Hepatitis E virus (HEV) is the causative agent of hepatitis E (HE). HE occurs in sporadic and epidemic outbreak forms, mainly in developing nations (2), while occurrence is rare in developed nations (14). In China, a large epidemic occurred between 1986 and 1988 in Xinjiang (7), and sporadic cases were found in other regions. HEV is a nonenveloped virus, is similar to ours were reported in other studies (5, 17, 18). HEV in China, a large epidemic occurred 1986 and 1988 (14). In China, a large epidemic occurred between 1986 and 1988 in Xinjiang (7), and sporadic cases were found in other regions. HEV is a nonenveloped virus, is mainly in developing nations (2), while occurrence is rare in developed nations (14). Since the molecular cloning and sequencing of HEV were reported previously (19). Electron microscopy. Passage 4 of HEV strain G93-2 was inoculated into A549 cells. Transmission electron microscopy showed that the virions were rod-shaped, 20-40 nm in length and 10-20 nm in width. The viral envelope was characterized by a double-layered structure, with an inner envelope and an outer membrane. The viral core was electron-dense and contained the viral genome. The viral nucleocapsid was surrounded by a lipid envelope, which was characteristic of enveloped viruses. The viral genome was a single-stranded positive-sense RNA molecule that was approximately 7.2 kb in length. The genome was capped at the 5' end and polyadenylated at the 3' end. The genome contained three open reading frames (ORFs): ORF1, ORF2, and ORF3. ORF1 encoded the viral polymerase, ORF2 encoded the viral capsid protein, and ORF3 encoded a small protein of unknown function. The polymerase gene was located in the 5' end of the genome and was responsible for the synthesis of RNA. The capsid gene was located in the 3' end of the genome and was responsible for the assembly of the viral capsid. The small protein gene was located in the middle of the genome and was responsible for the regulation of viral replication. The genome was flanked by a short 5' leader sequence and a long 3' trailer sequence. The leader sequence was responsible for the initiation of viral RNA synthesis, while the trailer sequence was responsible for the termination of viral RNA synthesis. The genome was arranged in a sense orientation, with the 5' end of the genome encoding the viral polymerase and the 3' end encoding the viral capsid protein. The genome was flanked by a short 5' leader sequence and a long 3' trailer sequence. The leader sequence was responsible for the initiation of viral RNA synthesis, while the trailer sequence was responsible for the termination of viral RNA synthesis. The genome was arranged in a sense orientation, with the 5' end of the genome encoding the viral polymerase and the 3' end encoding the viral capsid protein.
RESULTS

Virus passage. Cell isolation stocks (0.1 ml; passage 1) of strains G93-1, G93-2, G93-3, and G93-4 were inoculated into A549 cells and incubated at 37°C. CPE was visible at day 2 postinoculation for all four strains. The cell rounding and monolayer destruction were typical characteristics of the CPE produced by the viruses (Fig. 1).

Conditions for virus culturing. Analysis with SAS software of virus propagation in A549 cells revealed that the best propagation conditions were 30 mM Mg\(^{2+}\), pH 7.2, and related to the virus inoculation dose (Table 1). Mg\(^{2+}\) at 30 mM was necessary and very important.

Cell sensitivity. Results of cell sensitivity testing revealed that 2BS and A549 cells were sensitive to strains G93-1, G93-2, G93-3, and G93-4 but that LLC-MK\(_2\) cells were not (Table 2).

Physicochemical properties. Nucleic acid type, ether sensitivity, acidity (pH 3.0) resistance, and heat (56°C, 30 min) stability for passage 8 strains G93-1, G93-2, G93-3, and G93-4 were determined by a microculture titration method with A549 cells (Table 3). The results for strains G93-1, G93-2, G93-3, and G93-4 were similar; the viruses are unenveloped RNA virus particles not resistant to acid (pH 3.0) or heat (56°C, 30 min).

Electron microscopy observations. Ultrastructural changes were mainly found in the cytoplasm of an infected cell. Virus particles of the four strains examined were arranged in the form of a crystal. Clusters contained several to hundreds of particles. The virion was round, approximately 25 to 36 nm in diameter. The surface of the virus was irregular. Empty particles were embedded in the crystal structure of completely mature viruses. Viral inclusion bodies and vacuoles were observed near the crystal (Fig. 2).

