of multiple viruses on a high density semiconductor chip

Eleven uncharacterized isolates were barcoded and sequenced on the Personal Genome Machine using a high density semiconductor chip. Two (Taggert and Yacaaba viruses) will be described elsewhere (Huang and Warrilow, unpublished data). Genome sequences were obtained from another three (Maprik, Mapputta and Upolu viruses), but studies describing these were published by other groups in the interim (Briese et al., 2014; Gauci et al., 2015); therefore, they were not analysed further. One virus (Tzipori virus) was determined to be a strain of bovine ephemeral fever virus (data not shown), and was not analysed further. The sequence of fragments of the remaining 5 virus genomes (Fig. 1 and Table 1) was analysed here.

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