Murray Valley encephalitis and Kunjin viruses – the Victorian experience

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**Introduction**

- Murray Valley encephalitis (MVE) and Kunjin (KUN) viruses are both Arboviruses, from the genus Flavivirus, they are transmitted by infected mosquitoes biting humans and animals.
- Both viruses are maintained in a natural transmission cycle involving mosquito vectors and bird reservoir hosts.
- Humans and horses are believed to be incidental hosts.
- Culex annulirostris found across Australia.
Natural transmission cycle

Mosquito bites an infected bird & picks up the MVE virus

Infected mosquito bites a bird & passes on the MVE virus

Virus multiplies inside body cells

Mosquito bites an infected bird & picks up the MVE virus

Infected mosquito bites humans & non-host animals who are unable to pass on the virus
Infection with MVE virus

- MVE infection affects the central nervous system. It can be mild, severe or fatal.
- Most cases are subclinical\(^2\). However, clinical disease estimates range from 1:150 to 1:1000\(^3,4\) based on seroepidemiological studies.
- Clinical disease is often high impact with mortality rates as high as 20\% and severe neurological sequelae occurs in 40\% of survivors\(^4,5\).
- MVE disease is characterised by fever, headache, myalgia, malaise, anorexia, nausea followed by neurologic symptoms.
Infection with KUN virus

- KUN infection is typically associated with subclinical or mild febrile illness,\textsuperscript{6} often with arthralgia and rarely encephalitis. \textsuperscript{3}
MVE Epidemiology

- MVE virus is found in Australia, PNG and Irian Jaya.
- It is endemic in much of Northern Australia, in the Kimberley region of WA, top end of NT and northern QLD. 
- The virus is epizootic in the Pilbara and further south in Western Australia and the Northern Territory.
- It occurs rarely in SE Australia, predominately in the Murray River area.
- Occasional epidemics occur, the largest being in 1974.
- Outbreaks occur typically in the summer months after 2 consecutive years of heavy rainfall.
KUN Epidemiology

- Kunjin virus is found in Australia, PNG and Malaysia.
- It is closely related to West Nile virus (which is found in Africa, India, Europe and the USA\(^ {20}\)).
- It is endemic in the Kimberley region of WA and throughout northern Australia.
- It has a wider distribution than MVE extending over most of tropical Australia, eastern QLD and occasional spread into south-eastern Australia.\(^ {9}\)
History

- The first confirmed outbreak of MVE in Victorians was in early 1951, 40 severe cases (17 deaths).

- There were 3 prior outbreaks of encephalitis affecting humans in the Murray Valley region – all in the later summer months of:
  1) 1916-17,
  2) 1917-18 and
  3) 1924-25.10

- Kunjin virus first isolated in 1960.
The great mainland epidemic of 1974

- 58 clinical cases and 13 deaths nationally (20% mortality)\textsuperscript{11}
- 27 cases in Victoria, 22 of those admitted to Fairfield Infectious Diseases Hospital in Melbourne.
- Of the 22 hospitalised patients:
  - 4 died
  - 7 severe residual damage
  - 11 recovered almost completely
- National disease program set up to combat further outbreaks.\textsuperscript{11}
Victorian Arbovirus Task Force (VATF)

- Use three monitoring methods to try and predict the reappearance of MVE virus in south-eastern Australia, these are:
  1) serological testing of sentinel chicken flocks together with monitoring of vector mosquito species
  2) rainfall (Forbes hypothesis)\textsuperscript{13}
  3) barometric pressure (Nicholls hypothesis)\textsuperscript{14}
Objective

- VIDRL were asked by the VATF and the Victorian Government Department of Health to determine if there had been substantial undetected subclinical infection of MVE and Kunjin viruses since the last major outbreak in 1974.
Method

Three serosurveys were carried out:
1. 2002, VATF asked VIDRL to retrospectively test specimens collected as part of a Victorian Q Fever serosurvey
2. 2008 after 3 out of 10 sentinel chicken flocks showed reactivity to MVE for the first time in 30 years
3. 2011 following seroconversion in 5 out of 10 chicken flocks and environmental conditions being favourable to MVE spread.
Method (cont)

