J. Hyg., Camb. (1984), **93**, 139–146
Printed in Great Britain

Nephropathia epidemica in Norway: antigen and antibodies in rodent reservoirs and antibodies in selected human populations

By T. TRAAVIK*, ANN-INGER SOMMER*, R. MEHL†, B. P. BERDAL‡, K. STAVEM§, O. H. HUNDERI‖ and J. M. DALRYMPLE¶

* *Institute of Medical Biology, University of Tromsø and the Virological Laboratory, Regional Hospital of Tromsø, Norway,* † *Laboratory for Medical Entomology, National Institute of Public Health, Oslo, Norway,* ‡ *Norwegian Defence Microbiological Laboratory, Oslo, Norway,* § *Vefsn Hospital, Mosjøen, Norway,* ‖ *Ulleval Hospital, Oslo, Norway and* ¶ *U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, U.S.A.*

(*Received* 19 *September* 1983; *accepted* 21 *February* 1984)

SUMMARY

Nephropathia epidemica (NE) antigen was detected by IFAT (indirect fluorescent antibody technique) in the lungs of 14 of 97 bank voles (*Clethrionomys glareolus*) collected in three endemic areas. The distribution of antigen positive voles within an endemic location was scattered. Antibodies to Korean hemorrhagic fever (KHF) virus antigens were detected by IFAT in 12 of 14 NE antigen positive bank voles and in 15 of 83 that were antigen negative. NE antigen positive voles exhibited higher antibody titres. Antibodies to KHF were demonstrated in sera from *C. rutilus* and *C. rufocanus* collected more than 200 km north of the distribution area for *C. glareolus*. It appears likely that these vole species can serve as virus vectors for NE cases occurring north of the bank vole area. NE antibodies cross-reacting with KHF virus seem to diminish with time after infection in some NE patients, while for others such cross-reacting antibodies were detected up to 12 years after the disease. Antibodies to KHF were detected in eight of 106 healthy forestry workers with no clinical history of NE. No serological cross-reactions were detected between NE/KHF antigens and representative *Bunyaviridae* present in Norway. NE/KHF-like viruses appear widespread in Norway, both within and outside of the distribution area of the bank vole.

INTRODUCTION

The presence of an infectious disease which is clinically related to the hemorrhagic fever with renal syndrome (HFRS) complex has been known in Norway for more than 30 years (Knutrud, 1949). The term Nephropathia epidemica (NE) has been applied to this disease. Clinically similar conditions had been described earlier from Sweden and Finland (Myhrman, 1934; Zetterholm, 1934; Lahdevirta, 1971). HFRS was considered as a zoonotic disease with rodents as a reservoir. More recently, Korean hemorrhagic fever (KHF) and NE viruses have been detected in tissues from small rodents (Brummer-Korvenkontio *et al.* 1980; Lee, Lee &

Johnson, 1978) and it has become clear that the HRFS diseases in Asia, USSR and Scandinavia are caused by antigenically related viruses (Brummer-Korvenkontio *et al.* 1980; Lee *et al.* 1979; Svedmyr *et al.* 1979).

We recently reported the detection of antigens reacting with antibodies to KHF virus in the lungs of Norwegian bank voles (*Clethrionomys glareolus*). We detected antibodies in the sera from NE patients which reacted with both KHF virus in cell cultures and bank vole lung antigens. We also described epidemiological and ecological evidence that implicated reservoir animals other than the bank vole (Traavik *et al.* 1983).

The present investigations were undertaken in order to study ecological and epidemiological problems related to NE.

MATERIALS AND METHODS

Collection of small rodents

Two areas with a high number of NE-seropositive human cases were chosen for further rodent studies: Søgne in southernmost Norway and Hattfjelldal in northern Norway (Fig. 1). These locations are more than 800 km apart. Kirkenes, situated a further 800 km to the north east and far north of the distribution area for *C. glareolus* was included because a laboratory-confirmed case of NE was diagnosed in February 1982. Small rodents were captured alive in special traps after which they were transported alive to the laboratory or prepared in the field. They were lightly anesthetized with ether and blood samples taken by cardiac puncture. Lungs were removed aseptically. One lung was immediately frozen on dry ice in sealed tubes or in a −80 °C freezer for later isolation attempts. The other lung was cut in approximately 4 μm thick sections on a cryostat, acetone-fixed on slides and frozen in the −80 °C freezer until tested by the indirect fluorescent antibody technique (IFAT) (Traavik *et al.* 1983).

