Genetic and epidemiological characterization of Middle Point orbivirus, a novel virus isolated from sentinel cattle in northern Australia

Chris Cowled,1 Lorna Melville,2 Richard Weir,2 Susan Walsh,2 Alex Hyatt,1 Rosey Van Driel,1 Steven Davis,2 Aneta Gubala1 and David Boyle1

Correspondence
Chris Cowled
chris.cowled@csiro.au

1CSIRO Livestock Industries, Australian Animal Health Laboratory, East Geelong, Victoria 3220, Australia
2Northern Territory Department of Primary Industries, Fisheries and Mines, Berrimah Veterinary Laboratories, Berrimah, Northern Territory 0801, Australia

Middle Point orbivirus (MPOV) was isolated in 1998 from a healthy cow pastured at Beatrice Hill farm, Middle Point (formerly Coastal Plains Research Station), 50 km east of Darwin in Australia’s Northern Territory. The isolate could not be identified by using conventional serological tests, and electron microscopy indicated that it belongs to the family Reoviridae, genus Orbivirus. Genetic sequencing of segments 2 and 3 revealed that this virus is related to Yunnan orbivirus, an orbivirus known only from China and not previously associated with a vertebrate host. A real-time RT-PCR test was developed to study the epidemiology of this virus in the field. Over 150 previously unidentified viruses isolated from cattle between 1994 and 2006 were positively identified as isolates of MPOV. Serology was used to demonstrate the development of antibody responses to MPOV in cattle from multiple locations across the Northern Territory.

INTRODUCTION

Northern Australia has long been recognized as a hot spot for arbovirus activity, with summer monsoon rains triggering a massive proliferation of mosquitoes and Culicoides (biting midges) capable of transmitting viruses of the families Bunyaviridae, Togaviridae, Rhabdoviridae and Reoviridae (Standfast et al., 1984). Viruses previously unknown in Australia, such as bluetongue virus (BTV) serotypes 3, 9, 15, 16 and 23 (Gard et al., 1985, 1987a, b), have emerged on several occasions. These introductions are thought to be from countries located to the north and west of Australia, possibly by the prevailing winds during the monsoon season carrying infected insects to the Australian mainland (St George, 1992). The emergence of new viruses or introduction of exotic viruses poses a threat to animal health in Australia, and arbovirus activity is monitored by using a system of sentinel cattle herds known as the National Arbovirus Monitoring Program (NAMP, 2006). NAMP’s critical role in BTV monitoring has led to the adoption of virus-isolation protocols biased towards members of the genus Orbivirus.

The genus Orbivirus is one of 12 genera of the family Reoviridae, which includes 21 recognized species and 11 proposed species that are distributed worldwide (Mertens et al., 2004). Several species are known to cause serious disease in animals, and orbivirus infections associated with human disease have been documented (Libiková et al., 1978). Known vectors of orbiviruses include Culicoides midges, mosquitoes, phlebotomine sandflies and ticks (Roy, 2007). Orbivirus genomes consist of 10 segments of double-stranded RNA (S1–S10, largest to smallest), encased within a protein shell called the core, which is covered by a second protein layer called the outer capsid. The seven structural and three non-structural proteins are frequently referred to as VP1–VP7 and NS1–NS3, respectively, based on their order of migration on SDS-PAGE gels or as predicted from sequence data. VP2 and VP3 are reversed in some species as a result of this.

The outer capsid is made up of major outer capsid protein (VP2 or VP3), which is exposed on the virion surface and determines receptor specificity and cell entry, and minor outer capsid protein (VP5). The core layer consists of major subcore protein (VP2 or VP3, also known as T2), which binds genomic RNA and plays a role in T=2 symmetry, and core surface protein (VP7). The three proteins present at low levels inside the core are RNA-dependent RNA polymerase (VP1), capping enzyme (VP4) and helicase (VP6). The non-structural proteins are tubule protein (NS1), inclusion body protein (NS2) and virus release protein (NS3, NS3A) (Mertens et al., 2004).