- Sera were tested using an in-house epitope blocking EIA for total antibody developed according to the method described by Roy Hall from QLD Uni \(^{14}\) with a few modifications.
- Positives or equivocals were sent interstate to another laboratory for further testing.
- In 2002 an in-house immunofluorescence (IF) total Ab test and blocking EIA were used.
  - Subsequent testing done using EIA only.
- From 2011 an in-house IgM IF was introduced.
In-house epitope blocking EIA for MVE and KUN total antibody
2002 serosurvey

- 529 sera selected for testing from the Q Fever 2002 Victorian Pre-vaccination screening database (20,000 sera) according to the region they came from.
  - 306 (58%) specimens were from six towns along the Murray River and
  - 223 (42%) specimens from five regions (1-5) around Victoria. (For data analysis regions 1-5 were amalgamated because of the commonality of not being near the Murray River.)

- Sex and age data were also analysed.
- We also wanted to compare MVE and KUN Ab results by in-house blocking EIA and in-house IF test.
## In-house epitope blocking EIA vs in-house IF

<table>
<thead>
<tr>
<th>Results</th>
<th>Concordant</th>
<th>Discordant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POS</td>
<td>EQU</td>
<td>NEG</td>
</tr>
<tr>
<td><strong>MVE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near Murray River</td>
<td>14 (4.6%)</td>
<td>3 (1%)</td>
<td>288 (94%)</td>
</tr>
<tr>
<td>Other regions (1-5)</td>
<td>2 (1%)</td>
<td>1 (0.5%)</td>
<td>218 (98%)</td>
</tr>
<tr>
<td>Total</td>
<td><strong>16 (3.0%)</strong></td>
<td><strong>4 (0.8%)</strong></td>
<td><strong>506 (95.7%)</strong></td>
</tr>
<tr>
<td><strong>Kunjin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near Murray River</td>
<td>16 (5.2%)</td>
<td>2 (0.6%)</td>
<td>278 (90.8%)</td>
</tr>
<tr>
<td>Other regions (1-5)</td>
<td>3 (1.3%)</td>
<td>1 (0.4%)</td>
<td>217 (97.3%)</td>
</tr>
<tr>
<td>Total</td>
<td><strong>19 (3.6%)</strong></td>
<td><strong>3 (0.6%)</strong></td>
<td><strong>495 (93.6%)</strong></td>
</tr>
</tbody>
</table>
In-house epitope blocking EIA vs in-house IF copy

- More positives detected by IF than EIA.
- For MVE Positives: 19/529 by IF vs 16/529 by EIA
- For KUN Positives: 31/529 by IF vs 19/529 by EIA
- Discordant results for MVE and KUN Ab were 3 and 12 respectively out of 529 sera. All were IF Pos/EIA Neg, except for one MVE result that was IF Pos/EIA equivocal.
- 23 sera, representing a cross section of EIA and IF result combo’s. sent to David Smith’s Lab in WA for EIA & neut.
- Results from WA Lab showed majority of ‘false positives’ were by IF 18/23 (78%) with only 1/23 (4%) false positive by EIA (KUN strong Pos, MVE weak Pos) and 4/23 (17%) results agreed with ours.
- One KUN EIA ‘false neg’, on retest reacted with neg control cells.
- Subsequent testing in our lab was by EIA only, because high number IF false positives/equivocals. EIA more specific.
Blocking EIA Cutoff

- 20% inhibition used as the cutoff in Hall’s method. 50% better for our population?
- We looked at specimens within 20% - 50% inhibition range, the majority of specimens were IF negative.
- 8 spec’s sent to David Smiths lab, none confirmed positive by EIA or neutralisation.
- 50% cutoff appropriate for our low risk population in Vic.
## MVE & KUN total antibody results

