Isolation of Jamestown Canyon virus (California virus group) from vesicular lesions of a horse

Sudhir P. Sahu, John Landgraf, Nora Wineland, Doug Pedersen, David Alstad, Gary Gustafson

Jamestown Canyon (JC) virus is a member of California encephalitis virus group (Bunyaviridae). Antibody to JC virus has been detected in humans and various domestic and wild animals. White-tailed deer (Odocoileus virginianus) are generally recognized as the primary vertebrate reservoir host. The virus is transmitted by the bite of infective mosquitoes, mostly belonging to genus Aedes. An increase in deer population close to human residents has been implicated in the rise of human infection and an increase in the prevalence of antibodies to JC virus in many regions of the USA. A high prevalence of virus-specific antibody has also been found in horses in Wisconsin.

References

Table 1. Identification of virus isolate NVSL 97-29574 by serum neutralization test.

<table>
<thead>
<tr>
<th>Virus group</th>
<th>Results*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunyamwera</td>
<td></td>
</tr>
<tr>
<td>Cache Valley</td>
<td>negative</td>
</tr>
<tr>
<td>Lockern</td>
<td>negative</td>
</tr>
<tr>
<td>Northway</td>
<td>negative</td>
</tr>
<tr>
<td>Main Drain</td>
<td>negative</td>
</tr>
<tr>
<td>Talcotalpan</td>
<td>negative</td>
</tr>
<tr>
<td>Tensaw</td>
<td>negative</td>
</tr>
<tr>
<td>California encephalitis</td>
<td></td>
</tr>
<tr>
<td>Jamestown Canyon</td>
<td>positive</td>
</tr>
<tr>
<td>Keystone</td>
<td>negative</td>
</tr>
<tr>
<td>La Crosse</td>
<td>positive</td>
</tr>
<tr>
<td>San Angelo</td>
<td>positive</td>
</tr>
<tr>
<td>Snowshoe hare</td>
<td>positive</td>
</tr>
<tr>
<td>South River</td>
<td>negative</td>
</tr>
<tr>
<td>Trivittatus</td>
<td>negative</td>
</tr>
<tr>
<td>Simbu</td>
<td></td>
</tr>
<tr>
<td>Buttonwillow</td>
<td>negative</td>
</tr>
<tr>
<td>Turlock</td>
<td>negative</td>
</tr>
<tr>
<td>Turlock</td>
<td>negative</td>
</tr>
</tbody>
</table>

* A 1:10 dilution of antiserum to the viruses was used.

Epithelial tissues from vesicular lesions were homogenized in a tissue grinder with 10 ml of minimal essential medium (MEM) and centrifuged at 800 × g for 20 minutes, and the supernatant was used as inoculum. Vesicular fluid was diluted 1:10 with MEM and treated as above. Medium was discarded from 25-cm² tissue culture flasks of 48–72-hour-old Vero cells, and 1–2 ml of the supernatant was pipetted into 3 flasks. The inocula were allowed to adsorb onto Vero cells for 1 hour at 37 C; then the inoculum was discarded from each flask and 10 ml of MEM with 2% fetal calf serum was added to each flask. The flasks were incubated at 37 C and examined daily for cytopathic effect (CPE).

Sera from both horses were negative for antibody to VSV serotypes New Jersey and Indiana I and Indiana II. A virus (NVSL 97-29574) was isolated from vesicular fluid and epithelial tissues but did not react with antiserum to VSV (data not shown). Electron microscopic examination of the isolate showed the virus to be morphologically similar to the members of the Bunyaviridae family.

Tissue culture isolate NVSL 97-29574 was diluted 1:10 in phosphate-buffered saline containing 0.75% bovine serum albumin (pH 7.8), and 0.02 ml was inoculated into 20 4-day-old mice. Brain was harvested from mice that died 48 hours after intracerebral inoculation and was used to produce mouse ascitic fluid (MAF).18

Bunyavirus identification was done by a microtiter virus neutralization test. A 1:10 dilution of MAF to various bunyaviruses (Table 1) was mixed with 200 TCID₅₀ of the isolate virus. The microplates were incubated at 37 C for 1 hour, and then Vero cells were added. The plates were incubated at 37 C in a CO₂ incubator for 96 hours before evaluation for 90% protection of Vero cells.

A microtiter virus neutralization test was used as described earlier with the 4 known cross-reacting viruses, JC, La Crosse, snowshoe hare, and San Angelo, with 1:10 to 1:1,280 dilution of MAF against each virus. The plates were incubated at 37 C in a CO₂ incubator for 96 hours before evaluation for 90% protection of Vero cells.