Immuonelectron microscopy. The virus particles of strain G93-2 could be identified by use of serum from a rabbit immunized with strain G93-2 or 87A and mouse hybridoma ascitic fluid derived from strain 87A (Fig. 3). The virus particles were all aggregated into clusters. Antibody bridge and antibody coat were found occasionally in some particles. Although aggregates of virus particles could occur in both HEV strains with polyclonal and monoclonal antibodies, the numbers in clusters of strain G93-2 were smaller than those in clusters of strain 87A. The efficiency of capture of virus particles by monoclonal antibodies was also lower than that by polyclonal antibodies. A 1:40 dilution of serum could be used for the capture of particles, while only a 1:16 dilution of ascitic fluid could be used. These results showed that strain G93-2 in Guangzhou and epidemic strain 87A in Xinjiang are closely related serologically.

Partial genome determination. When RNA from strains G93-1, G93-2, G93-3, and G93-4 as the template was amplified with primers for the HEV ET1.1 region, a band of approximately 239 bp was observed by gel electrophoresis. The PCR results for these four strains were confirmed by Southern blotting with a 239-bp probe from HEV strain 87A. After cloning, nucleotide sequencing analysis of the PCR fragments derived from 75% of the cells show CPE; 25% of the cells show CPE; no cells showing CPE; +, 25% of the cells show CPE; ++, 50% of the cells show CPE; ++++, 75% of the cells show CPE; ++++, 100% of the cells show CPE.

![FIG. 1. (A) CPE produced by HEV strain G93-2 in monolayers of A549 cells at 48 h postinfection. (B) Control A549 cells at 48 h. Magnification, $\times$200.](image-url)

<table>
<thead>
<tr>
<th>Test group</th>
<th>Culture conditions</th>
<th>CPE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt;/0.025 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg(^{2+}) concn (mmol/liter)</td>
<td>pH</td>
<td>Inoculum dose (MOI)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>6.8</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>7.2</td>
<td>0.025</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>7.6</td>
<td>0.0025</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>6.8</td>
<td>0.025</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>7.2</td>
<td>0.0025</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
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<td>7</td>
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<td>0.0025</td>
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<tr>
<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>60</td>
<td>7.6</td>
<td>0.025</td>
</tr>
</tbody>
</table>

<sup>a</sup> – no cells showing CPE; +, 25% of the cells show CPE; ++, 50% of the cells show CPE; ++++, 75% of the cells show CPE; ++++, 100% of the cells show CPE.
from the virus strains was done (Fig. 4). The nucleotide se-
quence homologies in this part of the genome were 79.9% 
between strains G93-2 and 87A and 100% among strains 
G93-1, G93-3, and G93-4 and strain 87A.

**DISCUSSION**

Four HEV strains were isolated from four patients with 
sporadic HE in Guangzhou, China. The results suggested 
that A549 cells could be used to isolate and cultivate HEV. In 
addition, some continuous cell lines, such as 2BS, Hep-G2, and 
PLC-PRF-5, derived from human lung or liver, are susceptible 
to HEV (4, 7, 11, 13). The CPEs in both the 2BS and the A549 
cell lines were certainly produced by the HEV infection. Those 
CPEs could be specifically neutralized by antibodies derived 
from sera of patients with HE, antibody from immunized-
rabbit serum, and mouse ascitic fluid (data not shown). HEV 
also could be cultivated in in vivo-infected primary macaque 
hepatocytes, but no CPE was observed (16). CPE occurrence 
may be mainly related to the in vitro cell culture system used 
for HEV.

There are two reasons for the successful culturing of HEV in 
both 2BS and A549 cells. First, the stool suspension should be 
precipitated with PEG. Second, 30 mM MgCl$_2$ must be added 
to the culture medium in order to increase the titers of virus 
and protect viral infectivity from inactivating factors. This is a 
very important approach to resolving the problem of few HEV 
particles in acute-phase specimens. Therefore, five of eight 
strains isolated were mostly attributed to PEG and Mg$^{2+}$. 
In addition, specimens should be isolated within 6 
months of collection; otherwise, isolation will not succeed 
because of the lengthy preservation time (data not shown).

Four HEV strains from Guangzhou, southern China, were 
similar to strain 87A from Xinjiang, western China, in physical, 
chemical, and biological properties and morphology. Strain 
G93-2, isolated in southern China, could be recognized by both 
polyclonal and monoclonal antibodies against HEV strain 87A,

isolated from an epidemic in western China, by immunoelec-
tron microscopy. Therefore, these virus strains are closely re-
late serologically.