<table>
<thead>
<tr>
<th></th>
<th>POS</th>
<th>EQU</th>
<th>NEG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MVE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near Murray River</td>
<td>14 (4.6%)</td>
<td>3 (1%)</td>
<td>289 (94.4%)</td>
<td>306</td>
</tr>
<tr>
<td>Other regions (1-5)</td>
<td>3 (1.3%)</td>
<td>1 (0.4%)</td>
<td>219 (98.6%)</td>
<td>223</td>
</tr>
<tr>
<td>Total</td>
<td>17 (3.2%)</td>
<td>4 (0.8%)</td>
<td>508 (96.0%)</td>
<td>529</td>
</tr>
<tr>
<td><strong>Kunjin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<tr>
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<td>507 (95.8%)</td>
<td>529</td>
</tr>
</tbody>
</table>
Seropositivity: Murray vs Other regions

- MVE, the number of people positive in the Murray River region compared to other regions was 4.6% (14/306) vs 1.3% (3/223) respectively (p= 0.02).
- Similarly for KUN, 5.2% (16/306) vs 1.3% (3/223) respectively (p= 0.01).
- Overall seroprevalence
  - MVE Ab pos: 17/529, 3.2% (95%, CI 1.9-5.1)
  - KUN Ab pos: 19/529, 3.6% (95%, CI 2.2-5.6)
Age vs MVE & KUN seropositivity

- Age, related to being antibody positive, was more important for MVE than for KUN.

- We hypothesised that the difference between MVE and KUN was because MVE tends to occur as epidemics, therefore age is relevant whereas KUN is enzootic and age is not so important.

- As the last outbreak of MVE in Victoria was in 1974, positive results were analysed according to individuals < 40 years of age and > 40 years, MVE seropositivity was significantly different in the two age groups (p=0.049) but KUN seropositivity was not (p=0.38).
### Age vs MVE & KUN total Ab status

<table>
<thead>
<tr>
<th>Age(yrs)</th>
<th>MVE +</th>
<th>MVE -</th>
<th>MVE +</th>
<th>MVE -</th>
<th>KUN +</th>
<th>KUN -</th>
<th>KUN +</th>
<th>KUN -</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>20-39</td>
<td>0</td>
<td>101</td>
<td>0</td>
<td>51</td>
<td>3</td>
<td>98</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>40-64</td>
<td>10</td>
<td>154</td>
<td>2</td>
<td>136</td>
<td>9</td>
<td>157</td>
<td>2</td>
<td>136</td>
</tr>
<tr>
<td>&gt;65</td>
<td>4</td>
<td>18</td>
<td>1</td>
<td>26</td>
<td>4</td>
<td>17</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>?</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>290</td>
<td>3</td>
<td>221</td>
<td>16</td>
<td>289</td>
<td>3</td>
<td>220</td>
</tr>
</tbody>
</table>
Sex vs MVE & KUN seropositivity

- 76.5% of those tested were male.
- For MVE males were more likely to be Ab positive than females (4.4% vs 0.8%, p=0.06).
- For KUN males were more likely to be Ab positive than females (4.4% vs 1.6%, p=0.185)
2008 serosurvey

- In 2008, for the first time in 30 years, 3 sentinel chicken flocks in Victoria (Mildura, Kerang and Barooga) tested positive for MVE Ab.
2008 Serosurvey (cont)

- 121 specimens collected from volunteers 30 years or younger (born after 1974) from private path. in Kerang and Mildura.
- 1/121 MVE Ab positive, (0.8%, 95%, CI 0.02-4.6). Kunjin Ab not done.
- Main limitation small sample size, due to limited time frame, before next mosquito season.
2008 Serosurvey (cont)

- Spread of MVE virus may have been limited by unprecedented drought conditions in Victoria in 2008.
- There were low numbers of mosquito vectors, therefore amplification of MVE virus may have occurred in bird populations around remaining water sources but low vector numbers may have limited spread to humans\(^\text{17}\).
In the summer of 2010/2011, the Murray River region experienced 2 major flood events, 5 sentinel chicken flocks in Victoria tested positive for MVE Ab.
2011 Serosurvey (cont)

