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Prevalence of serum IgG antibodies to Puumala virus (haemorrhagic fever with renal syndrome) in Northern Sweden

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SUMMARY

A stratified and randomly-selected population sample was identified in 1990 in order to study the seroprevalence of nephropathia epidemica (haemorrhagic fever with renal syndrome) in Northern Sweden. Sera from 1538 subjects (750 men, 788 women), 25–64 years of age, were analysed for the presence of Puumala virus (PUV) specific-IgG by the indirect immunofluorescence antibody test. Specific IgG was detected in sera from 83 subjects (5·4%). Men and women had similar seroprevalence rates. The highest seroprevalences were found in subjects 55 years or older (8·0%) and among farmers and forestry workers (15·9%). The geographic distribution of seropositive individuals was uneven and there were significantly more seropositive persons in rural than in urban areas ($P < 0{\cdot}05$).

INTRODUCTION

Nephropathia epidemica (NE) is a viral zoonosis affecting humans in Scandinavia and several other countries in Europe [1–3]. NE and related diseases in Asia are designated as haemorrhagic fevers with renal syndrome (HFRS). Clinically NE is characterized by fever, abdominal- or back pain and renal impairment. The prognosis is generally good, but the long-term effects on renal function are not known [3]. The causative agent of NE is Puumala virus (PUV) [4], which is transmitted to humans from persistently-infected rodents, mainly bank voles (*Clethrionomys glareolus*). The annual incidence of human cases of NE varies in a cyclic fashion with peaks every third to fourth year, coinciding with the density of the vole population. In Sweden NE is present north of the 59th parallel [5, 6]. Although bank voles, the major rodent vector, occur throughout the country, no human cases have been reported from the south of Sweden. The highest incidence of clinical NE cases is found in Västerbotten county in the north of Sweden [6–8].

The aims of the present study were to establish the prevalence of antibodies to PUV in the two northernmost counties in Sweden, to study variations in

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Table 1. *Participation rates for 2000 randomly selected subjects in a Puumala virus seroprevalence study in Northern Sweden, 1990*

	Proportion of participants (%)	
	Men	Women
Age group (years)		
25–34	64·8	67·1
35–44	77·6	80·0
45–54	78·4	83·2
55–64	80·4	85·5
Areas		
Urban areas	74·6	79·4
Rural areas	77·6	77·1
Västerbotten county	75·9	78·5
Norbotten county	74·7	79·4

geographic distribution within the area and to identify risk factors of exposure to the virus. An additional aim was to see whether PUV seropositivity was associated with increased morbidity.

MATERIALS AND METHODS

Study subjects

Subjects were identified within the framework of the Northern Sweden MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Project [9]. This project includes the two northernmost counties of Sweden (Norrbotten and Västerbotten, 63·5°N–69°N) with a total population of 511 000 living in an area of 154 000 km^2, representing almost one third of the total area of the country. Within each age group (25–34, 35–44, 45–54 and 55–64 years), 250 men and 250 women were randomly selected from continuously updated population registers. Subjects were invited by letter to attend a medical examination including laboratory tests.

Of the 2000 subjects invited 1583 (79·2 %) participated in the study which was performed in January to April 1990. Table 1 shows the participation rates in different age groups and regions. The participation rate was lowest in the youngest age group (25–34 years) and for all age groups it was slightly higher among women than among men. The mean age did not differ more than $\pm 2{\cdot}5$ years in the occupational and geographic groups examined and therefore no standardization for age was performed. A questionnaire completed by the participants included questions on health, medication, occupation, and working conditions. In all, sera from 1538 individuals, 750 men and 788 women, were available for analysis. The Northern Sweden MONICA Project has been approved by the Research Ethics Committee of Umeå University, Umeå, and the data handling procedures by the National Computer Data Inspection Board.

