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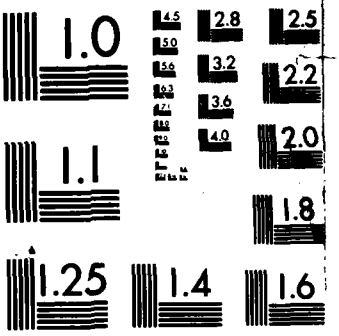

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Study of Nephropathia Epidemica in Sweden

Final Report

Bo Niklasson, M.D., Ph.D.

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An epidemiological study of human Nephropathia Epidemica (NE) in Sweden has shown the endemic area to be north of the 60th parallel. The bank vole, Clethrionomys glareolus, the host of NE virus, is widely distributed in Sweden. NE infected voles could only be found in endemic areas. Antibody prevalence in human populations from different parts of Sweden was evaluated by IFT using Korean hemorrhagic fever (KHF) infected A-549 cells. Antibody prevalence varied between 2.5-25% in endemic area and was 1% in non-endemic area.

The NE agent, a Bunyavirus, has been isolated from infected voles trapped in the endemic area. The virus has been adapted to Vero E6 cells. The earlier described partial one way cross between NE and KHF has been confirmed.

A Clethrionomys glareolus colony orginating from an non-endemic area has been established and proven to be susceptible to NE infection.

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SUMMARY

Nephropathia epidemica (NE) is a significant cause of human morbidity in Norway, Sweden and Finland. The disease is a member of the Hemorrhagic Fever with Renal Syndrome (HFRS) complex. An epidemiological study of human NE cases has shown the endemic area to be north of the 60th parallel. This zone also correspond with the Fennoscandia biotype. The bank vole, Clethrionomys glareolus, the host of NE virus, is widely distributed in Sweden except for the island Gotland where it is absent. NE infected voles could only be found in endemic areas. Antibody prevalence in human populations from different parts of Sweden was evaluated by IFT using Korean hemorrhagic fever (KHF) infected A-549 cells. Antibody prevalence varied between 2.5-25% in endemic area and was 1% in one non-endemic area. Gotland (the vole free island) had no antibody positive humans.

The NE agent, a Bunyavirus, has been isolated from infected voles trapped in the endemic area. The virus has been adapted to Vero E6 cells. The earlier described partial one way cross between NE and KHF has been confirmed.

A Clethrionomys glareolus colony orginating from an non-endemic area has been established and proven to be susceptible to NE infection. In a pilot study, wild trapped Clethrionomys from an endemic area have shown a lower susceptibility to NE infection compared to the colony animals. This experiment is being repeated under controlled conditions.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

For the protection of human subjects the investigator have adhered to policies of applicable Federal Law 45CFR46.

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Nephropathia Epidemica (NE), a syndrome with sudden chills, fever abdominal pain and renal involvment was first described in northern Sweden in 1934 (1). It was later found that NE is related to Hemorrhagic Fever with Renal Syndrome (HFRS) occuring in the USSR, China, Japan and Korea (2). NE is a significant cause of human morbidity in Sweden. It often results in severe illness where most recognized cases require hospitalization, a few, even intensive care with renal dialysis. However, the mortality is low (less than 1%) and no sequele have been reported (3).

The disease is belived to be maintained in nature in infected voles and transmission to man is thought to occur via aerosol of infectious vole excrement. The bank vole, Clethrionomys glareolus, the most prevalent vertebrate in Scandinavia is thought to be the principal host of NE in Scandinavia.

The objectives of the present study were the following:

1. To determine the geographic distribution of NE in Sweden. To conduct a seroepidemiological survey using sera from normal population from different regions of Sweden. To trap rodents from different parts of Sweden and assay them for HFRS infection.

2. To isolate the etiologic agent of NE from rodents or if possible from humans.

3. To develop rapid diagnostic tools, preferably ELISA, for NE antibody (IgG and IgM) as well as for NE antigen detection.