- Large mosquito vector numbers and detection of MVE Ab’s in horses.
- MVE virus was not detected in trapped mosquitoes.
- March 2011, a 69 year old man with encephalitis died within one week of being transferred from Mildura Base Hospital to ICU in Melbourne.\(^\text{18}\)
- Initial lab tests Day 4 – Day 8 MVE total Ab pos/IgM neg, PCR neg. Unfortunately he died Day 10 and an autopsy request was declined.
2011 serosurvey (cont)

- Victorian Health Department large opportunistic serosurvey to estimate population immunity to both MVE and KUN (total Ab) and determine evidence of recent infection (IgM).
- The study comprised 1,116 specimens, 523 serum specimens from a diagnostic laboratory in Mildura and 593 from blood donors from the Murray River region.
MVE and KUN total Ab seroprevalence
Diagnostic lab. vs Blood bank sera

<table>
<thead>
<tr>
<th>Results</th>
<th>MVE (N=1,115)</th>
<th>KUN (N=1,116)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POS</td>
<td>NEG</td>
</tr>
<tr>
<td>Diag. Lab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=523)</td>
<td>18 (3.4%)</td>
<td>500 (95.6%)</td>
</tr>
<tr>
<td>Blood Bank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=592)</td>
<td>6 (1.0%)</td>
<td>585 (98.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (2.2%)</td>
<td>1085 (97.3%)</td>
</tr>
</tbody>
</table>
MVE and Kunjin Ab Positives and Equivocals 2011 – Age breakdown for Mildura (no age data for Blood bank samples)

<table>
<thead>
<tr>
<th>Mildura</th>
<th>Positives</th>
<th>Equivocals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 37yrs (N=325)</td>
<td>&gt; 37 yrs (N = 198)</td>
</tr>
<tr>
<td>MVE</td>
<td>2 (0.6%)</td>
<td>16 (8.1%)</td>
</tr>
<tr>
<td>KUN</td>
<td>11 (3.4%)</td>
<td>19 (9.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>13 (4.0%)</td>
<td>35 (17.7%)</td>
</tr>
</tbody>
</table>
MVE seroprevalence

- 2.2% (24/1,115, 95% CI 1.3-3.0%) samples positive for MVE total Ab.
- More positives results in older than younger people (5.2% vs 0.4%, p<0.05).
- Seroprevalence higher in diagnostic lab. samples compared to blood bank (3.4% vs 1.0%, p<0.05).
- 2 positive results in patients less than 37 years of age, indicating exposure since 1974. Neither IgM positive suggesting recent exposure unlikely.
- All positive samples from people residing in Mildura (3.6%), Swan Hill (3.4%) or Greater Shepparton (0.6%) – all Murray Region.
KUN seroprevalence

- 3.1% (34/1,116, 95% CI 2.0-4.1%) samples positive for KUN total Ab.
- More positive results in older than younger people (5.1% vs 2.2%, p<0.05).
- Seroprevalence higher in diagnostic lab. samples than in Blood bank (5.0% vs 1.3%, p<0.05).
- 3/34 KUN total Ab positive samples also KUN IgM positive, suggesting recent exposure.
- Wider regional distribution of KUN positives (7 out of 8 regions tested) compared to MVE positives (3 out of 8 regions tested).
Conclusion

- Seroprevalence studies suggest minimal exposure to either MVE or Kunjin viruses in Victoria in recent decades, despite models predicting otherwise.
- People living near the Murray River and males were more likely to be positive.
- Age was more important for MVE seropositivity than for Kunjin.
- The absence of predicted disease circulation raises important questions about how existing models are used to predict MVE virus outbreaks.
- In an outbreak situation a large proportion of the Victorian population remain at risk of infection.
Acknowledgments

- James Fielding, Heath Kelly & Theo Karapanagiotidis & Alan Breschkin (VIDRL)
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References (1)