Serological test

Blood was collected by venepuncture and serum samples stored frozen at -20 °C until tested. PUV-specific IgG antibodies were demonstrated by an indirect immunofluorescence antibody technique (IFAT) using PUV Sotkamo

strain propagated in Vero E6 cells as antigen. The infected cells were trypsinized, washed, dried onto slides and fixed with acetone. Slides were stored at −70 °C until used. Sera were diluted 1/4 and incubated in a moist chamber for 1 h at room temperature on the antigen-coated slides. The slides were then incubated at 37 °C for 1 h with a fluorescein-labelled goat anti-human IgG ($F(ab)_2$ fragment; Kallestad Diagnostics, South Austin, TX, USA) diluted 1/40 in PBS plus 0·003% Evan's blue. The slides were read in an Olympus ultraviolet microscope at ×400 magnification. Readings were done blind and independently by two of the authors (C.A., M.L.). Samples that differed in the readings were retested and read by the senior virologist (P.J.). Specimens showing characteristic cytoplasmatic fluorescence at the 1/4 dilution were considered positive. Positive samples were titrated to determine the endpoint.

Data processing and statistical analyses

Data were processed and statistical analyses were performed with the software program SYSTAT® (SYSTAT Inc., Evanston, IL, USA). The occupation of all individuals was identified and grouped according to the Nordic standard occupational classification [10]. Categorization of geographical areas into urban and rural was done according to a modified delimitation of localities made by Statistics Sweden [11]. Chi-square test was used to compare proportions. Odds ratios according to serological status were calculated with 95% confidence intervals (CI) and *P*-values for demographic and morbidity variables with the software program GLIM® (General Linear Models 1985, Royal Statistical Society, London, UK).

RESULTS

In total, 83 of 1538 tested individuals (5·4%) had specific serum IgG antibodies to PUV (Fig. 1). Seropositive individuals were identified throughout the regions studied. The median titre of specific IgG was 16 (range 4–256) (Fig. 2). The seropositivity rate for men was 6·0% and for women 4·8%. The sex ratio (M:F) was 1·3:1. The age distribution showed significantly increasing seropositivity in relation to increasing age (Fig. 3). Among the oldest participants (55–64 years), 8% of the subjects were seropositive as compared to 1·8% in the youngest group (25–34 years) (odds ratio 4·66, CI 1·93–11·27, $P = 0·001$). The seroprevalence was higher among farmers and forestry workers; 15·9% (10/63) *v.* other groups 4·9% (73/1475); odds ratio 3·84; CI 1·77–7·41, $P = 0·002$. For medical personnel the seroprevalence was 3·6% (3/83), slightly lower than the mean (5·4%). There was a higher seroprevalence rate for persons living in rural *v.* urban areas (odds ratio 1·66, CI 1·02–2·70, $P = 0·048$). No significant differences of seroprevalence were demonstrated between the different communities and the two counties, neither between the coastal, inland, or mountain regions. The relative numbers of individuals with a history of diabetes mellitus, hypertension, myocardial infarction or cerebrovascular disease did not differ significantly between the seropositive and the seronegative group (data not shown). Odds ratios and confidence intervals for demographic and occupational data are summarized in Table 2.

