Leakey Virus: a New Hantavirus Isolated from *Mus musculus* in the United States

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**SUMMARY**

A hantavirus, designated Leakey virus, was isolated from a *Mus musculus* captured in Real County, Texas, U.S.A. in August 1986. Virus-specific fluorescence was first detected 13 days after inoculation of Vero-E6 cells with spleen tissue from the seropositive *M. musculus*. Ultrastructurally, the new isolate resembled other hantaviruses. Leakey virus induced a fatal meningoencephalitis in infant Fischer rats, with viral antigen detectable in brain, lung, liver, kidney and spleen. Serum dilution, plaque reduction neutralization tests indicated that Leakey virus was antigenically distinct from Hantaan, Seoul, Puumala and Prospect Hill viruses, and therefore constitutes a new serotype.

Several arvicolid (microtine) and murid rodent species serve as the natural reservoirs of hantaviruses, the aetiological agents of haemorrhagic fever with renal syndrome (HFRS) (Yanagihara & Gajdusek, 1988). Serological diagnosis of infection, using Hantaan viral antigen (Lee *et al.*, 1978), indicates that hantaviruses are widely distributed in rodent populations, even in geographical areas where HFRS has not been reported. In the United States, hantaviruses have been isolated from meadow voles (*Microtus pennsylvanicus*) (Lee *et al.*, 1985a) and urban rats (*Rattus norvegicus*) (LeDuc *et al.*, 1984; Tsai *et al.*, 1985; Childs *et al.*, 1987a). Furthermore, serological evidence for hantavirus infection has been found in other species of indigenous arvicolid and cricetid rodents captured in the United States, including *Microtus californicus*, *Clethrionomys gapperi*, *Peromyscus maniculatus*, *Peromyscus leucopus*, *Neotoma cinerea* and *Neotoma mexicana* (Tsai *et al.*, 1985; Yanagihara *et al.*, 1987; Childs *et al.*, 1987a), but confirmation by virus isolation has been lacking. We now report the isolation of a hantavirus from *Mus musculus* captured in Leakey, Texas, U.S.A.

Rodents were trapped alive in selected areas near Camp Creek and Athens (Mercer County) and in the Monongahela National Forest (Pocahontas County), West Virginia, and in Leakey (Real County) and near Del Rio (Kinney County), Texas. The trappings in Texas were conducted in the area where a human case of suspected HFRS had occurred (S. H. Norris, C. J. Gibbs, Jr & L. J. Baek, unpublished observations). None of the animals were trapped in dwellings. Captured animals were speciated and killed by cardiac puncture after chloroform anaesthesia. Sera and lung and spleen tissues were collected aseptically and stored at −70 °C until testing.

Sera from wild rodents, diluted 1:16 in 0.01 M-phosphate-buffered saline (pH 7.4), were tested for antibodies against Hantaan and Prospect Hill viruses by the indirect immunofluorescent antibody (IFA) technique (Lee *et al.*, 1985a), using virus-infected Vero-E6 cells (CRL 1586;
Short communication

ATCC) and eight antiglobulin units of fluorescein isothiocyanate-conjugated goat antibodies to rat or mouse IgG (Cappel Laboratories). Positive sera were then titrated in twofold increments and IFA titres were expressed as the reciprocal of the highest dilution of serum giving specific fluorescence.

A total of 403 arvicolid and cricetid rodents (174 *C. gapperi*, three *Microtus chrotorrhinus*, four *M. pensylvanicus*, 219 *P. leucopus*, three *P. maniculatus*) and 30 cricetid and murid rodents (five *Perognathus flavus*, eight *Perognathus hispidus*, 11 *Sigmodon hispidus*, six *M. musculus*) were captured in West Virginia and Texas, respectively, between July 1985 and October 1986. Antibodies against Prospect Hill and Hantaan viruses, ranging in titre from 32 to 4096, were found in sera from *C. gapperi* (11/174), *P. leucopus* (26/219), *P. maniculatus* (1/3) and *M. musculus* (1/6). The *M. musculus*, trapped along Leakey Creek, had an IFA titre to Hantaan virus of 64.

Virus-specific fluorescence was not detected in cryostat-cut lung sections from the seropositive rodents, but subconfluent monolayers of Vero-E6 cells were inoculated with 0.1 ml of 10% suspensions of lung and spleen tissues (Yanagihara *et al.*, 1984a). A hantavirus, designated Leakey virus, was isolated from spleen tissue of the seropositive *M. musculus*. Intracytoplasmic, virus-specific fluorescence, indistinguishable from that observed in Vero-E6 cells infected with other hantaviruses, was initially detected 13 days after inoculation. Neither c.p.e. nor viral inclusions were evident in infected cultures, and staining for reovirus antigen was negative. As evidenced by immunoperoxidase staining, Leakey virus produced plaques on Vero-E6 cell monolayers, which were somewhat smaller than those of Prospect Hill and Puumala viruses, measuring approximately 1.0 mm in diameter.

An attempt to reisolate Leakey virus from the remaining minute volume of spleen homogenate was unsuccessful, but the wild *M. musculus* had demonstrable antibodies against Leakey virus (IFA titre, 256). Unfortunately, serum from this animal was insufficient for testing for neutralizing antibodies. No virus was isolated from tissues of the seropositive *C. gapperi*, *P. leucopus* and *P. maniculatus*.