As described, T2 can be assigned as VP2 or VP3 in different species, depending on whether it is encoded on the second-largest RNA segment, S2, or the third largest, S3.
 Orbiviruses in which T2 is VP2 cluster separately in phylogenetic analysis from those in which T2 is VP3 (Attoui et al., 2005). The coding strands of all 10 genomic RNA segments are 5’-capped and methylated and all segments have conserved terminal sequences believed to be crucial for genome replication and packaging (Rao et al., 1983; Roy, 2007). Direct evidence of this is lacking for orbiviruses, but has been reported for other members of the family Reoviridae. For example, a recombinant gene segment can be incorporated into the genome of reoviruses, but only if it contains 96 nt from the 5’ end and 98 nt from the 3’ end of the wild-type segment (Ronin & Steele, 2007). Studies of rotavirus in cell-free systems have identified elements within the highly conserved terminal sequences essential for effective negative-strand RNA synthesis in vitro (Wentz et al., 1996).

The T2 gene is highly conserved and is typically used for phylogenetic comparison of different species within the genus Orbivirus. Attoui et al. (2001) suggested a cut-off point of 91 % similarity in the amino acid sequence of T2 for species determination. Conversely, the least-conserved gene encodes the dominant outer capsid protein, which has been used for phylogenetic analysis of variation within a species (Potgieter et al., 2003; Maan et al., 2007).

During the 1990s, viruses began to emerge through NAMP that could not be identified by conventional serology and, by 2006, almost one-third of all viruses isolated in the Northern Territory were unable to be identified. One of these isolates (DPP4440) was isolated from a healthy cow in May 1998 at around the time that these viruses first started to appear, and was believed to be a novel Australian virus. We have confirmed this by genetic sequencing and epidemiological studies, and suggest the name Middle Point orbivirus (MPOV).

**METHODS**

**Virus isolation and culture.** Viruses used in this study were isolated from sentinel cattle in the Northern Territory, Australia. Buffy coats from blood samples were inoculated onto monolayers of C6/36 Aedes albopictus (insect) cells in tubes containing 2 ml minimum essential medium with Earle’s salts and 10 % fetal calf serum (FCS). After 24 h, the inoculum was removed and replaced with fresh medium. After 14 days incubation at room temperature, samples were sonicated for 10 min at 4 °C to pellet cell debris. Supernatants were centrifuged at 100 000 g for 90 min at 4 °C to pellet virions. RNA was then extracted by using an RNeasy mini kit (Qiagen) following the manufacturer’s instructions and quantified by using a GeneQuant II DNA/RNA calculator (Pharmacia).

**PCR-Select suppression subtractive hybridization (PCR-Select).** Total MPOV RNA from TCSN (the ‘tester’) was analysed by using a PCR-Select cDNA subtraction kit (Clontech) following the manufacturer’s instructions, but with changes as detailed below. For the ‘driver’, we used total RNA from BTV 1 (Australia) cultivated in BSR cells. Briefly, RNA from both tester and driver samples was converted into double-stranded cDNA and digested with RsaI. Tester cDNA was divided into two portions and each portion was 5’-labelled with oligonucleotide adaptors. In the first of two hybridization steps, a large excess of unlabelled driver was mixed with each of the two labelled tester populations. The mixtures were then heat-denatured and allowed to anneal. In the second hybridization, the two primary hybridization products were combined (without further denaturation) and allowed to anneal overnight in the presence of oligomer denatured driver. In the resulting mixture, only those sequence fragments unique to the tester produced double-stranded cDNA hybrids with different adaptors at each end. The overhanging ends were then filled in and followed by two rounds of PCR amplification with Advantage II DNA polymerase (Clontech) to generate a cDNA library highly enriched with fragments of the MPOV genome (Diatchenko et al., 1996).

The cDNA-synthesis procedure was modified for double-stranded tester and driver RNA as follows: 2 μg RNA was combined with 0.5 μg random-hexamer oligonucleotide (1.5 μg ml−1; GeneWorks) and 0.5 μl formamide in a total volume of 5 μl. This mixture was denatured at 100 °C for 1 min in a heat block, chilled rapidly on ice and then used in a 10 μl reverse transcription reaction, following the PCR-Select kit instructions.