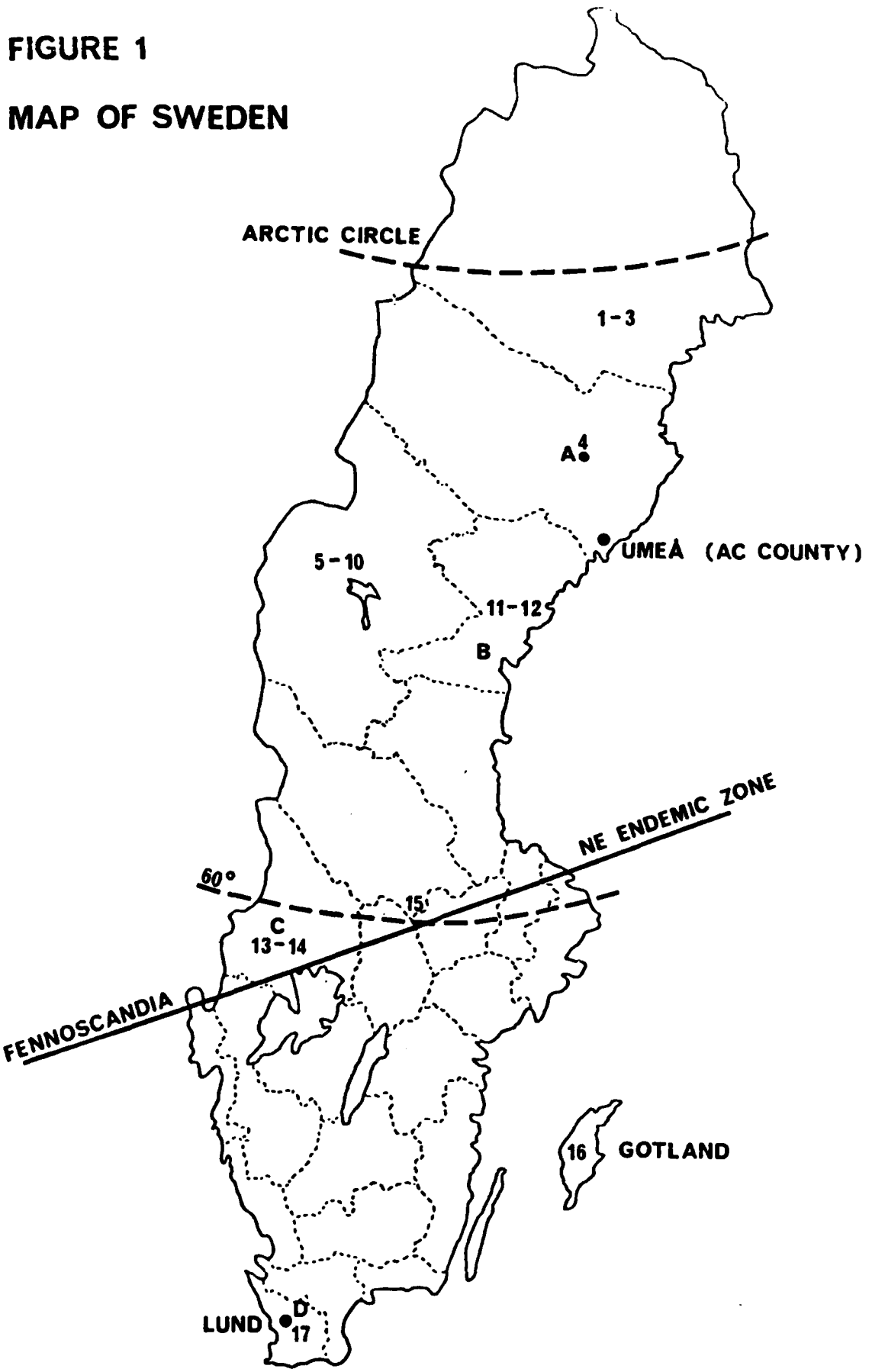
4. To establish a clean bank vole colony for virus isolation attempts and transmission experiments.

To determine the geographic distribution of NE in Sweden we have investigated all NE cases since 1981 that have come to our attention. Travel history has been obtained on all patients not coming from known endemic areas.

All serological confirmed NE patients either lived or had visited the endemic area marked on the map (fig. 1). We have asked the clincians, in several notes in the Swedish medical journal to report suspected NE cases from non-endemic areas, but no such cases have been reported. We have no indication that any human infection occurs south of the endemic zone in fig 1. The total number of NE cases is unknown, but one endemic county (AC county in the Umeå area) has reported as many as 103 clinical cases of NE in a year from a population of 235,000 (4). During 1984 and the first 15 weeks of 1985, 243 serological confirmed cases of NE had been reported to our laboratory from all of Sweden. We believe that the majority of all NE cases that receive medical care are not laboratory confirmed and therefore not reported.

