J. Hyg., Camb. (1984), **93**, 133–138
Printed in Great Britain

Evidence for flavivirus(es) outside of the distribution area for *Ixodes ricinus* in Norway

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(*Received* 5 *August* 1983; *accepted* 21 *February* 1984)

SUMMARY

Small rodent (vole) sera were collected from three different locations in Norway. One of these was within the distribution area for *Ixodes ricinus*, and a tick-borne encephalitis (TBE) virus strain had been isolated from ticks collected there (Traavik & Mehl, 1977). The two other locations were outside the *I. ricinus* area, one in southern Norway, the other at nearly 70° N. The sera were tested for TBE antibodies by hemagglutination inhibition (HI), hemolysis-in-gel (HIG) and complement fixation (CFT). All sera were also tested for HI antibodies to Uukuniemi (UUK) virus, and some positive TBE HI reactions were verified by separation of immunoglobulins and serum lipoproteins in NaBr gradients.

Animals containing TBE virus antibodies reacting in all three serological tests and animals with UUK HI antibodies were detected only from the location within the *I. ricinus* area. From the two locations outside the *I. ricinus* area we found animals which had antibodies reacting with TBE virus in HI and HIG, but not in CFT. Antibodies to UUK were not detected. The results indicate that flavivirus(es) related to, but not identical with TBE viruses are transmitted by other vectors than *I. ricinus* in parts of Norway.

INTRODUCTION

Tick-borne encephalitis (TBE) virus is an arbovirus classified within the genus Flavivirus of the family *Togaviridae* (Porterfield, 1975). The genus contains several tick-, but mainly mosquito-borne arboviruses.

The main vector for TBE virus is the tick *Ixodes ricinus*, which is distributed in the coastal areas of Norway, from the extreme south and nearly up to the Arctic circle. The most important hosts for this tick are small rodents, passerine birds and other wildlife, but it also feeds on domestic animals and humans if given a chance. In Norway these ecological circumstances are reflected by TBE virus isolations from *I. ricinus*, and the demonstrations of specific antibodies in wildlife,

cattle and human sera taken within the distribution area of the tick (Traavik, Mehl & Wiger, 1978; Traavik, 1979*a*).

Some early studies (Traavik, 1973) were justly criticized because control sera taken outside of the distribution area for *I. ricinus* were not included. Upon the examination of such sera, we found antibodies to TBE virus in wildlife caught in areas where *I. ricinus* does not exist (Traavik, 1979*b*). However, antibodies were detected by the hemagglutination inhibition (HI) test, a method with inherent specificity problems when applied to screening for TBE virus antibodies. First, antibodies to flavivirus hemagglutinins are widely crossreactive, and may not discriminate between different viruses. Secondly, improper removal of non-specific serum inhibitors (NSIs) may result in false positive results.

In this paper we present serological evidence for flavivirus activity outside the distribution area for *I. ricinus*. The design and results of the studies permit us to conclude that the activity in some areas of the country must be due to virus(es) antigenically different from TBE virus which is to date, the only flavivirus detected in Fennoscandia.

MATERIALS AND METHODS

Test sera and reference sera

Small rodents were trapped alive in the three locations indicated in Fig. 1. Locality 1 (Gaular) is within the *I. ricinus* area, and a TBE virus strain had earlier been isolated from ticks collected there (Traavik *et al.* 1978). Locality 2 (Kviteseid) is outside of the *I. ricinus* area in southern Norway, and locality 3 (Masi) is far north of the *I. ricinus* area. In Kviteseid and Gaular one other tick species on small rodents is present, *I. trianguliceps*. It is not found in Masi.

Small rodents were lightly anaesthetized with ether and bled by cardiac puncture. The sera were transported to the laboratory on dry ice and kept in a −20 °C freezer until tested.

