ISOLATION OF TWO STRAINS OF TICK-BORNE ENCEPHALITIS (TBE) VIRUS FROM IXODES RICINUS COLLECTED IN A MARSHY FOREST IN SWITZERLAND

H. MATILE(1), R. WYLER(1), A. AESCHLIMANN(2), W. BURGDORFER(3) and O. PETER(2)

(1) Institute of Virology, University of Zurich, Switzerland, (2) Zoology Institute of the University Neuchâtel, Switzerland, (3) Rocky Mountain Laboratory, Hamilton, Montana, USA.

Clinical and serological evidence of tick-borne encephalitis (TBE) in humans in Switzerland was first obtained in 1969 (1-2). In 1972, the virus was successfully isolated from the brain of a sick dog near Hallau in the canton of Schaffhausen (3), and the following year it was identified in ticks from the same region (4-5) indicating the existence of natural foci of infection in that area. Later, the virus was isolated from various tissues of Apodemus flavicollis (6) captured near Thun. These regions are shown in Fig. 1.

Clinical cases of TBE in humans have occurred most frequently in the regions of Thun and Schaffhausen but sero-epidemiological investigations have shown that foci of infection also exist in other parts of Switzerland (7). This report deals with the isolation of two strains of TBE virus from Ixodes ricinus collected in a marshy

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forest (Staatswald) on the Swiss plateau near Neuchâtel (Fig. 1).

METHODS

The ticks were obtained by flagging and were frozen at -20°C in Hanks albumin (HAzb) (Hanks BSS + 0.1% bovine serum albumin) containing 100 μg/ml Kanamycin and 125 μg/ml Ampicillin. For virus isolation, the ticks were separated according to stage and sex and divided into pools of 10 specimens, trituated in a cooled mortar and emulsified in 1 ml HAzb. Aliquots of 0.02 ml of this suspension were then inoculated intracerebrally (i.c.) into 1-2 day-old suckling mice (Zur-JCRZ-strain).

When clinical symptoms appeared, the mice were killed, their brains removed, pooled and homogenized in HAzb. This material was then passaged in a fresh litter by i. c. injection of 0.02 ml per mouse of a 10% brain suspension.

Hemagglutination tests were prepared following the method of Frisch-Niggemeyer (8) and were titrated according to Clarke and Casals (9). Neutralization tests were performed by incubating 10 TCID₅₀ (50% tissue culture infectious dose) of the virus at 37°C for 1.5 h with several TBE reference sera. After incubation, the preparations were added to Minipigkidney (MPK) cells in microtiter plates (Nunclon Delta Nr. 1480, Nunc Products, Roskilde, Denmark), 4 wells/serum dilution, 20'000 cells/well, 0.2 ml serum-virus suspension/well. The plates were examined for CPE 7 days after infection.

RESULTS

A total of 632 adult I. ricinus was examined. Virus was isolated
from two pools of 10 female ticks. During the first passage, the infection showed a latency period of 6-7 days. In the second passage, this period was reduced to 3-4 days. Mortality rate was 100%.

The virus titers in MPK-cell cultures reached up to $10^9$ TCID$_{50}$. Both isolates produced hemagglutination of goose erythrocytes at dilutions of 1:128 to 1:256.

The results of cross-neutralization tests (see Table 1) indicate that the two newly isolated strains were indistinguishable from TBE strain 140 which was used as TBE reference virus (5).

DISCUSSION

The Staatswald forest is located in the region of the three lakes Neuchâtel, Biel, and Murten, and represents the most western region in Switzerland where foci of TBE virus have been detected so far. Previous serologic investigation for this virus in that area was negative, and to the best of our knowledge there are no reports of confirmed human TBE cases from that area.

The Staatswald, like many other forests of the Swiss plateau, represents a relict or remnant of a former natural forest. Oaks (at the edge of the forest), ash-trees, birches and many black alders are the most common trees present, although conifers (Epinus) have been planted in many sectors. Underbrush and low vegetation is dense especially in clearings, ravines, and hollows where irrigation provides the rich and humid soil for many grasses and bushes (reeds, Carex sp., yellow irises, stinging nettles, etc.). There is an abundance of wild life including deer, wild boars, foxes, hares, rodents (Clethrionomys glareolus, Apodemus flavicolis, A. sylvaticus, and many others), insectivora, and many species of birds. The Staatswald provides the biologic and climatic conditions necessary for
massive populations of *I. ricinus*, the most common ixodid tick in Switzerland.

Distribution and infestations of this tick are not homogenous in the Staatswald but vary from heavy concentrations in small clearings to few specimens in areas covered by conifers. Ticks are especially abundant on low vegetation along the many roads and trails that penetrate the forest and provide with picnic grounds ample opportunities for recreational activities for the public from surrounding urban regions. There are very few cattle in the immediate vicinity, and the only domestic animal roaming through the woods are dogs.

*Ixodes ricinus* in the Staatswald forest has been and still is the subject of many ecological studies (10-13). Also, in 1978, a survey was initiated to determine the potential role this tick may play as a vector of viral and microbial agents. In addition to the two isolates of TBE virus reported here, a hitherto undescribed rickettsia of the spotted fever group, protozoa of the genus *Babesia* and *Trypanosoma*, as well as a microfilaria of the genus *Dipetalonema* were found associated with this tick (14-15). The significance of these pathogens and parasites as a cause of disease in man and/or animals is currently under investigation.

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<table>
<thead>
<tr>
<th>Virus Strain</th>
<th>Serum of a dog with TBE</th>
<th>Rabbit Anti-TBE Serum (courtesy of Prof. Kunz, Wien)</th>
<th>Human Serum, Laboratory Infection (Mat)</th>
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<tbody>
<tr>
<td>10 TCID 50</td>
<td>1:1015 *</td>
<td>1:1280</td>
<td>1:1280</td>
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<tr>
<td>Isolate 140</td>
<td>1:216</td>
<td>1:280</td>
<td>1:905</td>
</tr>
<tr>
<td>Isolate 1W3</td>
<td>1:450</td>
<td>1:1280</td>
<td>1:905</td>
</tr>
</tbody>
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* Endpoint titers calculated by the method of Reed and Muench.
Fig. 1. Map of Switzerland

Areas in Switzerland, where TBE-virus has been isolated
(SH: region Schaffhausen, TH: region Thun, SW: "Staatswald").