FIGURE 1

MAP OF SWEDEN



To determine the antibody prevalence of NE in different parts of Sweden, sera from normal populations comprised of out-patients regardless of previous history of infectious disease, have been collected and tested for the presence of antibody to Korean hemorrhagic fever (KHF). Serology was performed by the indirect immunofluorescence test (IFT) using Hantaan virus infected A-549 cells. A close antigenic relationship between KHF and NE has been reported using this method (5, 6, 7, 8). The geographic location where sera were collected is seen on the map in figure 1 and serological results are shown in table 1. In addition to data on geographic location, information on age, sex and occupation have also been collected. All these data are presently being computerized together with the serological results. In addition to the results presented in table 1, eight hundred sera with accompanying information on age, sex, geographic location and occupation have been collected but yet not tested. The antibody prevalence in the population tested varied between 2,5% and 28% within the endemic area. The AC-county reporting the most clinical cases also has the highest antibody prevalence. The Lund area which represents a non endemic area, had an antibody prevalence of 1%. No antibody was found in human sera from Gotland, the only vole free area known in Sweden. It is not clear if the 1% antibody prevalence in Lund is from people that have frequently visited endemic areas, or if the antibody reflects infection with an antigenically related virus not causing NE symptoms.

Rodents have been trapped at 4 different locations in Sweden (A, B, C, D map, fig 1). Three of the trapping sites were within endemic area and one site was south of the endemic zone. All animals trapped were identified to species, bled for serology and lung tissue was collected for detection of HFRS antigen. (A portion of the lung from each animal was also used for virus isolation attempts.) The results are summarized in table 2.

Three bank voles, trapped in the NE endemic area 80 km west of Umeå around the farm of a recent serologically confirmed NE patient, were found positive when lung cryostate sections were tested by IFT with human convalescent serum. Lung tissue from these voles was were used for virus isolation attempts. Lung tissue from the three animals was disrupted, suspended in tissue culture medium and seeded onto monolayer cultures of Vero E 6 cells. The cultures were incubated at 37°C for 14 days, trypsinized, one third of the cells were put into a new tissue culture flask. Subsequent to this the cells were trypsinized and one third were passed into a new flask every 7 days for 10 weeks. NE virus was isolated from 2 of the 3 animals. NE antigen was detected by IFT in the Vero cells 6 weeks after inoculation. Supernatants were successfully carried through six passages. No CPE were detected (9).

TABLE 1. Antibody prevalence to KHF in Swedish out-patients tested by IFT.

Geographic localization		No. of sera	Positive (%)
No. on map	Name of village	collected	
1	Niemisel	169	5 (11)
2	Vistträsk	180	17 (9)
3	Svensbyn	221	10 (5)
4	Vindeln	200	50 (25)
5	Vassnäs	108	14 (13)
6	Bratteggen	93	10 (11)
7	Kall	151	16 (11)
8	Järpen	121	8 (7)
9	Högarna	152	10 (7)
10	Kövra	246	6 (2)
11	Överammer	151	13 (9)
12	Mårdsjön	123	20 (16)
13	Arvika	165	7 (4)
14	Torsby	200	5 (3)
15	Fagersta	99	4 (4)
16	Gotland	200	0 (0)
17	Lund	200	2 (1)
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TABLE 2. Rodents trapped and assayed for NE antigen and NE antibodies. Geographic localization A, B, C and D are seen on map figure 1

Area	No. of animals	Antigen and antibody pos.	Only antibody positive
A. Umeå area			
C. glareolus	94	9	1
M. musculus	4	-	-
B. Sundsvall			
C. glareolus	35	7	1
M. musculus	1	-	-
C. Arvika			
C. glareolus	36	-	5
M. musculus	9	-	-
D. Lund			
C. glareolus	74	-	6
Apodemus	51	-	-
Rattus Norvegicus	8	-	2

One of the new NE isolates was used to prepare spot-slides for serology following the same procedures as for KHF but using Vero E 6 cells instead of A 549.

Acute and convalescent sera from 5 patients with typical NE symptoms, from 100 out-patients living in the vole trapping area, and one convalescent serum from a patient with KHF, were tested using both NE and KHF antigen. In the 5 patients with NE symptoms, antibody titers were 2-8 fold higher using NE virus as antigen as compared to results using KHF (table 3). These results support the previously reported one-way cross-reaction between NE and KHF (8). The antibody prevalence among out patients living in the trapping area was 30% using NE as antigen, and 25% with the KHF antigen. All 25 sera positive by KHF were also positive by NE.