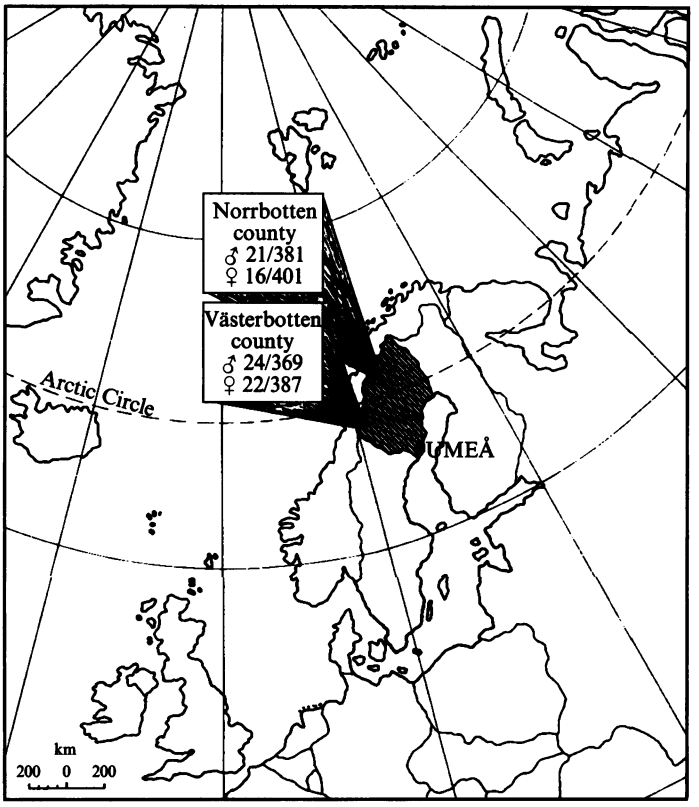

Fig. 1. Number of seropositive/number of examined individuals in a seroprevalence study of Puumala virus in 1538 randomly selected subjects in Norrbotten and Västerbotten counties, Sweden, 1990.

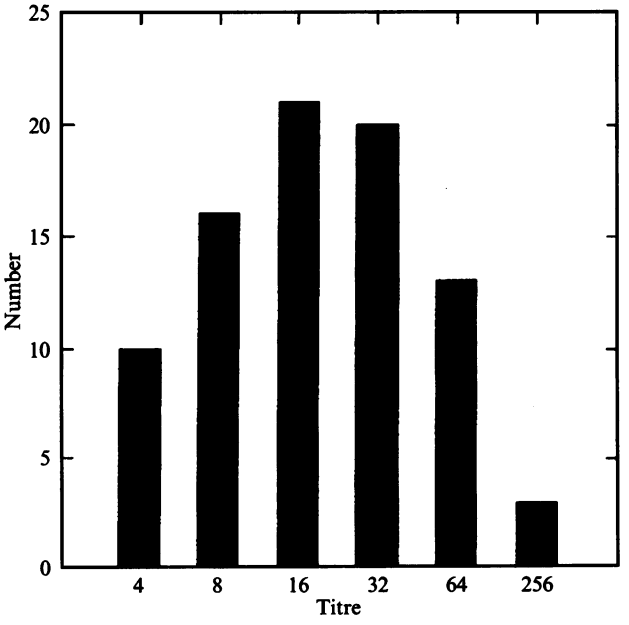

Fig. 2. Distribution of serum IgG titres against Puumala virus in 83 seropositive individuals in Northern Sweden, 1990.