The virus isolate NVSL 97-29574 was neutralized by antisem to JC, San Angelo, La Crosse, and snowshoe hare viruses (Table 1). MAF to NVSL 97-29574 gave a neutralizing titer of 1:320 with its homologous virus and 1:640 with JC virus (Table 2). MAF to JC virus produced a similar titer with its homolog and with the isolate virus NVSL 97-29574; however, La Crosse, San Angelo, and snowshoe hare viruses gave a titer of ≤1:40 with the isolate virus NVSL 97-29574 and with JC virus. When serum samples from the 2 horse

Table 2. Cross-neutralization tests for various virus isolates and mouse ascitic fluid (MAF) antisera.

<table>
<thead>
<tr>
<th>Antiserum (MAF)</th>
<th>NVSL 97-29574</th>
<th>Jamestown Canyon</th>
<th>La Crosse</th>
<th>San Angelo</th>
<th>Snowshoe hare</th>
<th>Normal MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVSL 97-29574</td>
<td>1:320</td>
<td>1:320</td>
<td>1:10</td>
<td>1:40</td>
<td>1:20</td>
<td>&lt;1:10</td>
</tr>
<tr>
<td>Jamestown Canyon</td>
<td>1:640</td>
<td>1:320</td>
<td>1:20</td>
<td>1:80</td>
<td>1:40</td>
<td>&lt;1:10</td>
</tr>
<tr>
<td>La Crosse</td>
<td>1:40</td>
<td>1:10</td>
<td>1:640</td>
<td>1:40</td>
<td>1:160</td>
<td>&lt;1:10</td>
</tr>
<tr>
<td>San Angelo</td>
<td>1:10</td>
<td>1:20</td>
<td>1:20</td>
<td>1:640</td>
<td>1:160</td>
<td>&lt;1:10</td>
</tr>
<tr>
<td>Snowshoe hare</td>
<td>1:40</td>
<td>1:40</td>
<td>1:80</td>
<td>1:160</td>
<td>1:640</td>
<td>&lt;1:10</td>
</tr>
</tbody>
</table>

sin,6 California,2,5 Michigan,13 and the Delmarva area17 in the USA. Of 5 ponies experimentally infected with JC virus remained normal, none developed detectable viremia but 3 developed at least a 4-fold rise in antibody titers to JC virus.6 Here, we report the isolation of JC virus from vesicular lesions on coronary band, tongue, and inner lip of a horse from northern Colorado.

In July 1997, a ranch outfitter in Colorado purchased 2 horses. Approximately 1 month later, the rancher observed that 1 of the new horses was anorexic and had lost approximately 45 kg. The second horse remained normal. The attending veterinarian noticed that the affected horse also had vesicles on the coronary band and ruptured vesicular lesions on the tongue and inside of the lower lip. At the time these signs were noticed, there was a vesicular stomatitis virus (VSV) outbreak in the southwestern USA. Because VSV was part of the differential diagnosis, local and federal officials were notified, and fluids from the vesicle, epithelial tissues from lesions on the tongue and the lower lip, and blood were collected. Blood was also collected from the companion horse. All samples were submitted to the National Veterinary Services Laboratories, Ames, Iowa, for VSV evaluation.
were tested for neutralizing activity against all 4 bunyavi-

rus es and NVSL 97-29574, only serum from the nonaffected

horse neutralized JC virus and NVSL 97-29574 at 1:20 di-

lution (Table 3).

Antibodies to JC virus have been reported from many do-

mestic and wild animals, but the virus had only been isolated

from a fawn.\textsuperscript{11} Isolation may be difficult because the virus

is fragile and is inactivated during transportation to the lab-

oratory, there is a low level of viremia, or the virus has an

affinity for tissues other than blood cells. Recently, JC virus

from the whole blood of 4 deer was isolated by diluting the

samples with equal volumes of medium 199 with 10% fetal
calf serum and freezing them immediately on dry ice before

transporting them to the laboratory (Grimstad, personal com-

munication). This finding suggests that the virus is fragile, and

freezing samples immediately on dry ice may have pro-
tected the virus from inactivation. At the National Veterin-

ary Services Laboratories, JC virus was isolated from vesicula-

r fluid and vesicular epithelial tissues. The samples were kept

at 4°C for 4 days before virus isolation was attempted. The

Vero cells showed CPE 36 hours after inoculation and had

3 logs of the virus per 25 μl of culture fluid, suggesting an

affinity of the virus for epithelial cells. No attempt was made

to isolate virus from the horse blood. A comparison of the

present results with those of earlier studies suggests the pres-

ence of perhaps 2 genetically different types of JC viruses.\textsuperscript{6,11}

One type is asymptomatic, circulates in the blood, and is

sensitive to changes in temperature. The other type has af-

finity for epithelial cells, produces vesicular lesions, and is

less sensitive to degradation at refrigeration temperature.

California serogroup viruses are important causes of en-

cephalitis (nuchal rigidity, tremors, convulsions) in humans. In-creased reports of JC virus infections in humans are asso-

ciated with expanding deer populations in the northwestern

USA and have led many scientists to implicate JC virus as an

etiologic agent of the emerging human disease.\textsuperscript{7} How-

ever, the isolation of JC virus from human patients also re-

mains elusive. The diagnosis is usually based on a 4-fold in-

crease in the virus neutralization titer of the patient. Other

than in humans, no visible signs of clinical disease have been

reported in domestic or wild animals infected with JC virus.

