Genetic Characterization of Tilligerry and Mitchell River Viruses

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Viruses that are normally safely contained within their host species can emerge due to intense livestock farming, trade, travel, climate change and encroachment of human activities into new environments. The unexpected emergence of bluetongue virus (BTV), the prototype species of the genus *Orbivirus*, in economically important livestock species (sheep and cattle) across the whole of Europe (since 1998), indicates that other orbiviruses represent a potential further threat to animal and human populations in Europe and elsewhere. The genus *Orbivirus* is the largest within the family *Reoviridae*, containing 22 virus species, as well as 14 unclassified orbiviruses, some of which may represent additional or novel species. The orbiviruses are transmitted primarily by arthropod vectors (e.g. *Culicoides*, mosquitoes or ticks).

Viral genome sequence data provide a basis for virus taxonomy and diagnostic test development, and make it possible to address fundamental questions concerning virus biology, pathogenesis, virulence and evolution, that can be further explored in mutation and reverse genetics studies. Genome sequences also provide criteria for the classification of novel isolates within individual *Orbivirus* species, as well as the identification of different serotypes, topotypes, reassortants and even closely related but distinct virus lineages.

Full-length genome characterization of *Tilligerry virus* (TILV), a member of the *Eubenangee virus* species, and *Mitchell River virus* (MRV), a member of the *Warrego virus* species, have revealed highly conserved 5′ and 3′ terminal hexanucleotide sequences. Phylogenetic analyses of orbivirus T2 'sub-core-shell' protein sequences reinforce the hypothesis that this protein is an important evolutionary marker for these viruses. The T2 protein shows high levels of amino acid (AA) sequence identity (> 91%) within a single *Orbivirus* species / serogroup, which can be used for species identification. The T2-protein gene has therefore been given priority in sequencing studies. The T2 protein of TILV is closely related to that of *Eubenangee virus* (~91% identity), confirming that they are both members of the same *Eubenangee virus* species. Although TILV is reported to be related to BTV in serological assays, the TILV T2 protein shows only 68-70% AA identity to BTV. This supports its current classification within a different serogroup (Eubenangee).

Warrego virus and MRV are currently classified as two distinct members (different serotypes) within the *Warrego virus* species. However, they show only about 79% AA identity in their T2 protein (based on partial sequences). It is therefore considered likely that they could be reclassified as members of distinct *Orbivirus* species. The taxonomic classification of MRV will be reviewed after generating full length sequences for the entire genomes of both viruses. The taxonomic status of each of these viruses will also be tested further by co-infections and attempts to create reassortants between them (only viruses belonging to the same species can reassort their genome segments). TILV and MRV are the first viruses from their respective serogroups / virus species to be genetically fully characterized, and will provide a basis for the further characterization / identification of additional viruses within each group / species. These data will assist in the development of specific diagnostic assays and potentially in control of emerging diseases. The sequences generated will also help to evaluate current diagnostic [reverse transcriptase - polymerase chain reaction (RT-PCR)] tests for BTV, African horse sickness virus, epizootic haemorrhagic disease virus, etc., *in silico*, by identifying any possibility of cross reactivity.

Keywords: *Tilligerry virus – Mitchell River virus – Orbivirus.*

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