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Sindbis and West Nile virus infections in the Witwatersrand-Pretoria region

P. G. JUPP, N. K. BLACKBURN, D. L. THOMPSON, G. M. MEENEHAN

Summary

From mid-December 1983 until mid-April 1984, there was an epidemic of Sindbis (SIN) virus infection in the Witwatersrand-Pretoria region in which hundreds of human cases were diagnosed clinically. Twenty-eight of these diagnoses were confirmed in the laboratory by seroconversion as being infections with SIN virus, and 5 cases of infection with West Nile (WN) virus were also found. Attempts to isolate virus from 66 patients, mainly from serum specimens, were unsuccessful. Infection rates for the mosquito vector Culex univittatus, collected at localities on the Witwatersrand and in February and March, were mostly higher for both SIN and WN viruses than in previous years. The highest rates determined were 5.4 (SIN) and 9.6 (WN) per 1000 mosquitoes. It is concluded that an epizootic of both viruses occurred which was manifested by a high level of viral activity in the feral Cx. univittatus-bird transmission cycle. Cx. univittatus efficiently transferred infection of SIN virus from this cycle to man to cause the epidemic of infection with that virus but it is unclear why there were apparently only a few cases of WN virus infection.


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rates for both viruses during the epidemic. This was done to investigate whether the high frequency of human cases of SIN virus infection was also expressed in the feral cycle by a higher infection rate in Cx. univittatus.

The human infection and Cx. univittatus infection with both viruses is reported.

Methods

Virology

On arrival in the laboratory blood from acute human cases was centrifuged and the sera stored at -70°C, while sera from blood of convalescents were stored at -20°C. In an attempt to isolate the virus, sera and mosquito suspensions were inoculated into suckling mice, 1 - 3 days old, intracerebrally. A few suspensions prepared from skin vesicle scrapings were also inoculated. For identification of virus isolates complement-fixing antigens were prepared from the brains of sick suckling mice, either by sucrose-acetone extraction of the brains or from a lightly centrifuged 20% buffered saline suspension. These antigens were tested in complement-fixation tests against hyperimmune mouse ascitic fluids.

All human sera were tested for antibodies in microhemagglutination-inhibition (HI) tests against antigens of SIN, WN, Chikungunya, Wesselsbron and Rift Valley fever viruses. Ten-fold dilutions of serum over the range 1/20 - 1/2560 were tested against 8 units of viral antigen. Those sera which reacted against both WN and Wesselsbron antigens were also tested for neutralizing antibodies by microneutralization tests using vero cells.

Mosquitoes

Up to 20 portable battery-operated light traps baited with solid carbon dioxide were set overnight for 15 nights during February and March 1984. The trap used was a National Institute for Virology light suction trap designed and made by the senior author (P. G. J.). Collections were made at four localities on the Witwatersrand chosen because of the presence of permanent water for mosquito breeding and for their concentrations of birds. These localities were Germiston (Rondebult Lake), Rietfontein (Jukskei River), Modderfontein (several dams) and Florida (Florida Lake).

In order to collect as many Cx. univittatus as possible the large number of traps was necessary, because Cx. univittatus was invariably greatly outnumbered by Culex pipiens and Culex theileri. The Cx. univittatus were either extracted from the catches on the day of collection, pooled in 25s and stored at -70°C, or all mosquito species were stored at -70°C and subsequently the Cx. univittatus were extracted and stored again at -70°C.

Results

There were 28 cases of infection with SIN virus and 5 with WN virus shown by seroconversion in the HI test. These may be compared with only 2 such seroconversions for SIN virus and none for WN virus among paired sera received and tested the previous summer (1982/1983). All WN virus infections were confirmed by the neutralization test. No viruses were isolated from 63 sera from acutely-ill humans or from the skin lesions of 6 patients.

The numbers of isolations of SIN and WN viruses obtained from Cx. univittatus collected at four Witwatersrand localities in 1984 are shown in Table I. As a comparison the same data obtained during five previous summers at Olifantsvlei in the southern Witwatersrand are included.

Discussion

The significant increase in the number of human infections with SIN virus in the 1983/1984 summer compared with 1982/1983 confirmed clinical reports which indicated that an epidemic of this virus had occurred. The increase in the number of human infections with WN virus over the previous season was only slight. More of the SIN cases were not confirmed in the laboratory because suitable specimens were not obtained. In infection by either SIN or WN virus the viraemia is of a low level and transient, the patient usually seeking medical attention only when the maculopapular rash appears. The rash indicates the commencement of antibody formation which usually precludes successful virus isolation. Therefore the demonstration of a rise in antibody titre — seroconversion — is the only practical method of laboratory diagnosis and it depends on paired sera — acute and convalescent — being available from each patient. The first or 'acute' serum should be obtained as early in the illness as possible and the second or 'convalescent' serum 2 - 3 weeks or longer afterwards. From 50 or more patients in this study with antibodies to one or both of the viruses only 1 serum sample was collected. Therefore, any rise in antibody level indicative of a recent infection could not be demonstrated.

