Reservoir animals for nephropathia epidemica in Norway: indications of a major role for the bank vole (*C. glareolus*) in comparison with the woodmouse (*A. sylvaticus*)

By ANN-INGER SOMMER, T. TRAAVIK

Institute of Medical Biology, University of Tromsø, and the Virological Laboratory, Regional Hospital of Tromsø, Norway

R. MEHL

Laboratory for Medical Entomology, National Institute of Public Health, Oslo, Norway

B. P. BERDAL

Norwegian Defence Microbiological Laboratory, Oslo, Norway

AND J. M. DALRYMPLE

U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Fredrick, Maryland, U.S.A.

SUMMARY

Small rodents were collected live in two different locations within a nephropathia epidemica (NE) endemic area, and tested for both antiviral serum antibodies and viral antigens in lung sections. In one location, only *Apodemus sylvaticus* (woodmice) were found in the traps, in the other, both *A. sylvaticus* and *Clethrionomys glareolus* (bank voles) were collected.

Among the woodmice from the former location the prevalence of NE virus markers was significantly lower than for either woodmice or bank voles from the other location, and no NE antigen-positive animals was found. The woodmice co-existing with bank voles had a lower prevalence of NE antigen and antibodies than the bank voles, and fewer woodmice had both antibodies and antigen. The results emphasize the important role of bank voles as a major NE virus reservoir and probable source of human infections.

INTRODUCTION

Close aetiologic relations have been proved, in several different investigations, between agents of the haemorrhagic fever with renal syndrome (HFRS) disease complex in Scandinavia, European U.S.S.R. and Asia (Friman *et al.* 1980; Lee *et al.* 1979; Svedmyr *et al.* 1979). As a result, antigen from Korean haemorrhagic fever (KHF) virus can be used for detection of serum antibodies against the scandinavian nephropathia epidemica (NE) virus.

Epidemiological and ecological studies indicate that these diseases are zoonoses, with small rodents acting as reservoirs (Läthevirta, 1971; Lee *et al.* 1981). The bank vole (*Clethrionomys glareolus*) has been reported as probably the main source of NE virus, in Finland by Brummer-Korvenkontio *et al.* (1980) and in Norway by Traavik *et al.* (1983). No other virus reservoir animal has been reported from Finland and Sweden, while in Norway antibodies to KHF virus have been demonstrated in sera from *C. rutilus* and *C. rufocanus* (Traavik *et al.* 1984). In addition to these rodent species, we have now detected NE antigen and antibodies in the woodmouse (*Apodemus sylvaticus*). In the U.S.S.R., Gavrilovskaya *et al.* (1983) have found HFRS antigen in small mammals of eight species, including the same species found as host animals for NE virus in Norway. Recent evidence suggests that domestic rodents like *Rattus norvegicus* and *Rattus rattus* are also reservoirs for HFRS-related virus (LeDuc *et al.* 1982; Tsai *et al.* 1982). The virus carrier state among the different species may vary, as well as their importance as reservoir animals for human infections.

In this article we report on the occurrence of NE virus markers among *A. sylvaticus* caught within a NE endemic area, under circumstances indicating either a close or a more distant or sporadic contact with NE carrying *C. glareolus*.

MATERIALS AND METHODS

Animals and collection sites

Small rodents of the species *C. glareolus* (bank vole) and *A. sylvaticus* (woodmouse) were captured using live-traps (Ugglan special) in NE foci at Søgne in southern Norway. The two stations selected were situated approximately 5 km apart. The trapping was done under similar conditions and at the same time at both stations. At station A, a total of 49 woodmice and no bank vole were captured, while 66 woodmice and 51 bank voles were caught at station B.

Markers of NE infection

Animals were considered to have been infected with the virus of NE if either NE antigen, or KHF antibodies, or both, were present.

Samples of blood were obtained by cardiac puncture of animals anaesthetized with ether. The sera were tested for KHF antibodies by indirect fluorescent antibody technique (IFAT) using KHF spot slides (Friman *et al.* 1981) by methods that we have earlier described in detail (Traavik *et al.* 1983).

Lungs were removed aseptically, and a piece of each lung was cut in 4 μm thick sections on a cryostat (five sections per slide). The lung sections were fixed for 15 min in cold acetone and tested for NE antigen by IFAT (Traavik *et al.* 1983).

NE antigen and KHF antibodies are referred to as 'NE markers' in the following text.

Statistics

The results were evaluated by the Chi-square test.

RESULTS

There were fewer woodmice with NE markers collected at station A than at station B (Table 1), the difference being statistically significant ($P < 0.025$). At station B, bank voles were positive about twice as often as woodmice.