Control hyperimmune sera and ascitic fluids against the TBE virus prototype strain Hypr, The Norwegian TBE isolate E672 (Traavik *et al.* 1978) and the Norwegian Uukuniemi (UUK) virus strain E50 (Traavik & Mehl, 1977) were produced in rabbits and laboratory mice as earlier described (Traavik, 1977*b*).

Viral antigens

TBE and E672 antigens were produced by PEG 6000/NaCl precipitation of cell culture media from infected KB cells, and UUK E50 antigens likewise from infected BHK 21/c13 media as earlier described (Traavik, 1977*a*).

Serological methods

HI tests were performed by a conventional microtitration method, but otherwise essentially as described by Clarke & Casals (1958). We used chicken erythrocytes and 4 HA units of antigen in all experiments. For TBE viruses, the final pH was 6·2 and incubation took place at 4 °C, for UUK E50 we used pH 5·8 at 37 °C (Traavik, 1977*a*).

Kaolin absorption of NSIs was carried out according to Clarke & Casals (1958) and acetone extraction as described by Hammon & Sather (1969).

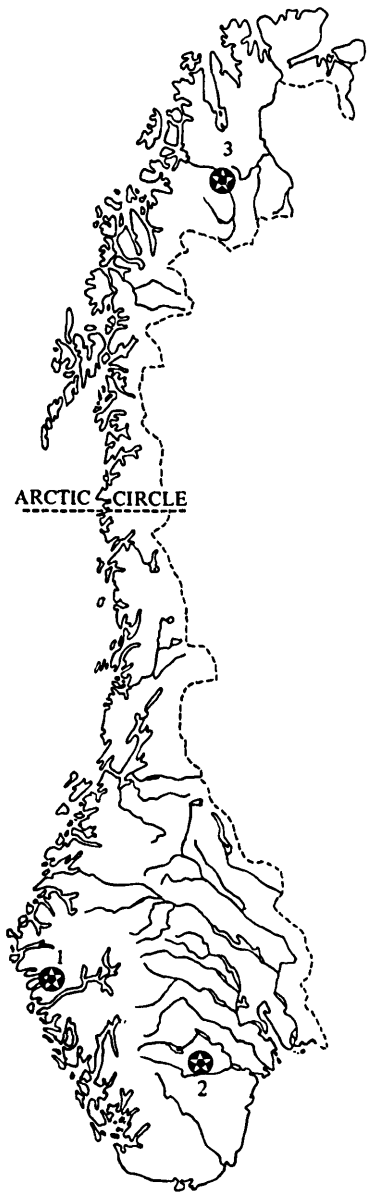

Fig. 1. Map of Norway showing the Collection sites for small rodents. 1 denotes Gaular, 2, Kviteseid and 3, Masi. Location no. 1 is within, the two others outside the distribution area for *Ixodes ricinus*.

Positive HI reactions were further controlled by separation of immunoglobulins from NSIs (lipoproteins) in NaBr gradients (Blom & Haukenes, 1974), when the limited serum volumes permitted this. Since the NaBr interfered with the settling pattern for chick erythrocytes, the bottom fractions containing the immunoglobulins were dialysed against two changes of borate-saline buffer before they were

tested by HI. The complement fixation test (CFT) was performed by a standard procedure (Hammon & Sather, 1969).

Hemolysis-in-gel (HIG) for TBE and E672 viruses was executed exactly as described by Duca *et al.* (1979). Preliminary experiments with the positive reference sera demonstrated good correlation between HI titers and HIG diameters. The small serum volumes which are obtainable from voles prohibited employment of serum neutralization tests.

RESULTS

The main results are compiled in Table 1. Only the data for TBE virus strain Hypr are shown, but identical figures were obtained with the Norwegian TBE strain E672.

Antibodies to TBE virus were detected by HI and HIG in four of 44 gray redbacked voles (*Clethrionomys rufocanus*) from Masi and for three of these animals it was shown by NaBr separation that the HI reactions were due to antibodies. No antibodies were detected in six northern red-backed voles (*C. rutilus*) tested. Positive CFT reactions against TBE virus were not seen for any of these 50 animals, nor did any have HI antibodies against UUK virus.