**Cloning.** The enriched cDNA library generated by cDNA subtraction was treated with DNA polymerase I–Klenow large fragment (New England Biolabs) to generate blunt ends and then ligated into the PCR-Blunt-II-TOPO vector and electroporated into OneShot TOP-10 Escherichia coli by using a Zero-Blunt-II-TOPO PCR cloning kit (Invitrogen). Transformants were grown on Luria–Bertani (LB) agar plates supplemented with 50 μg kanamycin ml−1 (Gibco). Colonies were picked at random and grown overnight in 5 ml cultures for plasmid DNA isolation using a Qiagen spin miniprep kit.

**5’–3’ ligation RACE (rapid amplification of cDNA ends).** 5’–3’ ligation RACE was carried out following the method of Mandl et al. (1991). Briefly, tobacco acid pyrophosphatase (EPICENTRE Biotechnologies) was used to remove the 5’-cap structure from 2 μg RNA, and T4 RNA ligase (New England Biolabs) was used to circularize the RNA. The reaction was carried out overnight at 4 °C,
then phenol/chloroform-extracted and ethanol-precipitated. Circular RNA was resuspended in 2 μl RNase-free H2O, combined with 1 μl formamide, denatured at 100 °C for 1 min in a heat block and then chilled on ice. The denatured RNA was then used in a 20 μl reverse transcription reaction containing 200 units Stratascript reverse transcriptase (Stratagene) and 20 units RNase inhibitor (Invitrogen). This was followed by two rounds of PCR, using nested primers designed by using sequence obtained through the PCR-Select cDNA subtraction method. Secondary PCR products were cloned and sequenced with vector-specific primer.

**Sequencing and sequence analysis.** PCR products and plasmids were sequenced by using ABI BigDye Terminator sequencing reagents and an ABI Prism DNA sequencer (Perkin Elmer). Sequencing primers were obtained from GeneWorks. DNA sequences were trimmed and assembled by using SeqMan (version 7; Lasergene, DNASTAR). Sequences were identified by using the online BLAST search engine (http://www.ncbi.nlm.nih.gov/BLAST/). PCR primers and sequencing primers were designed by using Clone Manager (version 8; SciEd Software). Multiple sequence alignments were created by using CLUSTAL W (Thompson et al., 1994) and a phylogenetic tree was prepared by using the distance-matrix and neighbour-joining method in PHYLIP software BioManager by ANGIS (http://www.angis.org.au) and TreeView (Page, 1996). Amino acid sequences were analysed by using PredictProtein (http://www.predictprotein.org/) (Rost et al., 2004).

**Real-time PCR.** Real-time PCR was carried out on a Corbett Rotorgene 6000 real-time PCR machine, using a SYBR green One-Step RT-PCR kit (Invitrogen), targeting a 151 bp region of the conserved T2 gene sequence (segment 2, bp 987–1137). HPLC-purified virus-specific primers were obtained from GeneWorks (forward primer, AATGCGGCTTAGGATTTCC; reverse primer, TTGGAACATCCGGCATGAC) and used at a final concentration of 250 nM each. A mastermix containing enzymes, SYBR green reagent and primers was prepared on ice. For each sample, total RNA was extracted from 100 μl infected TCSN by using a Qiagen RNeasy mini kit and eluted in 30 μl H2O. RNA (5 μl) was denatured at 100 °C for 1 min in a heat block, then combined with 15 μl mastermix on ice. Cycling parameters were 50 °C for 3 min, 95 °C for 5 min, 40 cycles of 95 °C for 15 s, followed by 60 °C for 30 s. Melt-curve analysis was performed, including a 90 s pre-melt step at 37 °C.

**Virus-neutralization test (VNT).** Serum samples were initially diluted 1:4 in cell-culture medium. Aliquots (50 μl) of diluted serum were placed into duplicate wells of 96-well flat-bottom tissue-culture plates and combined with 50 μl virus pre-titrated to contain 100 TCID50 or culture medium only (serum control). Plates were then incubated at 37 °C and 5% CO2 for 1 h. BSR cell suspension (100 μl) containing 2 × 105 cells ml−1 was added to every well and plates were returned to the incubator. Back-titration of the pre-titrated virus was performed on a control plate and the test was read when the control plate read 100 TCID50 (at 5 days post-infection). Positive sera were titrated in duplicate using serial 2-fold dilutions and titres were defined as the reciprocal of the highest dilution causing complete inhibition of CPE.

