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Genomic characterisation of Trubanaman and Gan Gan viruses, two bunyaviruses with potential significance to public health in Australia

Penelope J. Gauci [a,*], Jane McAllister [a], Ian R. Mitchell [a], Richard P. Weir [b], Lorna F. Melville [b], Aneta J. Gubala [a]

[a] Land Division, Defence Science & Technology Group, 506 Lorimer St Fishermans Bend, VIC 3207, Australia
[b] Berrimah Veterinary Laboratories, Department of Primary Industry and Fisheries, Makagon Rd, Berrimah, NT, Australia

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ABSTRACT

Trubanaman (TRUV) and Gan Gan (GGV) viruses are members of the tentatively assigned Mapputta group of the genus *Orthobunyavirus* within the family Bunyaviridae. Despite reported associations with an acute polyarthritis-like illness in Australia, TRUV and GGV have remained genetically uncharacterised. Here we report the complete genome sequences of TRUV and GGV which were originally isolated from mosquitoes in 1966 and 1970, respectively. Sequence and phylogenetic analyses indicate close relationships to other characterised viruses within the Mapputta group. These viruses exhibit the same characteristic features observed in other viruses in the group including the absence of the NSs (non-structural) ORF and an apparent absence of glycosylation sites on the Gn protein of GGV. We comment on the distribution of these viruses based on the available seroprevalence data and vector feeding preferences. The significance of this group of viruses to public health, in terms of unidentified polyarthritic disease, warrants further investigation.

1. Introduction

The family Bunyaviridae, incorporating five genera, is comprised of a large number of tripartite negative sense single-stranded RNA viruses, and includes pathogens of humans, animals and plants. Bunyaviruses are arthropod borne, with the exception of the rodent transmitted viruses of the genus *Hantavirus*. The human diseases caused by bunyaviruses have been reported worldwide and include febrile illness, haemorrhagic fevers and central nervous system disorders (Soldan and González-Scarano, 2005).

The Mapputta group of viruses is a taxonomically unclassified group in the Bunyaviridae family consisting of four serologically related viruses isolated in Australia and Papua New Guinea; Mapputta (MAPV), Trubanaman (TRUV), Maprik (MPKV) and Gan Gan (GGV) (Plyusnin et al., 2012). The previous suggestion that this group likely belongs to the genus *Orthobunyavirus* (Newton et al., 1983) was recently confirmed by the genomic sequencing of MAPV, MPKV and a new Mapputta group member, Buffalo Creek virus (BUCV) (Gauci et al., 2015). Two viruses recently sequenced directly from mosquitoes, Murrumbidgee (MURBV) and Salt Ash (SASHV), also belong to this group (Coffey et al., 2014; Gauci et al., 2015). GGV has been associated with an acute epidemic polyarthritic-like illness in three patients and it is suggested that TRUV may also cause a similar illness

* Corresponding author.
E-mail address: Penny.Gauci@dsto.defence.gov.au (P.J. Gauci).

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(Boughton et al., 1990). Of the recognised viruses in the Mapputta group, TRUV and GGV are the only viruses that have been associated with human disease; however, there is no genetic information publicly available for either of these viruses.

TRUV was first isolated in February 1966 from a pool of *Anopheles annulipes* mosquitoes collected in October 1965 near the Mitchell River Mission in Cape York Peninsula, Queensland (QLD) as part of an arbovirus monitoring initiative of local Aboriginal communities (Doherty et al., 1968). Complement-fixation and neutralisation testing indicated a relationship to MAPV and not any other known Australian arbovirus (Doherty et al., 1968). TRUV has subsequently been isolated from *An. annulipes* in the Northern Territory (NT) (Weir, 2002), Victoria (VIC), New South Wales (NSW) and Western Australia (WA), from *Culex annulirostris* in WA and NSW (reviewed in (Mackenzie et al., 1994)) and from *Anopheles meraukensis* in the NT (Weir, 2002). The first isolation of GGV occurred in May 1970, from a pool of *Aedes vigilax* collected a month earlier in portable light traps set up in bushland South West of Nelson Bay, NSW, following small recurrent outbreaks of epidemic polyarthritis in the area (Gard et al., 1973). Complement-fixation testing indicated that GGV was related to the Mapputta group but further testing distinguished it from other members of this group and demonstrated that antigenically it is more closely related to MPKV than to MAPV or TRUV (Marshall et al., 1980). GGV has subsequently been isolated from a pool of *Culex* spp. in NSW (Vale et al., 1986), and *Tripteroides atripes* (Skuse) and *Coquillettidia linealis* (Skuse) mosquitoes in NSW (Russell et al., 1991).

