Growth of Venezuelan Encephalitis Virus in Embryonic Cell Cultures of Wild Birds

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Primary embryonic cell cultures made from nine species of wild and domestic birds were infected with Venezuelan encephalitis virus and reproduced each strain to moderately high levels.

Although the natural cycle of Venezuelan encephalitis (VE) virus (a group A arbovirus) is recognized to principally involve terrestrial mammals (3, 5), the virus to date has been isolated from seven species of wild birds and antibodies have been detected in 21 species of naturally infected wild birds (2). Fifteen species of birds, in addition to domestic chickens, have been infected experimentally, all developing detectable levels of viremia (Dickerman, unpublished data; reference 1). Still, little has been reported on the comparative ability of animals to become infected with VE virus. This study reports the relative ability of avian embryonic cells from birds of diverse taxonomic ranks to replicate Venezuelan encephalitis virus.

Tissue cultures. Primary cultures were made from 8- to 15-day-old embryos by the method used for chicken embryonic cell culture (CEC) (4). Cells were grown with 5 ml of liquid media in square culture bottles with a surface of about 18 cm². Monolayers of CEC in these bottles contain 2.0 × 10⁶ to 2.7 × 10⁶ cells (3), and monolayers of snowy egret cultures contained 2.2 × 10⁶ cells as counted by hemocytometer after trypsinization. Chicken and Japanese quail eggs were from domestic sources. Others were from wild or semi-wild birds and were collected from natural nestings. At 24, 48, and 72 hr post-inoculation, 0.5 ml of supernatant fluid was harvested from each of two replicate cultures, pooled, and then divided into two samples and frozen at −70 C until tested. Peak virus levels were found in the 48-hr harvests and those data are reported. Virus concentrations in inocula and fluid harvests were measured in terms of plaque-forming units (PFU) in CEC with an agar overlay as previously described (5).

Viruses. Four strains of VE virus were used representing three varieties of subtype I (6). Strains 64A87 isolated from Culex opisthopus mosquitoes in Mexico in 1964 and 68U201 isolated from a sentinel hamster in Guatemala in 1968 represent endemic Central American variety I-E. Strain 69Z1 isolated from a sick human in Guatemala in 1969 is variety I-B and PMC Ho5 isolated from a sick human in Venezuela is variety I-C. The first three strains were used as brain suspensions from first or second passages in suckling mice. PMC Ho5 was employed after one passage in Vero cultures and one passage in suckling mouse brains. Inocula varied from 10⁴.¹ to 10⁵.¹ PFU/cell culture bottle.

Four strains of VE virus, two isolated from sick humans during epidemics and two isolated from endemic situations, grew in primary embryonic cell cultures of each of six species of birds, whereas the two epidemic and one endemic strain grew in four additional species. The 10 species belong to six orders of birds (Table 1). An occasional batch of cells produced low levels of viruses to all strains (for example, rock dove) but for each species one or more cell preparations produced high titers of virus. In Table 1, titers under 4.0 resulting from low-producing lots of cells are not included in averages. At least one primary embryonic cell culture of each species had the ability to replicate any one virus to approximately equivalent yields. (Compare especially maximum yields in each vertical column: 64A87, 6.0 to 8.2; 68U201, 6.2 to 8.6; 69Z1, 7.4 to 9.1; PMC Ho5, 7.8 to 8.6.) In contrast, except for 68U201 in Japanese quail cells, and in the single batch of herring gull cells and for 64A87 in a single batch of starling cells, mean and maximum titers in any one type of cells were produced by infection with the two epidemic strains of VE virus, 69Z1 and PMC Ho5.

The ability of embryonic cell cultures to amplify VE virus to high titers may not correlate with in vivo systems. Still, it is interesting to note the uniformity in maximal viremia titers among five species of birds belonging to two orders (Columbiformes and Passeriformes) following inoculation or infection by bite of experimentally infected mosquitoes with one epidemic
Table 1. Yields of Venezuelan encephalitis virus in avian embryonic cell culture supernatant fluids 48 hr after inoculation and incubation at 37°C

<table>
<thead>
<tr>
<th>Avian order and species</th>
<th>Yields of VE virus in avian embryonic cell cultures&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>64A87</td>
</tr>
<tr>
<td>Ciconiiformes</td>
<td></td>
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<tr>
<td>Common egret</td>
<td>6.3</td>
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<tr>
<td>(Egretta alba)</td>
<td>(5.2-7.2; n = 3)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Snowy egret</td>
<td>6.7</td>
</tr>
<tr>
<td>(Egretta thula)</td>
<td>(6.5-7.1; n = 3)</td>
</tr>
<tr>
<td>Black-crowned night heron</td>
<td>7.0</td>
</tr>
<tr>
<td>(Nycticorax nycticorax)</td>
<td>(3.5, 5.8-7.5; n = 5)</td>
</tr>
<tr>
<td>Anseriformes</td>
<td></td>
</tr>
<tr>
<td>Mallard duck</td>
<td>8.1</td>
</tr>
<tr>
<td>(Anas platyrhynchos)</td>
<td></td>
</tr>
<tr>
<td>Galliformes</td>
<td></td>
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<tr>
<td>Japanese quail</td>
<td>7.7</td>
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<tr>
<td>(Coturnix coturnix)</td>
<td>(7.4-8.1; n = 3)</td>
</tr>
<tr>
<td>Domestic chicken</td>
<td>6.4</td>
</tr>
<tr>
<td>(Gallus gallus)</td>
<td>(6.1-7.1; n = 4)</td>
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<tr>
<td>Charadriiformes</td>
<td></td>
</tr>
<tr>
<td>Herring gull</td>
<td>7.8</td>
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<tr>
<td>(Larus argentatus)</td>
<td></td>
</tr>
<tr>
<td>Columbiformes</td>
<td></td>
</tr>
<tr>
<td>Rock dove</td>
<td>7.8</td>
</tr>
<tr>
<td>(Columba livia)</td>
<td>(2.6 and 6.0; n = 2)</td>
</tr>
<tr>
<td>Passeriformes</td>
<td></td>
</tr>
<tr>
<td>Starling</td>
<td>8.2</td>
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<tr>
<td>(Sturnus vulgaris)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Log<sub>10</sub> plaque-forming units/0.1 ml of fluid harvest.

<sup>b</sup> First number indicates mean, numbers in parenthesis indicate maximum yields, and n indicates number of cell cultures tested.

strain of VE virus (Trinidad). These five titers were 3.8, 4.2, 4.3, 4.8, and 5.0 weanling mouse LD<sub>50</sub>/0.02 ml (1). Further, whereas all four strains reported above destroyed cell cultures by 48 to 72 hr, strains 64A87, 69Z1, and PMC Ho5 showed relatively low levels of lethality to newly hatched White Leghorn chickens (R. W. Dickerman and C. M. Bonacorsa, submitted for publication), and strain 64A87 has been experimentally inoculated into nine other species of wild birds without significant mortality.

These results demonstrate the ability of primary embryonic cell cultures of 10 species of birds belonging to six orders of birds to support the growth of three (or four) strains of VE virus to approximately equivalent titers. Variation in mean and maximal titers produced was dependent in part on viral strain and in part on the vigor of a particular batch of cells rather than on the taxa of avian cells in which the virus was grown.

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LITERATURE CITED