Among bank voles (*C. glareolus*) collected in Kviteseid, 21 of 200 had antibodies to TBE by HI and HIG tests. Serum volumes which were large enough to permit NaBr centrifugation were available from only six animals having antibodies. HI activity was detected in the immunoglobulin fractions of all of these sera. However, all 200 voles were negative in CFT and HI with the UUK virus. Fifty bank voles from Gaular were tested and nine of these had TBE antibodies by HI and HIG. In seven of these sera antibodies were also detected using CFT. NaBr separation was performed on the seven positive sera, and HI activity was found in the immunoglobulin fraction of all of these. In six of 50 bank voles HI activity to UUK virus was detected.

Table 1. *Antibodies to TBE and Uukuniemi viruses in sera from small rodents*

Antibody test...	Kaolin HI		Acetone HI		HIG	NaBr/HI	CFT
Virus...	TBE	UUK	TBE	UUK	TBE	TBE	TBE
Place of collection							
Masi	8/50*	2/50	4/50	0/50	4/50	3/3	0/50
Kviteseid	39/200	4/200	21/200	0/200	21/200	6/6	0/200
Gaular	14/50	7/50	9/50	6/50	9/50	7/7	7/50

* Number antibody positive/number tested.

DISCUSSION

These investigations have shown for the first time that flavivirus(es) related to, but not identical with TBE virus are circulating in parts of Fennoscandia. This conclusion is based upon the following findings. First in one locality (Gaular) within

a known *I. ricinus* and TBE virus endemic area, the small rodents had HI, HIG and CFT antibodies to TBE virus. The prevalence was as expected (Traavik, 1979*a*). Rodents captured outside the *I. ricinus* area (Kviteseid and Masi), had antibodies to TBE detectable by HI and HIG but not by CFT. The latter method is known to be more virus type specific than HI (Hammon & Sather, 1969). Second for some of the animals collected both within and outside of the *I. ricinus* area it was shown by NaBr centrifugation that the positive HI reactions were due to immunoglobulins. Third UUK virus seems to be very strongly adapted to *I. ricinus* (Saikku, 1974). In the present investigations no animals with UUK antibodies were detected outside of the *I. ricinus* area. This contradicts non-specific serum factors as a source for false positive flavivirus reactions.

Flaviviruses may be transmitted by ticks or mosquitoes. In addition to *I. ricinus*, the tick *I. trianguliceps* is a potential vector of flaviviruses to small rodents (Nosek & Grulich, 1967). This tick is present in Kviteseid and theoretically might be involved in flavivirus transmission in that locality. None of these two tick species is present in Masi. Thus, a hypothetical explanation for the flavivirus activity observed there is transmission by mosquitoes. Gamasid mite species, many of which are found on small rodents all over the country (Edler & Mehl, 1972), may also be arbovirus vectors. And, finally, the recent demonstration (Varelas-Wesley & Calisher, 1982) of flaviviruses without any known arthropod-vector relationships is of obvious relevance.

Our serological results demonstrate that kaolin treatment is unreliable for removal of NSIs from small rodent sera. The same conclusion was made for pretreatment of human sera (Traavik, 1979*b*). HIG may be a method of choice for seroecolological arbovirus studies. This method is simple, economical, requires small serum volumes and is not influenced by NSIs. A plaque neutralization test for TBE virus, for instance the sensitive and specific version described by de Madrid & Porterfield (1969), might have given indications about the degree of relatedness to TBE virus for the flaviviruses circulating outside the *I. ricinus* area. Unfortunately, the small serum volumes available did not admit this to be carried out in addition to the tests employed.

These studies were supported by grants from the Norwegian Council for Science and the Humanities, and Fridjof Nansen's and affiliated funds for the advancement of Science and the Humanities. The expert technical assistance of Sissel Andreassen and Inger Furre is gratefully recognized.

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