### RESULTS

#### Electron microscopy

Ultrathin sections of MPOV-infected BSR cells were examined by transmission electron microscopy (Fig. 1). Virus particles of 64 ± 4 nm (n=16) in diameter were observed in the cytoplasm and extracellular spaces. Within the cytoplasm, viruses were observed as individual particles associated with prominent, semi-electron-dense inclusion bodies (VIBs) (Fig. 1a). The VIBs varied in size, were of irregular appearance and density and had a matrix that was both fibrillar and granular. Viruses were associated along the periphery and within the matrix. Within the cytoplasm, individual viruses were not associated with vesicles or any membrane systems. Viruses appeared as double-shelled particles with electron-dense cores (24 ± 3 nm, n=12) and, in some profiles, revealed an icosahedral symmetry (Figs 1a, b). Low numbers of putative viral tubules (20 ± 2 nm in

![Fig. 1. Transmission electron micrographs of MPOV-infected BSR cells. (a) Viral inclusion body (VIB) located in the cytoplasm. Arrows indicate viruses. (b) Higher magnification of MPOV from (a), showing an electron-dense core (arrow). (c) Extracellular MPOV particles (arrow). (d) Aggregate of MPOV-associated tubules (T) in the cytoplasm. V indicates a virus. Bars, 500 nm (a, c); 250 nm (b, d).](http://vir.sgmjournals.org)
Genetic sequencing
Infected TCSN were subjected to PCR-Select cDNA subtraction to generate an enriched cDNA library containing fragments of viral nucleotide sequence. Random sequencing of 50 clones from the enriched library produced sequence from eight of ten gene segments, amounting to approximately one-third of the estimated 19 500 bp genome (Fig. 2). Fragments ranged in size from approximately 100 to 1500 bp in length. BLAST searches on these fragments showed high similarity to the recently described Yunnan orbivirus (YUOV) (Attoui et al., 2005). The most- and least-conserved genes (encoding T2 and major outer capsid protein, respectively) were then sequenced fully. Gaps between fragments were filled in by PCR, and the 5′-3′ ligation method of Mandl et al. (1991) was used to obtain the end sequences. The entire sequence of both gene segments was confirmed by PCR, with a minimum of 2-fold coverage in both directions.

Sequence analysis
The gene encoding the major subcore protein (T2) is the most conserved of the orbivirus genes and, in MPOV, is located on segment 2. MPOV segment 2 is 2900 nt in length and has 85 % nucleotide identity to that of YUOV, although the first 58 nt and last 31 nt are identical between the two viruses. The 5′-untranslated region (UTR) of MPOV segment 2 is 11 nt in length, whilst the 3′-UTR is 66 nt in length (Fig. 3a). One large open reading frame (ORF) of 2820 bases, encoding a protein of 940 aa, was identified in the coding strand. Two additional ORFs, 131 and 104 aa in length, were observed in the complementary strand of MPOV segment 2; however, neither of these is present in YUOV and no additional features were found to suggest functional roles. At the amino acid level, MPOV T2 is 99 % identical to YUOV T2, with just 17 substitutions; however, a hypervariable region was observed, containing seven substitutions within a 16 aa stretch near the N terminus (aa 27–42) (Fig. 4). MPOV T2 has a predicted molecular mass of 107 kDa and contains 10 cysteine residues; however, none of these are predicted to participate in disulphide bonding (as determined by bioinformatic analysis using PredictProtein). A phylogenetic tree based on the amino acid sequence of T2 places MPOV alongside YUOV in a cluster that includes Peruvian horse sickness virus, Corriparta virus, the tick-borne Broadhaven virus and Wongorr virus (Fig. 5).

The gene encoding the major outer capsid protein is the least conserved of the orbivirus genes and, in MPOV, is encoded on segment 3. MPOV segment 3 is 2686 nt in length. It has 70 % nucleotide identity to YUOV segment 3 and the first 8 nt and last 3 nt are identical between the two viruses. The 5′-UTR is 18 nt in length, whilst the 3′-UTR is 46 nt in length, which is 2 nt shorter than that of YUOV (Fig. 3b). MPOV segment 3 contains a single ORF, encoding a protein of 873 aa with a predicted molecular mass of 101 kDa. At the amino acid level, MPOV dominant outer capsid protein is 78 % identical to that of YUOV and they are more similar towards the ends than in the middle (Fig. 6). MPOV dominant outer capsid protein contains 14 cysteine residues, all of which are predicted to engage in disulphide bonding.