Small rodent sera

Sera from *C. glareolus* collected in Søgne and Hattfjelldal and *C. rutilus* collected in Kirkenes, were tested for antibodies to KHF virus by IFAT. In addition, we had sera from collections in Rana (Fig. 1) in 1980. Two *C. glareolus* from these collections had NE antigen in their lungs (Traavik *et al.* 1983). During arbovirus investigations in 1975, sera were obtained from small rodents collected in Masi (69° 26′ N, 23° 39′ E. Fig. 1) (Traavik, Mehl & Wiger, 1984). This is far north of the distribution area for *C. glareolus* in Norway, and the samples consisted mainly of two other vole species, *C. rutilus* (northern red-backed vole) and *C. rufocanus* (gray, red-backed vole).

Human sera

Sera from patients with suspected NE were sent to our laboratory from physicians and hospitals all over the country. Sera from forestry workers living within an earlier established endemic NE area in Kongsvinger (Traavik *et al.* 1983) were collected in 1981. None of these forestry personnel had a history of or remembered having had symptoms consistent with NE.

A panel of sera from patients with recent NE, a history of clinical NE 1–12 years

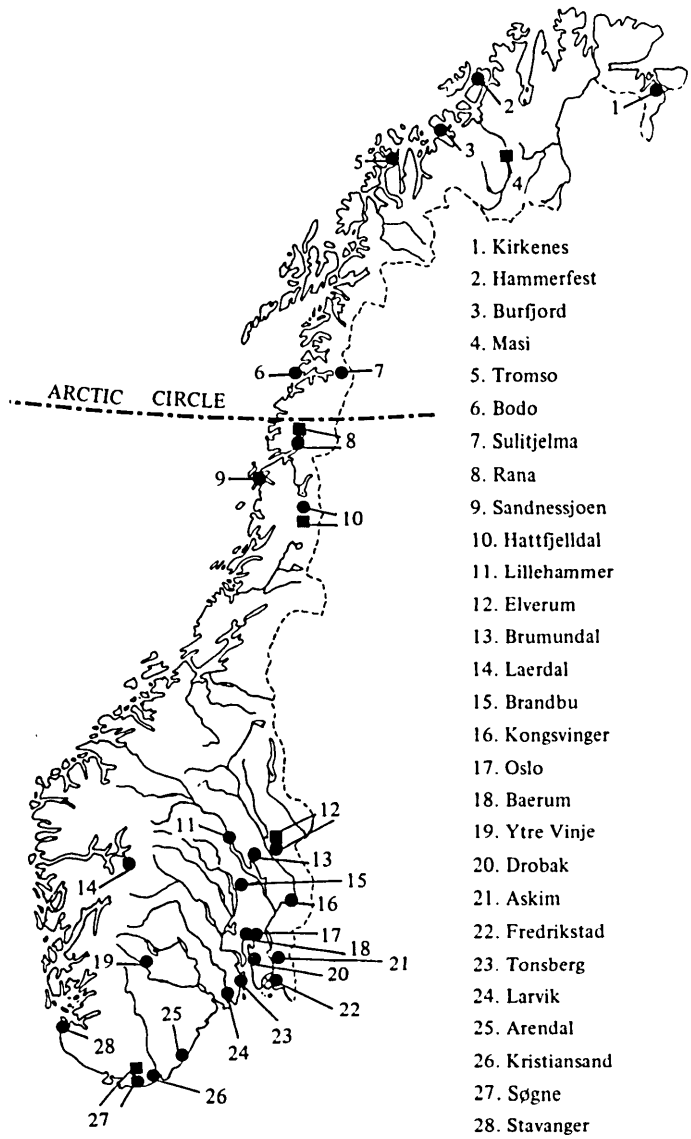

Fig. 1. Map of Norway showing locations where evidence for the presence of NE virus has been demonstrated. The dots indicate detection of antibodies in patients, the squares antigen and/or antibodies in small rodents.