Table 1. *Total numbers of animals with NE markers (either antigen, antibody or both) in the two collection sites*

	Station A		Station B	
Species	Number tested	Number (%) positive	Number tested	Number (%) positive
A. sylvaticus	49	5 (10)	66	18 (27)
C. glareolus	—	—	51	29 (57)

The pattern of the NE infection markers differed for the two stations. Antigen was never detected in woodmice caught at station A, whereas about one-third of the woodmice caught at station B were antigen-positive (Table 2). Only one bank vole carried antigen without also having antibody, and about one-third of the bank voles had both antigen and antibody.

Table 2. *The distribution of the different NE markers among the two rodent species at each location*

	Station A	Station B	
NE marker	A. sylvaticus	A. sylvaticus	C. glareolus
Antibody	5 (100%)	11 (61%)	19 (66%)
Antigen	—	5 (28%)	1 (3%)
Both antibody and antigen	—	2 (11%)	9 (31%)
Total number positive	5	18	29

Titration of 12 NE-positive sera from woodmice and 11 NE-positive sera from bank voles showed a marked difference in titre range. The bank voles demonstrated much higher values. Most of the bank voles with titres > 80 were also antigen-positive. This correlation did not apply to the woodmice (Table 3).

Table 3. *NE antibody titres in sera from* 12 A. sylvaticus *and* 11 C. glareolus

	NE antibody titre range				
	5–20	20–40	40–80	80–60	≥ 640
Number of A. sylvaticus	5 (1)*	5	1	1	
Number of C. glareolus	1	2		3 (2)	5 (5)

* Numbers of NE antigen-positive animals are in parentheses, the rest are antigen-negative.

Lung sections from antigen-positive woodmice contained fewer antigen-positive cells, and the intensity of fluorescence per cell was less than in positive bank voles.

DISCUSSION

The bank vole (*C. glareolus*) is obviously not the only host animal for NE virus in Norway, as NE-positive patients are found outside bank vole distribution areas. Other host animals outside these areas may include *C. rutilus* and *C. rufocanus*, species closely related to *C. glareolus* (Traavik *et al.* 1983, 1984). One might nevertheless suspect bank voles to be the major source of NE infection in Norway, as the main endemic foci in this country tend to be within bank vole distribution areas (Traavik *et al.* 1983). The results from station B show that within the same location bank voles are more frequently infected than woodmice.

Gavrilovskaya *et al.* (1983) reported that infection with HFRS virus in wild rodents produces no symptom of overt animal disease. They demonstrated the presence of virus in many visceral organs of *C. glareolus*, which may facilitate excretion into the environment. Lee *et al.* (1981) found that small rodents may become persistently infected with KHF virus. Animals carrying both NE antigen and antibodies may represent a persistent infection or reinfection. If we consider animals carrying antigen without detectable antibodies as recently infected, then those possessing antibodies and no antigen may have been infected earlier but are virus-free animals. We have, however, not tested for circulating immune complexes either in antigen- or in antibody-positive animals.

At station A there were no recently infected woodmice, using the criteria described above. All NE-positive individuals possessed only antibodies. Likewise most of the NE-positive woodmice at station B only possessed antibodies, but about a third of the woodmice at this station were recently infected. There were also a few woodmice that were probably persistently infected or reinfected individuals with both antibodies and antigen. A higher proportion of the bank voles with NE markers were persistently infected or reinfected. These marked differences indicate that bank voles may be important as a virus reservoir and virus transmitters to humans.

The results also suggest that the woodmice may be able, to a greater extent than the bank voles, to limit the infection. The much higher antibody titres found in bank vole sera, especially from those carrying antigen, support the theory of a persistent infection or frequent reinfections in these animals. LeDuc *et al.* (1982) have earlier reported that rats which were KHF antigen-positive also showed the highest antibody titres. This did not apply to the woodmice, where the titres were much lower, and those with the highest antibody titres were not carrying antigen.

We have considered the possibility that the antibodies produced in the two species may react with different affinity to the KHF antigen on the spot slides used for IFAT. We presume that this cannot be the explanation of the lower antibody titres found in woodmice, as NE antigen investigations on lung sections showed the same pattern. There were fewer infected cells, and these had a lower antigen content in the woodmouse than in the bank vole lung sections. Gavrilovskaya *et al.* (1983) have also reported similar differences in antigen content in the two species. The use of anti-mouse FITC-conjugated IgG does not favour the detection

of bank vole antibodies. Gel precipitation tests showed that woodmouse sera reacted slightly better than bank vole sera with anti-mouse IgG (unpublished results).

A persistently infected animal may excrete intact as well as defective virus particles (Traavik, 1979). Nevertheless, the importance of such animals in viral transmission is evident, because of the continuing viral excretion. The importance of the woodmouse as a virus host seems to depend on the presence of infected bank voles in the same biotopes.

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