**Fig. 2.** Sequencing results following PCR-Select cDNA subtraction of the MPOV double-stranded RNA genome. The 10 gene segments of MPOV are represented as horizontal lines, with lengths relative to their predicted length in nucleotides and putative gene products indicated (based on the sequence of YUOV). Shaded bars indicate fragments of sequence data obtained from sequencing 50 individual clones picked at random. No sequence data were obtained for segments 8 or 10.
residues are conserved between the dominant outer capsid proteins of MPOV and YUOV (which has two fewer cysteines). PredictProtein was used to identify 39 potential post-translational modification sites, 17 of which were conserved between MPOV and YUOV. These are N-glycosylation sites (positions 197 and 308), cAMP- and cGMP-dependent protein kinase phosphorylation sites (positions 79 and 859), protein kinase C phosphorylation sites (positions 50, 71, 78, 161, 814 and 823), casein kinase II phosphorylation sites (positions 48, 78, 89, 555 and 821), a tyrosine kinase phosphorylation site (position 372) and an N-myristoylation site (position 58).

The first 7 nt and last 3 nt are conserved between segments 2 and 3 of MPOV (Fig. 3c). Kozak consensus sequences for strong eukaryotic translation, (A/G)XXAUGG, flank the initiation codons (underlined) of both genes, as indicated in Fig. 3(c) (Kozak, 1997).

The first 7 nt and last 3 nt are conserved between segments 2 and 3 of MPOV (Fig. 3c). Kozak consensus sequences for strong eukaryotic translation, (A/G)XXAUGG, flank the initiation codons (underlined) of both genes, as indicated in Fig. 3(c) (Kozak, 1997).

Fig. 3. Alignment of terminal UTRs of gene segments from MPOV and other orbiviruses. Bases matching exactly with MPOV are highlighted. Bases that have been omitted for clarity are indicated in parentheses. (a, b) Alignment of UTRs from gene segments encoding (a) the major subcore protein (T2) and (b) the major outer capsid protein (OUTER), comparing MPOV with other orbiviruses. (c) Alignment of UTRs from gene segments encoding the major subcore protein (T2) and major outer capsid protein (OUTER) from MPOV, illustrating conservation between different segments of the virus. Positions -3 and +4 relative to the first letter of the AUG start codon conform to the Kozak consensus sequence for strong eukaryotic translation. GenBank accession numbers: MPOV: T2, EF591620; OUTER, EF591621; YUOV: T2, NC_007657; OUTER, NC_007658; PHSV (Peruvian horse sickness virus): T2, NC_007749; OUTER, NC_007750; BRDV (Broadhaven virus): T2, M87875; SCRv (St Croix River virus): T2, NC_005998; OUTER, NC_005999; CHUV (Chuzan virus): T2, NC_005996; OUTER, NC_005986; AHSV (African horse sickness virus): T2, NC_006001; OUTER, NC_005996; EHDV (epizootic hemorrhagic disease virus): T2, AB078629; OUTER, AB078632; BTV (bluetongue virus): T2, NC006014; OUTER, AJ585145.

Fig. 4. Alignment of MPOV (GenBank accession no. EF591620) and YUOV (GenBank accession no. NC_007657) major subcore protein (T2) sequences (first 237 aa only). Differences are highlighted to illustrate the hypervariable region.
The partial sequences obtained for other gene segments aligned with their homologous counterparts in YUOV, with amino acid identities ranging from 91% (NS1) to 98% (VP4).

**Virus identification by real-time PCR**

Real-time PCR was used to screen unidentified viruses isolated from cattle in the Northern Territory of Australia between 1994 and 2006. In total, 152 separate isolates of MPOV were identified, which included 112 from 2006. This represented almost one in three viruses isolated through the Northern Territory sentinel programme in 2006, and >95% of the unidentified viruses for that year. Two isolates collected in 1994 were identified as MPOV, making them the earliest examples to date. MPOV has been isolated from cattle located at Beatrice Hill farm (1994–2006), Darwin (2000), Katherine (2003), Douglas Daly (2000) and Tennant Creek (2001) (Fig. 7). Fifteen of 23 cattle infected with MPOV in 2006 were viraemic for 6 weeks or more, up to a maximum of 15 weeks in one animal.