earlier, other kidney diseases (not living within endemic areas) and healthy persons living within an endemic area were collected in 1982. These sera were tested under code. Human control sera used were (*a*) a convalescent human serum to KHF (titre 1280) received from U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland (*b*) a pool composed of sera from 11 NE patients. The anti-KHF titre was 320 and (*c*) a serum from a person with no history of NE in which no antibodies to KHF were detectable by IFAT.

https://doi.org/10.1017/S0022172400061027 Published online by Cambridge University Press

Reference antiviral sera and FITC-conjugated antisera

Hyperimmune mouse ascitic fluids to various viruses within *Bunyaviridae* were received from NIAID, Bethesda, Maryland. Hyperimmune ascitic fluids to the Norwegian Uukuniemi (UUK) virus strains E 50 and E 82, the Norwegian California encephalitis virus strain S 586 and the European CE prototype Tahyna were produced as described elsewhere (Traavik, Mehl & Kjeldsberg, 1977).

Goat anti-human IgG and IgM and anti-mouse IgG antisera conjugated with fluorescein isothiocyanate (FITC) were purchased from Bionetics, Kensington, Maryland, Earlier experiments had shown that these anti-mouse preparation reacted with IgG from the three vole species tested (Traavik, Mehl, & Wiger, 1984).

KHF spot slides. Spot slides of the human cell line A549 and Vero E6 cells infected with the KHF virus strain 76–118 (Hantaan virus) were produced as earlier described (Traavik *et al.* 1983).

Indirect fluorescent antibody techniques (IFAT)

The IFAT methods used for detection of vole lung NE antigen and human antibodies to KHF and NE virus have been described in detail (Traavik *et al.* 1983). Tests for specific IgM antibodies were performed after immunoabsorption of IgG from the sera. This was done in order to eliminate false positive results due to rheumatoid factors and false negatives due to competitive inhibition of IgM by specific IgG antibodies. Vole antibodies to KHF virus were detected on spot slides incubated with sera diluted 1 in 5, sandwiched with FITC-conjugated anti-mouse IgG diluted 1 in 20. The specificity of positive vole reactions was examined by blocking tests, the anti-mouse FITC-conjugate being first passed through a column with human IgG in order to eliminate any cross-reactions. KHF slide spots were then incubated with undiluted pooled human NE positive sera, NE negative serum or PBS. The vole sera were titrated on the pre-incubated spots. Inhibition of the fluorescence by positive human serum, but not by negative serum or PBS was taken as an indication of specificity, i.e. that the fluorescence with vole sera were due to antibodies reacting with KHF antigen. Small serum volumes prohibited fractionation of the vole sera and further tests for specificity.

RESULTS

NE antigen in bank voles

Among 69 bank voles collected in Søgne in October, 1981, nine (13%) had NE antigens in their lungs. Small rodents were collected in three different stations, some 5 km apart (A, B, C; Table 1). Eight NE antigen-positive voles were captured at station A, situated around the house of a family in which both wife and husband had a history of clinical NE some months earlier. IFAT with convalescent sera from these people obtained during our vole-collecting expedition confirmed the diagnosis. Stations B and C were chosen at random, B gave one positive vole.

No antigen-positive voles were found among 15 animals collected in Hattfjelldal in June, 1981, whereas all three bank voles caught at this location in May 1982 had NE antigen in their lungs (Table 1).

