

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

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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 Neuchatel (Fig. 1).

METHODS

The ticks were obtained by flagging and were frozen at -20°C in Hanks albumin (HA2b) (Hanks BSS +0.1 % bovine serum albumin) containing 100 $\mu\text{g/ml}$ Kanamycin and 125 $\mu\text{g/ml}$ Ampicillin. For virus isolation, the ticks were separated according to stage and sex and divided into pools of 10 specimens, triturated in a cooled mortar and emulsified in 1 ml HA2b. 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 HA2b. This material was then passaged in a fresh litter by i. c. injection of 0.02 ml per mouse of a 10 % brain suspension.

Hemagglutininins 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 (Nuncion 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 TCID₅₀. 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 Neuchatel, 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 (*Epicea*) 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 flavicollis*, *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.

REFERENCES

- (1) KRECH, U., JUNG, F. and JUNG, M. 1969: Zentraleuropäische Zeckenzephalitis in der Schweiz. *Schweiz. med. Wschr.* 99: 282-285.
- (2) SPIESS, H., MUMENTHALER, M., BURKHARDT, S. and KELLER, J. 1969: Zentraleuropäische Enzephalitis (Zeckenzephalitis) in der Schweiz. *Schweiz. med. Wschr.* 99: 277-282.

- (3) WANDELER, A., STECK, F., FANKHAUSER, R., KAMMERMANN, B., GREŠTKOVÁ, M. and BLAŠKOVIČ, D. 1972: Isolierung des Virus der zentral-europäischen Zeckenzephalitis in der Schweiz. *Pathol. Microbiol.* 38: 258-270.
- (4) RADDI, A., SCHMIDTKE, W. and WANDELER, A. 1974: Virus der Frühsommer-Meningoenzephalitis. Nachweis des Virus der FSME in Ixodes ricinus aus dem Kanton Zürich. *Zbl. Bakt. I. Abt. Orig. A* 299: 268-272.
- (5) WYLER, R., SCHMIDTKE, W., KUNZ, CH., RADDI, A., HENN, V. and MAYER, R. 1973: Zeckenzephalitis in der Region Schaffhausen: Isolierung des Virus aus Zecken und serologische Untersuchungen. *Schweiz. med. Wschr.* 103: 1487-1492.
- (6) MATILE, J. et al., in preparation.
- (7) MUNZINGER, J., MAERKI, H., MODDE, H., HUBOLD, P., SCHNEIDER, M.V. and WYLER, R. 1977: Seroepidemiologische Untersuchungen zum Vorkommen der Zeckenzephalitis-virus-Infektion in der Schweiz. *Schweiz. Rundschau Med. PRAXIS* 66: 968-972.
- (8) FRISCH-NIGGENMEYER, W. 1966: Inaktivierung der Hämagglutinationsfähigkeit des FSME Tick-borne Encephalitis -Virus. *Arch. Ges. Virusforschung*, 18: 163-171.
- (9) CLARKE, D.H. and CASALS, J. 1958: Techniques for hemagglutination and hemagglutination-inhibition with Arboviruses. *Amer. J. trop. med. Hyg.* 7: 561-573.
- (10) AESCHLIMANN, A. 1972: Ixodes ricinus, Linné, 1758 (Ixodidae; Ixodidae). Essai préliminaire de synthèse sur la biologie de cette espèce en Suisse. *Acta tropica* 29: 321-340.
- (11) HERMOD, C., AESCHLIMANN, A. and GRAF, J.F. 1973: Ecologie et éthologie d'Ixodes ricinus L. en Suisse. Première note: Fluctuations numériques. *Acarologia* 15: 197-205.

- (12) MERMOD, C., AESCHLIMANN, A. and GRAF, J.F. 1974: Ecologie et éthologie d'Ixodes ricinus L. en Suisse. Deuxième note: Comparaison de la population 1972 et 1973. *Acarologia* 16: 612-620.
- (13) MERMOD, C., AESCHLIMANN, A. and GRAF, J.F. 1975: Ecologie et éthologie d'Ixodes ricinus L. en Suisse. Quatrième note: Comparaison de deux populations d'altitude différente. *Acarologia* 17: 442-451.
- (14) BURGDORFER, W., AESCHLIMANN, A., PETER, O., HAYES, S.F. and PHILIP, R.N. 1979: Ixodes ricinus: vector of a hitherto undescribed spotted fever group agent in Switzerland. *Acta tropica* (in press).
- (15) AESCHLIMANN, A., BURGDORFER, W., MATILE, H., PETER, O. and WYLER, R. 1979: Aspects nouveaux du rôle de vecteur joué par Ixodes ricinus L. en Suisse. Note préliminaire. *Acta tropica* 36 (in press).

TABLE 1. CROSS NEUTRALIZATION IN MINIPIG CELL CULTURES

virus strain 10 TCID 50	Serum	Serum of a dog with TBE	Rabbit Anti- TBE Serum (courtesy of Prof. Kunz, Wien)	Human Serum, Laboratory infection (Mat)
Isolate 140		1:1015 *	1:1280	1:1280
Isolate 1W3		1:216	1:280	1:905
Isolate 1C7		1:450	1:1280	1:905

* 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").