Malherbe et al. managed to isolate virus from the skin lesions of 1 patient but McIntosh et al. in a later study failed

<table>
<thead>
<tr>
<th>Locality/</th>
<th>Year</th>
<th>Cx. univittatus</th>
<th>No. pools</th>
<th>No. Isolations of SIN</th>
<th>Infection rate</th>
<th>Isolations of WN</th>
<th>Infection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germiston</td>
<td>1984</td>
<td>1980</td>
<td>79</td>
<td>5</td>
<td>2.5</td>
<td>19</td>
<td>9.6</td>
</tr>
<tr>
<td>Rietfontein</td>
<td>1984</td>
<td>817</td>
<td>33</td>
<td>2</td>
<td>2.4</td>
<td>3</td>
<td>3.7</td>
</tr>
<tr>
<td>Modderfontein</td>
<td>1984</td>
<td>558</td>
<td>23</td>
<td>3</td>
<td>5.4</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Florida/</td>
<td>Rietfontein</td>
<td>1984</td>
<td>22</td>
<td>1</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Olifantsvlei*</td>
<td>1972</td>
<td>4259</td>
<td>177</td>
<td>3</td>
<td>0.7</td>
<td>10</td>
<td>2.3</td>
</tr>
<tr>
<td>Olifantsvlei*</td>
<td>1970</td>
<td>6943</td>
<td>221</td>
<td>2</td>
<td>0.3</td>
<td>7</td>
<td>1.0</td>
</tr>
<tr>
<td>Olifantsvlei*</td>
<td>1969</td>
<td>1670</td>
<td>51</td>
<td>1</td>
<td>0.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Olifantsvlei*</td>
<td>1967</td>
<td>2875</td>
<td>114</td>
<td>7</td>
<td>2.4</td>
<td>11</td>
<td>3.8</td>
</tr>
<tr>
<td>Olifantsvlei*</td>
<td>1966</td>
<td>3522</td>
<td>164</td>
<td>1</td>
<td>0.3</td>
<td>3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*From McIntosh et al.*

†Infection rate is number infected per 1000 tested.
TABLE II. MONTHLY RAINFALL AND TEMPERATURES FOR 1983/1984 COMPARED WITH NORMAL MEANS FOR THE SAME MONTHS CALCULATED FROM 20 YEARS OF PREVIOUS RECORDINGS

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall (mm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Current</td>
</tr>
<tr>
<td>October</td>
<td>65.0</td>
<td>90.4</td>
</tr>
<tr>
<td>November</td>
<td>120.2</td>
<td>131.9</td>
</tr>
<tr>
<td>December</td>
<td>113.4</td>
<td>167.3</td>
</tr>
<tr>
<td>January</td>
<td>129.2</td>
<td>60.5</td>
</tr>
<tr>
<td>February</td>
<td>91.1</td>
<td>13.1</td>
</tr>
<tr>
<td>March</td>
<td>88.7</td>
<td>77.6</td>
</tr>
</tbody>
</table>

to do this with several patients. Similarly, no virus was isolated from skin vesicle scrapings in our study.

The infection rates (IRs) for *Cx. univittatus* determined in 1984 were compared with those determined in previous years at Olifantsvlei. The IR for SIN virus at Modderfontein and for WN virus at Germiston were much higher, while IRs for SIN virus at Germiston and for both viruses at Rietfontein equaled the highest rate previously recorded at Olifantsvlei. These high IRs indicate that an epizootic of both viruses occurred which was manifested by a high level of viral activity in the feral maintenance cycle between birds and *Cx. univittatus*. The unusually high rainfall early in the summer together with higher than normal temperatures throughout the mosquito season were probably important. These environmental factors probably contributed towards the occurrence of these epizootics and the subsequent epidemic of SIN virus infection. Table II shows the rainfall and temperatures from October to March 1984, compared with the normal averages usually expected during the same months. The rainfall pattern favoured mosquito breeding early in the summer so that densities of *Cx. univittatus* would have been high by December. The high temperatures are thought to have favoured viral infection in the mosquito and also to have enhanced the subsequent transmission of virus: Jupp\(^4\) has demonstrated how increased temperature can enhance the transmission of WN virus by *Cx. univittatus*.

Although *Cx. univittatus* evidently successfully transferred infection of SIN virus from the feral cycle over to man, hence causing the epidemic of infection with that virus, it is unclear why such transfer only occurred to a limited extent with WN virus.

A grant from the South African Medical Research Council towards this work is acknowledged. We are grateful to Mrs N. Aldridge and Miss L. Ben Joseph for technical assistance and to the following for allowing us to set mosquito traps in areas under their jurisdiction: the Director of Parks, Germiston and Florida Municipalities; the Senior Administrative Officer, Rietfontein Hospital; and African Explosives and Chemical Industries, Modderfontein. We thank the Director-General of National Health for permission to publish.

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