**VNT**

VNT assays were performed to assess exposure to MPOV in sentinel cattle herds at three locations between 1995 and 2005. Seropositive animals were identified at Beatrice Hill farm (all years), Berrimah farm (1998 and 2004 only) and Katherine (1998 and 2002 only). VNT assays were then performed to determine the frequency of exposure to MPOV in sentinel cattle at six locations in 2006. Seropositive animals were identified at Beatrice Hill farm (21 positive of 23 tested), Douglas Daly (three positive of 18 tested) and Victoria River (one positive of 20 tested). Two animals with trace levels of antibody were identified at Katherine, whereas no evidence of MPOV exposure was seen in sentinel animals from Alice Springs or Berrimah farm in 2006. Sera from the 2006 Beatrice Hill farm sentinel cattle herd were then analysed in detail by VNT, showing the development of MPOV-specific antibody responses over a period of 8 months (Table 1). Traces of activity were detected in early January (summer wet season), but the first significant titre was not observed until March. Two animals had neutralizing titres by April, four by May and, by the beginning of June (winter dry season), most of the herd were producing antibodies, although all of the measured titres were considered to be low. Animals 5 and 21 each appeared to have two antibody-response peaks; however, in both cases, the peaks are at the borderline of sensitivity for this assay, and we do not interpret this as these animals being infected twice.

**Fig. 5.** Phylogenetic tree of the genus *Orbivirus* based on the major subcore protein sequence. Many of the sequences available are incomplete, so the analysis was performed on a 254 aa region relative to aa 357–610 of MPOV. Bootstrap values >50 (out of 100 replications) are shown at the branch points and the scale bar represents relative genetic distance. GenBank accession numbers: MPOV, EF591620; YUOV, NC_007657; PHSV, NC_007749; BRDV, M87875; CORV (Corriparta virus), AF530086; WGRV (Wongorr virus), U56994; SCRV, NC_005998; AHSV, NC_006017; CHUV, NC_005989; WARV (Warrego virus), EF213555; EHDV, AB078829; BTV, NC006014; EUBV (Eubenangee virus), AF530087; WALV (Wallal virus), AF530084.
DISCUSSION

MPOV is related to, but distinct from, YUOV and infects an economically important species in Australia. It has been isolated on more than 150 occasions from Australian domestic cattle and grows well in the mammalian BSR cell line. This is in sharp contrast to YUOV, which was isolated from Culex mosquitoes in the Yunnan province of China and has not yet been associated with any vertebrate species (except for experimentally infected mice) or propagated in any mammalian cell line tested (L929, BHK-21, Vero, BGM, HEp-2 and MRC-5) (Attoui et al., 2005). Based on the criteria of Attoui et al. (2001), MPOV should be classified as a novel genotype of the YUOV species. Whether it is a distinct serotype remains to be determined, but this seems likely, due to the unique amino acid sequence of the outer capsid protein. The availability of sequence data from these two closely related viruses opens a window of opportunity to study the biology of both in greater detail than would be possible with either sequence alone. MPOV appears to have emerged recently in this country and the serological evidence presented here suggests that the frequency of infection has increased over the last decade. We believe that this justifies further investigation of MPOV, even though it has not been associated with clinical disease.

To our knowledge, this is the first time that the PCR-Select cDNA subtraction hybridization technique has been applied successfully to a double-stranded RNA virus. The success of this approach shows that a large amount of

Fig. 6. Alignment of MPOV (GenBank accession no. EF591621) and YUOV (GenBank accession no. NC_007658) major outer capsid protein sequences. Differences are highlighted.
sequence data can be generated quickly from a very basic preparation of RNA, without the need for complex virus-purification methods. The 5’–3’ ligation method of Mandl et al. (1991) was effective in generating up to 1000 bases of terminal sequence of a gene segment and obtaining both 5’- and 3’-end sequences simultaneously.