Efforts have also been made to get an NE isolate direct from humans. Early sera from 15 NE patients have been inoculated into Vero E-6 and cells have been passed for 3 months using the same technique as described above without success. Vero E-6 cells have also been sent weekly to 3 hospitals regularly seeing NE patients with instructions to inoculate the cell cultures with whole blood from acutely ill NE patients (bedside) and send the flasks to SBL for incubation and passage for 3 months. Ten such specimens have been received but no NE agent has been recovered. We are presently trying to pass all virus isolation material in bank voles in an attempt to get a human NE isolate.

Reagents needed for the development of an ELISA system for antibody (IgG and IgM) and antigen have been partially completed. Rabbits have been immunized against NE using NE-infected Vero E-6 supernatants in Freund's complete adjuvant as well as by affinity bead immunization (10) using the same supernatant as antigen. The affinity bead immunization proved superior. An ELISA for both antigen and antibodies has been developed. Both tests have acceptable background after reagents being absorbed with affinity chromatography. The signal in the antibody ELISA is too weak as a result of low antigen concentration, and we will try to purify and concentrate NE antigen to overcome these difficulties.

During the past year we have established a colony of Clethrionomys glareolus in our laboratory. The colony originates from Lund which is a non-endemic area. Initially we had difficulties getting the colony to breed. After changing to larger cages (40 x 20 x 15 cm) and keeping the animals on a photoperiod of 18 hours of light and 6 hours of dark, reproduction started. The original 19 animals as well as 25 of their offspring have been killed and tested for NE specific serum antibody and NE specific antigen in lung tissue. All were negative.

TABLE 3. Indirect immunofluorescence titers* in human sera

		NE infected cells	KHF infected cells
NE patient 1	A	256	64
	C	1024	256
NE patient 2	A	32	8
	C	128	64
NE patient 3	A	2048	256
	C	1024	256
NE patient 4	A	1024	512
	C	1024	256
NE patient 5	A	8	8
	C	64	32
KHF patient	C	8	256

*Titers are expressed as reciprocal of highest dilution giving a positive immunofluorescence test.

A = Acute serum C = Convalescent serum

We have completed the safety training of personnel working with NE infected animals. The animals are kept in a negative pressured HEPA filtered risk unit in the animal house. All personnel wear a full face respirator and protective clothing while working with infected or potentially infected animals.

In an initial experiment we infected bank voles using both infectious lung and cell culture material. Both virus sources gave almost 100% infection rates, but it took nearly 8 weeks before all animals became antibody and antigen positive.

Through contacts with the Zoology department in Uppsala we have determined that a great deal of genetic variation occurs among bank vole populations in different regions of Sweden. Some of these markers seems to correlate very well with the distribution of NE. The border of NE follows the landmass of Fennoscandia. There is a marked difference in biotype between Fennoscandia and the area to the south. Rodent populations, including bank voles, are cyclical in the Ferroscandia area, but are apparently stable south, of this region in Sweden.

Our initial intent was to use the vole colony from Lund for transmission experiments. Since we now know that there is a marked genetic difference between our colony and the animals from NE endemic areas (naturally infected with NE), we have completed an infectivity study using both voles from the south and north. In this pilot study we infected 40 bank voles from the Lund colony and 39 wild trapped bank voles from Umeå. Both groups were infected with a lung suspension shown earlier to be infectious to bank voles from Lund and Vero E-6 cells. After an incubation period of 2 months all animals were killed and assayed for NE antigen. All Lund voles but one were antigen positive, but only 24 of 39 voles from Umeå were positive. These results indicate there may be a difference in susceptibility for NE infection in the two groups. This experiment needs to be repeated using laboratory confirmed HFRS-negative animals in both groups. For this purpose we have started 2 new bank vole colonies. Animals for one are recently trapped in an endemic area and the other colony is comprised of animals initially trapped in endemic area, but they have been colonized for several years. We are presently working on getting the 2 colonies to breed and confirming that they are NE negative. We plan to do a comparative infectivity study using all 3 bank vole colonies.

Human NE occurs north of the polar circle. An outbreak of at least 10,000 cases among German troops stationed in Salla in the Finnish Lappland was recorded during the second world war (11). Bank voles are distributed throughout Sweden; however, in the most northern regions population levels are low and other rodents, such as Clethrionomys rofucanus, Clethrionomys rutilus and lemmings are more prevalent. A

colony of Clethrionomys rofacanus and of lemmings have recently been established at our laboratory and we intend to study their susceptibility to NE under laboratory conditions. We intend to trap Clethrionomys rutilus during 1985 field trips to include this species in our comparative study.

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