Table 1. *NE virus antigens in lung and serum antibodies to KHF virus in bank voles* (C. glareolus)

Place of collection	Date of collection	Number tested	Number with		
			Antigen	Antibodies	Both
Søgne (27)*	Oct. 1981	69	9	16	8
Station A		12	8	10	7
Station B		50	1	6	1
Station C		7	0	0	0
Hattfjelldal (10)	June 1981	15	0	7	0
	May 1982	3	3	3	3
Rana (8)	Nov. 1980	10	2	1	1

* Numbers refer to the markings in Fig. 1.

Antibodies to KHF virus in bank voles (C. glareolus)

Antibodies were detected in the sera of eight of nine antigen-positive voles collected in Søgne in October 1981. Antibodies were demonstrated in sera from eight of 60 antigen-negative voles from the same Søgne collection. Of the 10 bank voles collected in Mo i Rana in November 1980, two had NE antigens in their lungs (Traavik *et al.* 1983). One of these voles had serum antibodies reacting with KHF virus. None of the eight antigen-negative voles were antibody positive.

Seven out of 15 antigen-negative voles from Hattfjelldal in June 1981 had serum antibodies reacting with KHF, and all three antigen-positive voles caught in May 1982 had antibodies. These results are summarized in Table 1. Titration of antibody-positive vole sera indicated that the antigen-positive animals had considerably higher anti-KHF titres than the negative ones. One of five antigen-positive voles had a titre of 640, two had titres of 320 and one each had a titre of 160 and 80. One antigen-negative vole had a titre of 40, two voles had a titre of 20 and two had a titre of 10.

Antibodies to KHF virus in northern red-backed and gray red-backed voles

Antibodies reacting with KHF virus were detected in six of 10 gray red-backed voles (*C. rufocanus*) and in one of three northern red-backed voles (*C. rutilus*) collected in Masi in 1975. Antibodies were also detected in four out of 16 *C. rutilus* collected in Kirkenes in June 1982, none of which had NE antigens in their lungs. The antibody titres were between 10 and 40 for all these animals.

Antibodies to KHF virus in patients with recent disease

From October 1, 1981 until September 15, 1982 we tested 136 sera from 112 patients suspected of NE. Scarcity of antigens prohibited titration of positive sera. IgG antibodies to KHF were detected in one or more sera from 46 of these patients. Seroconversions were demonstrated for 14 patients. For all these the first (negative) serum was taken during the first 8 days of illness. For 11 of the seroconverting patients specific IgM antibodies were detected in the first (IgG negative) serum (data not shown). Specific IgM antibodies were detected in one or more sera from 38 of the 46 positive patients.

Table 2. *Antibodies to NE and KHF viruses in sequential sera from patients with prior disease*

Patient	Months after acute disease	IgG antibodies to NE	IgG antibodies to KHF
O.H.J.	4	+	+
	13	+	−
S.O.L.	1	+	+
	11	+	−
G.S.	2	+	+
	8	+	+

Antibodies to NE/KHF viruses in patients with prior disease

For three patients who had serologically confirmed NE in 1981, we received serum samples taken 8, 11 and 13 months respectively after the symptoms were recognized. As shown in Table 2, antibodies to NE virus were still detectable in all samples, while antibodies to KHF virus could no longer be detected in the sera taken 11 and 13 months after the acute stage of disease. Among individuals with a history of clinical NE 1–12 years earlier, and tested under code together with controls, IgG antibodies to KHF were detected in the sera from 25 persons, while three were negative. The positive samples were taken between 2 weeks and 12 years after the disease. The negative samples were taken 5 months, 5 years and nearly 8 years after the disease. Among eight patients with kidney disease not diagnosed as NE, one patient had KHF IgG antibodies, and among ten healthy control subjects from an endemic area, three had antibodies.

Antibodies to KHF in healthy forestry workers.

Sera from 106 men working in the forests within an earlier established endemic NE area in Kongsvinger (Fig. 1) were tested for IgG antibodies to KHF virus. Antibodies were detected in the sera from eight (7·5%) of these workers.

Antigenic relationship with Bunyaviridae

Neither KHF virus in cell cultures nor NE virus in vole lung sections reacted in IFAT with antibodies against UUK viruses E 50 or E 82, Bunyamwera group, California encephalitis group or California encephalitis strains Tahyna or S586.