Leakey virus was inactivated by ether (Mallinckrodt, St Louis, Mo., U.S.A.) and was unaffected by 40 µg/ml of 5-bromo-2'-deoxyuridine (Sigma). By negative-stain electron microscopy, Leakey virus appeared pleomorphic like other hantaviruses (Martin *et al.*, 1985; Tsai *et al.*, 1985), with spherical and elongated forms, the former measuring 60 to 150 nm in diameter and the latter 175 nm or longer in length, and exhibited prominent surface projections, arranged in distinctly demarcated rows. In addition, tubular structures, measuring up to 1 µm, were occasionally observed.

Infant (1- to 2-day-old) Fischer rats, inoculated intracerebrally with 10^2 p.f.u. of Leakey virus, developed a fatal meningoencephalitis, resembling that seen in infant rats experimentally infected with Hantaan and Seoul viruses, with viral antigen detectable in brain, lung, liver, kidney and spleen. Suckling (5-day-old) bank voles also developed an encephalitic illness following intracerebral inoculation, and viral antigen was similarly widespread in tissues. By contrast, infant mice developed an asymptomatic, persistent infection, reminiscent of Prospect Hill virus infection in meadow voles.

Extensive cross-reactivity was found by simultaneous cross-IFA tests using virus-specific rat antiserum prepared against Leakey virus and Hantaan, Seoul, Puumala and Prospect Hill viruses (Table 1), with antiserum to Leakey virus reacting to equally high titre to Puumala virus. By cross-plaque reduction neutralization (PRN) tests (Lee *et al.*, 1985b), however, Leakey virus was easily differentiated from the other hantavirus serotypes (Table 1). Antiserum to Leakey virus failed to neutralize Hantaan, Seoul, Puumala and Prospect Hill viruses, and conversely antisera against these viruses exhibited low PRN titres to Leakey virus. Furthermore, Leakey virus was not neutralized by convalescent-phase sera from patients with Korean haemorrhagic fever or nephropathia epidemica (data not shown), suggesting that Leakey virus represents a new hantavirus serotype.

Because of a severe, fatal haemorrhagic illness with renal insufficiency, of suspected hantavirus aetiology, in an illegal Mexican immigrant to Texas, wild rodents were trapped in the general vicinity and along the route travelled by this index case and their sera were tested for evidence of hantavirus infection. A virus, serologically related to, but antigenically distinct
Table 1. Antigenic relationship between Leakey virus and Hantaan virus (strain 76-118), Seoul virus (strain 80-39), Puumala virus (strain Hallinäs) and Prospect Hill virus (strain Prospect Hill-I)*

<table>
<thead>
<tr>
<th>Rat antiserum</th>
<th>Fluorescent antibody titre</th>
<th>Neutralizing antibody titre</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HTN</td>
<td>SEO</td>
</tr>
<tr>
<td>Hantaan (HTN) virus</td>
<td>1024</td>
<td>4096†</td>
</tr>
<tr>
<td>Seoul (SEO) virus</td>
<td>1024</td>
<td>1024</td>
</tr>
<tr>
<td>Puumala (PUU) virus</td>
<td>1024</td>
<td>256</td>
</tr>
<tr>
<td>Prospect Hill (PH) virus</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Leakey (LEA) virus</td>
<td>256</td>
<td>64</td>
</tr>
</tbody>
</table>

* Virus-specific rat antisera were tested for antibodies using the indirect IFA and PRN techniques. IFA titres are expressed as the reciprocal of the highest dilution of serum giving specific fluorescence, and neutralizing antibody titres are expressed as the reciprocal of the highest dilution of serum capable of reducing the number of virus plaques by 80% or more.
† Antibody titre to the homologous virus.

from, prototype Hantaan virus and other hantavirus serotypes (namely, Seoul, Puumala and Prospect Hill viruses), was isolated from the spleen of a seropositive M. musculus captured in Leakey, Texas. Although antibodies against hantaviruses have been reported previously in M. musculus captured in the United States (Baltimore) (Childs et al., 1987a) and in European U.S.S.R. (Tkachenko et al., 1983), hantaviruses have not been recovered from such rodents. The isolation of Leakey virus from the common house mouse augments our concepts of the epizootiology of hantavirus infection in commensal rodents and raises important questions about the prevalence of Leakey virus infection in feral and laboratory mice. Specifically, whether the world-wide distribution of Leakey virus in Mus populations resembles that of Seoul virus in Rattus populations (Lee et al., 1982) needs clarification.

Several antigenically distinct hantaviruses are known to circulate among rodent species sharing the same ecological habitat in HFRS endemic regions. This accounts partially for the differences in clinical severity of HFRS within a given area. In non-endemic regions, such as the United States, hantaviruses have been isolated from M. pennsylvanicus, R. norvegicus, and now M. musculus, all species which are occasionally sympatric and synchronistic (Lee et al., 1985a; Childs et al., 1987a). This raises the possibility of genetic reassortment among the hantaviruses, which may result in differences in pathogenicity.

Serological evidence of infection with Hantaan and related viruses has been found in American mammalogists (Yanagihara et al., 1984b) and in individuals exposed to laboratory or wild rodents in the United States (Tsai et al., 1985; Yanagihara et al., 1985; Childs et al., 1988). However, clinically apparent hantavirus infection, resembling the acute nephropathy found in Far East Asia, Scandinavia and Europe, has not yet been unequivocally demonstrated in permanent residents of the United States. To what extent differences in virulence of the American hantavirus strains is responsible is unknown. Studies are needed to determine whether Leakey virus causes human infection and disease.

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REFERENCES


Short communication


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