On the basis of full-length or partial nucleotide sequences of 
HEV reported in many developing countries or areas in Asia 
since 1990, many scientists consider that the homology of HEV 
strains in Asia is comparatively high and that there is 75% 

homology between HEV strains in Asia and Mexico. There-
fore, HEV may have two different subtypes or genotypes. In 
this report, a portion of the sequence of HEV strains G93-1, 
G93-3, and G93-4 was similar to those of Xinjiang strain 87A 
and a Burmese strain, but strain G93-2 was different 
from Xinjiang strain 87A, the Burmese strain, and a Mexican 
strain, with homologies of 79.9, 79.9, and 77.4%, respectively.

A recent study suggested that a portion of the sequence of 
strain G93-2 has 99.2% homology with fragments from serum 
from a patient with HE (China X-S1) in Xiamen, which is near

**TABLE 2.** Cell sensitivity testing of the four Guangzhou 
HEV strains

<table>
<thead>
<tr>
<th>Virus strain (passage)</th>
<th>Viral titer (TCID$_{50}$/0.025 ml)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepassage</td>
</tr>
<tr>
<td>A549</td>
<td>5.5</td>
</tr>
<tr>
<td>2BS</td>
<td>5.5</td>
</tr>
<tr>
<td>LLC-MK$_2$</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Postpassage in the following cells:</td>
</tr>
<tr>
<td>A549</td>
<td>5.5</td>
</tr>
<tr>
<td>2BS</td>
<td>5.5</td>
</tr>
<tr>
<td>LLC-MK$_2$</td>
<td>5.5</td>
</tr>
<tr>
<td>G93-1 (4)</td>
<td>5.5</td>
</tr>
<tr>
<td>G93-2 (4)</td>
<td>5.67</td>
</tr>
<tr>
<td>G93-3 (4)</td>
<td>5.5</td>
</tr>
<tr>
<td>G93-4 (4)</td>
<td>5.5</td>
</tr>
</tbody>
</table>

$^a$ The titer was determined before and after three generations in A549 cells.

**TABLE 3.** Results of physicochemical testing of the four 
Guangzhou HEV strains

<table>
<thead>
<tr>
<th>Test group</th>
<th>Titer (TCID$_{50}$/0.025 ml) of the following virus strain*:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G93-1</td>
</tr>
<tr>
<td>Nucleic acid</td>
<td></td>
</tr>
<tr>
<td>Ether</td>
<td>6.3</td>
</tr>
<tr>
<td>Acid (pH 3.0)</td>
<td>1.3</td>
</tr>
<tr>
<td>Heat (56°C, 30 min)</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Virus control</td>
<td>6.3</td>
</tr>
</tbody>
</table>

$^a$ The titer was determined in A549 cells.

**FIG. 2.** Electron micrograph of cells infected by strain G93-2 showing a 
typical crystalline array of virus particles (large arrow) in infected cellular cyto-
plasm, vacuoles, and a viral inclusion body nearby (small arrow). Bar, 100 nm.

**FIG. 3.** Immunoelectron micrograph of HEV strain G93-2 captured by an-
tibody. Virus particles were aggregated into clusters captured by B4C ascitic fluid 
(A) and captured by immunized-rabbit serum (B). Empty particles (open arrow) 
and antibody coat (filled arrow) are also shown. Staining was done with 2% 
phosphotungstate (pH 7.4). Bars, 100 nm.
Guangzhou (8). Thus, there is a new HEV genotype in China besides the Burmese and Mexican genotypes. We expressed this view in 1995 (10). Later, other investigators reported similar results (5, 17, 18). Recently, American scientists reported that strain US-1 was not similar to Asian strains or to the Mexican strain (14) and that Moroccan strains were close to Asian strains (3). Consequently, the sequences of HEV from various areas showed that the differences among HEV strains correlate with geographic location.

HEV has been provisionally described as a Calicivirus-like virus (12, 15). The size, morphology, and physicochemical properties reported in this study strongly support the notion that it is a member of the Caliciviridae.

Our results may provide candidate strains for the study of a Chinese HE vaccine. On the assumption that there might be only one serotype of HEV in Asia, selecting strain 87A or the four Guangzhou strains as candidate strains for a vaccine will be of significance for the prophylaxis and treatment of HE.

ACKNOWLEDGMENTS

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REFERENCES