The distribution of NE virus in Norway

The locations where NE virus activity has been demonstrated are marked in Fig. 1. The map is based on the findings reported in our earlier (Traavik *et al.* 1983) and the present study.

DISCUSSION

The efforts to detect NE antigen in bank voles collected in three different, adjacent locations within an endemic area, indicated that NE virus activity may be focal and unevenly distributed in the bank vole populations. Likely explanations for this are not obvious. We caught NE antigen-positive voles in the immediate surroundings of a house where two adult family members had contracted NE some

months earlier. According to the patients' own account they were bothered not only by 'mice' in their house, but also by rats. In accordance with the recent reports of KHF-related antigens in rats (Tsai *et al.* 1982), one may speculate as to whether or not an ecological link exists between rats and free living small rodents in the transmission of NE virus.

Our efforts to isolate NE virus from antigen-positive vole lungs have failed so far (unpublished results). Some antigen-positive animals did not have any detectable antibodies. Whether the difficulty of isolating NE virus from vole lungs is related to the concommitant presence of specific antibodies is not known yet. Antibodies were not recorded during the isolation of KHF (Hantaan) virus in cell culture (French *et al.* 1981), and neither during the unsuccessful efforts to isolate the Finnish NE virus (Oker-Blom *et al.* 1979). Recent publications (Donets *et al.* 1982; McCormick *et al.* 1982; White *et al.* 1982) indicate that Hantaan virus is a member of the *Bunyaviridae*, morphologically similar to Uukuniemi (UUK) virus, and such viruses easily give rise to defective interfering particles (Traavik, 1979). During our experiments we could, however, not detect any antigenic relationship between NE/KHF and members of the *Bunyaviridae*.

The findings of KHF antibodies in two other vole species, show, as earlier indicated (Traavik *et al.* 1983) that the bank vole (*C. glareolus*) is not the only host animal for NE virus in Norway. In European USSR, NE virus related antigens have previously been detected in *C. rutilus* (Gavrilovskaya *et al.* 1980). Our investigations seem to include a new species, *C. rufocanus*, among the host animals for NE virus.

The studies on antibody prevalence for humans with recent, prior and no earlier history of NE revealed some interesting features. New endemic NE areas were detected. Demonstration of NE antibodies in sera negative for KHF indicate that cross-reacting antibodies may disappear with time, and that a true estimate of antibody prevalence can only be made using antigens of the homologous virus. Detection of KHF antibodies in humans without a history of NE in the past, indicate that infections may follow a subclinical or atypical course. One case of atypical infection was reported earlier (Traavik *et al.* 1983).

A distribution map for NE virus in Norway based on antigen detection and serology in small rodents and serology in humans, shows that the virus is widespread throughout the country both within and outside the distribution area for *C. glareolus* which thus far is the only host animal recognized in Sweden and Finland (Brummer-Korvenkontio *et al.* 1980; Lahdevirta, 1971; Oker-Bloom *et al.* 1979).

These investigations were supported by grants from the Norwegian Council for Science and the Humanities, and Fridtjof Nansen's and affiliated funds for the advancement of Science and the Humanities. The authors wish to thank Sissel Andreassen for her expert technical assistance.

REFERENCES

Brummer-Korvenkontio, M., Vaheri, A., von Bonsdorff, C. H., Vuorimies, J., Manni, T., Penttinen, K., Oker-Blom, N. & Lahdevirta, J. (1980). Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. *Journal of Infectious Diseases* **141**, 131–134.

DONETS, M. A., REZAPKIN, G. V., KOROLEV, M. B. & TKACHENKO, E. A. (1982). Taxonomic features of causative agent of hemorrhagic fever with renal syndrome. *Lancet* **i**, 794–795.

FRENCH, G. R., FOULKE, R. S., BRAND, O. A., EDDY, G. A., LEE, H. W. & LEE, P. W. (1981). Propagation of etiologic agent of Korean hemorrhagic fever in a cultured continuous cell line of human origin. *Science* **211**, 1046–1048.