Limited seroprevalence data exists for TRUV and GGV, but it appears that both are likely maintained by macropods as key host species, with horses and to a lesser extent cattle, also involved in the transmission cycle of TRUV (Doherty et al., 1970; Johansen et al., 2005; Vale et al., 1991). Studies in QLD, NSW and WA indicate the prevalence of neutralising antibodies for TRUV in the studied human populations was generally low (up to 1.4%), although prevalence in Aboriginals in Cape York Peninsula have been reported at 13% (Boughton et al., 1990; Johansen et al., 2005; Vale et al., 1991). A sero-epidemiological study, on sera from different areas of NSW, indicated the overall prevalence of neutralising antibodies for GGV in humans was 4.7%, however, the prevalence was more than 18% in the North West Plains region (Boughton et al., 1990). Antibodies against the related BUCV and MAPV have also been reported in humans (Doherty et al., 1970; Weir, 2002). Here we report the first complete genome sequences for TRUV and GGV and discuss the potential significance of the Mapputta group to human health.

2. Material and methods

2.1. Virus propagation and cDNA preparation

TRUV (isolate MRM3630) and GGV (isolate NB6057) were obtained from the Berrimah Veterinary Laboratories (BVL), Darwin, NT, Australia. These viruses were included in the CSIRO Long Pocket Laboratory (LPL) virus collection that was transferred to BVL with Steven Davis when the LPL laboratory closed. Both viruses were originally isolated through suckling mouse brain and were subsequently propagated through cell culture. For this study, viruses were propagated through BSR cells (a subclone of the BHK-21 cell line), harvested, total RNA extracted and converted to double stranded cDNA as previously described (Gauci et al., 2015). All procedures were performed according to the Australian Standard AS/NZS 2243.3:10 Safety in Laboratories Part 3: Microbiological Safety and Containment.

2.2. Full genome sequencing

The cDNA material was prepared for high-throughput sequencing using the TruSeq CHIP-seq (Illumina) protocols and standard multiplex adaptors. A paired-end, 250-base-read protocol was used for sequencing on an Illumina MiSeq instrument, at the University of New South Wales, Sydney, NSW. Primary assembly of raw data and generation of consensus sequences, followed by the RACE method to obtain genome termini, were all performed as described previously (Gauci et al., 2015). The resulting PCR products were sequenced directly using virus specific primers on the Genetic Analyser 3130*xl* (Applied Biosystems).

2.3. Sequence and phylogenetic analysis

Predictive ORF and protein analyses were performed as previously described (Gauci et al., 2015). To support the group designation of the Mapputta group, MEGA5 software was used to calculate inter-group and intra-group p-distances at both the nucleotide and amino acid level using pairwise deletion for the Mapputta group viruses and a large set of representative orthobunyaviruses (a total of 60–80 viruses for each protein) (Tamura et al., 2011). Phylogenetic trees were constructed using complete RNA dependent RNA polymerase (RdRp), polyprotein and nucleocapsid (N) protein sequences of bunyaviruses, representing all five genera, accessed from GenBank and the cognate TRUV and GGV protein sequences. Sequences were aligned using the MUSCLE 3.6 algorithm (Edgar, 2004) and Maximum likelihood (ML) and Bayesian analyses were conducted as previously described (Gauci et al., 2015).

2.4. GenBank sequences used for phylogenetic analysis

Accession numbers for L, M and S segments accessed from GenBank are: AGUV, Aguacate virus, NC_015451, NC_015450, NC_015452; AINOV, Aino virus, NC_018465, NC_018459, NC_018460; AKAV, Akabane virus, NC_009894, NC_009895, NC_009896; AMBV, Anhembi virus, (L,M), JN572062, JN572063; ANAV, Anopheles A, (S), FJ660415; ANBV, Anopheles B, (S), FJ660417; ANDV, Andes virus, NC_003468, NC_003467, NC_003466; BATV, Batai virus, (M,S), JX846596, JX846595; BeNMV, Bean necrotic mosaic