**Table 1.** Antibody responses to MPOV infection in 23 sentinel cattle pastured at Beatrice Hill farm (Middle Point) over 8 months during 2006, as determined by VNT

Titres are defined as the reciprocal of the highest dilution of serum causing complete inhibition of CPE. –, Negative; +, positive, with titre indicated; I, incomplete inhibition at lowest serum dilution.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+5</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+10</td>
<td>+16</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+10</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>+1</td>
<td>–</td>
<td>+5</td>
<td>–</td>
<td>–</td>
<td>+5</td>
<td>+8</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+5</td>
<td>–</td>
<td>+5</td>
<td>+8</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>+5</td>
<td>–</td>
<td>+20</td>
<td>+16</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+5</td>
<td>–</td>
<td>+8</td>
<td>+10</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+8</td>
<td>–</td>
<td>+8</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+10</td>
</tr>
<tr>
<td>11</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+5</td>
</tr>
<tr>
<td>12</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+5</td>
</tr>
<tr>
<td>14</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+8</td>
<td>–</td>
<td>+8</td>
<td>+5</td>
</tr>
<tr>
<td>16</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+8</td>
<td>–</td>
<td>+8</td>
<td>+8</td>
<td>+10</td>
</tr>
<tr>
<td>17</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+5</td>
<td>–</td>
<td>+8</td>
<td>+8</td>
<td>+8</td>
</tr>
<tr>
<td>18</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+8</td>
<td>+8</td>
<td>+16</td>
</tr>
<tr>
<td>19</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>+8</td>
<td>+8</td>
<td>+8</td>
</tr>
<tr>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>21</td>
<td>–</td>
<td>–</td>
<td>+5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>22</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+8</td>
<td>+5</td>
<td>–</td>
</tr>
<tr>
<td>23</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+8</td>
<td>+5</td>
<td>–</td>
</tr>
</tbody>
</table>
The coexistence of suitable vector and host species at the same location is essential for the completion of arbovirus infection cycles and determines the potential range of virus distribution. Domestic cattle are bred in most parts of Australia, although MPOV has so far only been detected in the Brahmin breed. The arthropod vector(s) of MPOV has not yet been identified, but as its close relative YUOV was isolated from *Culex tritaeniorhynchus* mosquitoes (Attoui et al., 2005), it seems reasonable to anticipate a mosquito species performing this role for MPOV.

The ultrastructural characteristics of MPOV are consistent with those described for viruses belonging to the family *Reoviridae*, genus *Orbivirus* (Mertens et al., 2004). The diameter of the tubules observed in MPOV-infected cells (20 nm) was similar to that described for Wongorr virus (18–20 nm) and much narrower than those described for BTV (68 nm), epizootic hemorrhagic disease virus (54 nm) or Broadhaven virus (60 nm) (Gould & Hyatt, 1994; Nuttall et al., 1981).

The hypervariable region observed in the alignment of MPOV and YUOV T2 sequences has not been reported for any other orbivirus. Genome packaging and assembly of the segmented double-stranded RNA viruses in general have been explained inadequately and, for the genus *Orbivirus*, it is clear that T2 is critically involved, as it binds directly to the genomic RNA segments and forms the core structure in which they are packaged. The alignment of T2 sequences from all sequenced orbivirus species reveals that the T2 proteins of MPOV and YUOV have particularly long 5’ ends and that the hypervariable region lies beyond the 5’ ends of the other species. Sequence hypervariability can be explained either by strong selection pressure to mutate or by weak selection pressure to be conserved. The fact that most of the changes are conservative (e.g. K→R) indicates a selection pressure to maintain the general physical properties of the region.

The real-time PCR assay that was used targeted a highly conserved region of the T2 gene. This was done deliberately so that viruses with sequence variations from the prototype MPOV (outside the PCR target region) could also be detected. Consequently, it is possible that the 152 viruses identified as MPOV may include variants and this possibility is under investigation.

Overall, the characterization of MPOV has revealed a virus new to Northern Australia, widespread in domestic cattle and related closely to a mosquito-borne virus from China. MPOV apparently emerged here during the 1990s and induces antibody responses in host animals. The development of a rapid diagnostic test has already made possible the identification of over 150 previously unidentified viral isolates, and can now be used for the routine identification of future isolates.

ACKNOWLEDGEMENTS

The authors would like to thank Sandra Crameri for assistance with taking electron micrographs. This work was supported by a research grant from the Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease (AB-CRC).

REFERENCES