GAVRILOVSKAYA, I. N., APEKINA, N. S., GARBACHKOVA, E. A., MYASNIKOV, YU YU. A., RYLEVA, E. V. & CHUMAKOV, M. P. (1980). Detection by enzyme-linked immunosorbent assay of hemorrhagic fever with renal syndrome virus in lung suspensions of rodents from European USSR. *Lancet* **ii**, 1050.

KNUTRUD, O. (1949). Nehropathia epidemica. *Tidsskrift for den Norske Laegeforening* **69**, 259–261 (in Norwegian).

LAHDEVIRTA, J. (1971). Nephropathia epidemica in Finland. A clinical, histological and epidemiological study. *Annals of Clinical Research* **3**, suppl. 8.

LEE, H. W., LEE, P. W. & JOHNSON, K. M. (1978). Isolation of the etiologic agent of Korean hemorrhagic fever. *Journal of Infectious Diseases* **137**, 298–308.

LEE, H. W., LEE, P. W., LAHDEVIRTA, J. & BRUMMER-KORVENKONTIO, M. (1979). Aetiological relation between Korean hemorrhagic fever and nephropathia epidemica. *Lancet* **i**, 186–187.

MCCORMICK, J. B., SASSO, R., PALMER, E. L. & KILEY, M. P. (1982). Morphological identification of the agent of Korean hemorrhagic fever as a member of the Bunyaviridae. *Lancet* **i**, 765–768.

MYHRMAN, G. (1934). En njursjukdom med egenartad symptombild. *Nordisk Medisinsk Tidsskrift* **7**, 793–794.

OKER-BLOM, N., VON BONSDORFF, C. H., BRUMMER-KORVENKONTIO, M., HOVI, T., PENTTINEN, K., SAIKKU, P., VAHERI, A. & LAHDEVIRTA, J. (1979). Nephropathia epidemica in Finland. In *Arctic and Tropical Arboviruses* (ed. Kurstak), pp. 197–209. New York: Academic Press.

SVEDMYR, A. H., LEE, H. W., BERGLUND, A., HOORN, B., NYSTROM, K. & GAJDUSEK, D. C. (1979). Epidemic nephropathy in Scandinavia is related to Korean hemorrhagic fever. *Lancet* **i**, 100.

TRAAVIK, T. (1979). Tick and mosquito-associated viruses in Norway. Thesis, University of Tromsø.

TRAAVIK, T., MEHL, R., BERDAL, B. P., LUND, S. & DALRYMPLE, J. M. (1983). Nephropathia epidemica in Norway: description of serological response in human disease and implication of rodent reservoirs. *Scandinavian Journal of Infectious Diseases* **15**, 11–16.

TRAAVIK, T., MEHL, R. & KJELDSBERG, E. (1977). 'Runde' virus, a coronavirus-like agent associated with seabirds and ticks. *Archives of Virology* **55**, 25–38.

TRAAVIK, T., MEHL, R. & WIGER, R. (1984). Mosquito-borne arboviruses in Norway: further isolations and detection of antibodies to California encephalitis viruses in human, sheep and wildlife sera. *Journal of Hygiene*, submitted for publication.

TSAI, T. F., DAUER, S. P., SASSO, D. R., MCCORMICK, J. B., BRADFORD, H., CARAWAY, C. T., MCFARLAND, L. M., MEDRANO, O. & SAULIE, G. (1982). Preliminary evidence that Hantaan or a closely related virus is enzootic in domestic rodents. *New England Journal of Medicine* **307**, 623.

WHITE, J. D., SHIREY, F. G., FRENCH, G. P., HUGGINS, J. W., BRAND, O. M. & LEE, H. W. (1982). Hantaan virus, aetiological agent of Korean hemorrhagic fever has Bunyaviridae-like morphology. *Lancet* **i**, 768–771.

ZETTERHOLM, S. (1934). Akuta nefriter simulerande akuta bukfall. *Lakartidningen* **31**, 425–429 (In Swedish).