In humans, JC virus affects mostly adults, and the central

nervous system infection is the most common diagnosis. En-

cephalitis is correlated with high antibody titers to JC virus.

A single human case with multiple red vesicular papules in

the genital region of an individual following a camping trip

has been described.\textsuperscript{7} The papules were clinically determined

not to be of herpesvirus etiology. There was a significant

increase in antibody titer to JC virus between the initial and

second serum samples of the patient (1:16 vs. 1:256), sug-

gesting JC virus as the cause of the papules.

The horse without lesions had an antibody titer of 1:20 to

JC virus, and these antibodies may have protected the horse

during lesions. The horse from which JC virus was

isolated had an antibody titer of <1:10 and possibly devel-

oped lesions because it lacked protection. The horse with

lesions became extremely emaciated and weak and was eu-

thanized. Convalescent serum samples from the unaffected

were not be obtained.

In summary, a bunyavirus, JC virus (NVSL 97-29574), of

the California virus serogroup was isolated from fluid of a

vesicle on the coronary band and epithelial tissues from ve-

sicular lesions on the tongue and inside of the lower lip of

1 of 2 horses in northern Colorado. Serum from the affected

horse was negative for antibodies to the isolate, JC, La

Crosse, San Angelo, and snowshoe hare viruses. Serum from

a clinically normal companion horse had an antibody titer

of 1:20 to the isolate and to JC virus. This is the first report

of the isolation of JC virus from a horse, which had vesicular

lesions on the coronary band, tongue, and lip, and the second

report of isolation of JC virus from mammals.\textsuperscript{11} The clinical

presentation of JC virus infection in this horse is similar to

the clinical presentation of horses infected with VSV. Con-

sequently, VSV and JC virus infections should be consid-

ered in the differential diagnosis of vesicular lesions in horses.

Acknowledgements. We thank Suzette Anderson and

Mary Goecke for their help in preparing the manuscript.

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| Table 3. | Neutralization titers for various viruses and sera from infected and companion horses. |
|---|---|---|
| **Viruses** | **MAF antiserum** | **Horse sera** |
| | NVSL 97-29574 | Jamestown Canyon | Snowshoe hare | Infected horse | Companion horse | Normal horse |
| NVSL 97-29574 | 1:640 | 1:640 | 1:40 | <1:10 | 1:20 | <1:10 |
| Jamestown Canyon | 1:640 | 1:640 | 1:40 | <1:10 | 1:20 | <1:10 |
| Snowshoe hare | 1:80 | 1:40 | 1:640 | <1:10 | <1:10 | <1:10 |
| La Crosse | ND‡ | ND | ND | <1:10 | <1:10 | <1:10 |
| San Angelo | ND | ND | ND | <1:10 | <1:10 | <1:10 |

* MAF = mouse ascitic fluid.

† ND = not done.
Improved plating media for the detection of *Salmonella* species with typical and atypical hydrogen sulfide production

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Abstract. The *Salmonella* detection ability of 2 surfactant-supplemental media, xylose–lysine–tergitol (Niaproof) 4 (XLT4) and Miller-Mallinson (MM) agar, was compared against that of several commonly used plating media. XLT4 and MM appeared to be the most efficient in detecting *Salmonella* in meat products and food animal environments. MM was superior to XLT4 in detecting those increasingly more prevalent strains of *Salmonella* possessing weak to ultraweak H$_2$S production characteristics.

The accurate, efficient detection of *Salmonella* in red and white meat products and in the food animal environment is of paramount concern to veterinary microbiologists. The avoidance of false-positive plating reactions significantly reduces the time and effort needed to establish a diagnosis. Further, the avoidance of false-negative test results can be of critical importance to epidemiologists whose analyses are compromised when positive samples are misclassified as negative. As a consequence, the choice of plating media used for *Salmonella* detection has broad implications.

Many commonly used *Salmonella* selective plating media are formulated to detect *Salmonella* species on the basis of H$_2$S production. Such media include bismuth–sulfite (BS), Hektoen enteric (HE) and xylose–lysine–desoxycholate (XLD) agar. New surfactant-supplemented media have recently been introduced that also signal the presence of H$_2$S production. These include xylose–lysine–tergitol 4 (XLT4) and Miller-Mallinson (MM) agars. Both XLT4 and MM are uniquely formulated with the surfactant tergitol (Niaproof) to enhance the specificity and sensitivity of *Salmonella* detection and the reduction of competing Enterobacteriaceae other than *Salmonella*.

MM agar (Table 1) is particularly useful in detecting *Salmonella* species with weak H$_2$S production characteristics that may be missed when using XLT4 or the other selective agars. Such *Salmonella* species have been reported to rep-