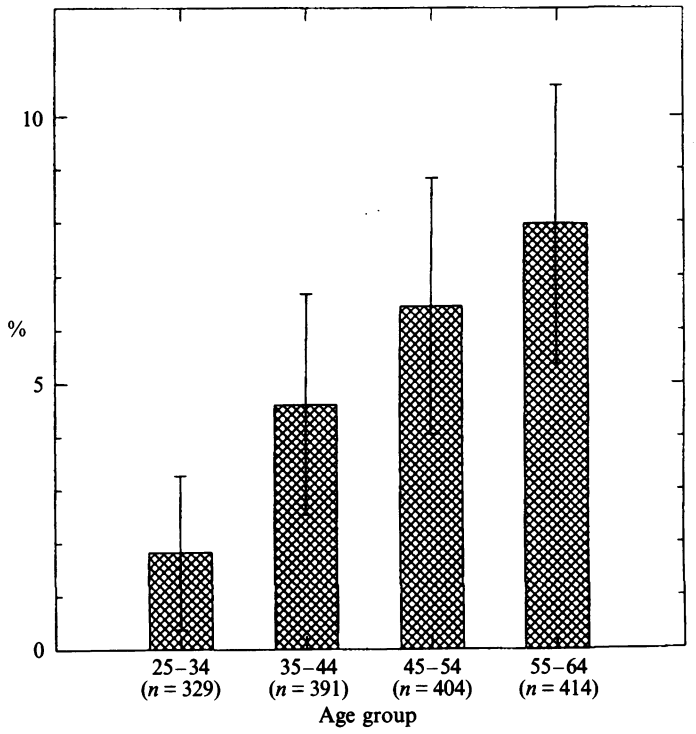

Fig. 3. Seroprevalence rates of IgG antibodies to Puumala virus in different age groups of 1538 randomly selected subjects in Northern Sweden, 1990 (including 95% confidence intervals).

Table 2. *Odds ratios and confidence intervals according to seropositivity to Puumala virus in 1538 randomly selected subjects in Northern Sweden, 1990*

Variable	Odds ratio	CI*	*P*-value
Men (45/750)† *v.* women (38/788)	1·26	0·81–1·96	0·307
Age group 55–64 years (33/414) *v.* 25–34 years (6/329)	4·66	1·93–11·27	0·001
Farmers and forestry workers (10/63) *v.* others (73/1475)	3·84	1·77–7·41	0·002
Health workers (3/83) *v.* others (80/1455)	0·65	0·20–2·09	0·435
Rural (25/325) *v.* urban areas (58/1213)	1·66	1·02–2·70	0·048
Västerbotten (46/756) *v.* Norrbotten county (37/782)	1·30	0·84–2·04	0·240

* CI, Confidence interval of 95%.

† Number of positive subjects/number tested are given in parentheses.

DISCUSSION

Previous studies from Sweden of the seroprevalence rate to PUV reported 4–19% seropositivity [5, 7, 8]. In Norway a seroprevalence rate of 24% (23/97 subjects) was reported from a small but highly endemic region [12]. Finland has reported an average seroprevalence of 5% and in certain areas in Lake and East Finland more than 20% [13, 14]. In the present study the overall prevalence of IgG antibodies to PUV was not higher than 5·4% despite the fact that a population from the most endemic area in Sweden was investigated. This

discrepancy is most probably explained by the mode of selection of the study populations. In previous Swedish studies [5, 7, 8] sera from selected populations in specific endemic areas were analysed. In contrast, our study was performed on a random sample. It may be argued that the method used to detect specific IgG to PUV (IFAT) was less sensitive and that this could account for the relatively low seroprevalence. However, using the same IFAT method an IgG titre of 8 was detected in a patient with a proven clinical history of NE more than 50 years earlier [15]. A serum dilution as low as 1/4 could be used without producing non-specific fluorescence when using a $F(ab)_2$-fragment of the conjugated antibody with an effective counter-stain. Thus, the IFAT was sensitive enough to fulfil the requirements for this study.

In several clinical studies of NE, men predominate with sex ratios (M:F) of 2·0–3·5:1 [5, 6, 16]. In our study there was no statistically significant difference in seroprevalence rate between the sexes, indicating a similar risk of PUV infection for men and women. The finding that more men have clinical NE could be explained by a less severe or subclinical illness in women.

Physicians in Sweden are obliged to report confirmed cases of NE to the National Bacteriological Laboratory, Stockholm. From 1987–91 the annual incidences of NE cases for the whole country were 131, 251, 68, 172 and 289 cases, respectively. From these data the mean incidence rates in Västerbotten and Norrbotten counties were obtained (21 and 31 cases/100000 inhabitants, respectively). These incidence rates were compared with the calculated seroprevalence figures to estimate the ratio of clinical versus inapparent PUV infections (data not shown). The results showed that approximately one in eight PUV infected individuals had symptoms severe enough to seek medical attention. It is not known if the inapparent PUV infections represent mild, atypical and/or subclinical cases. If one extrapolates the seroprevalence rate to the corresponding population in this region, it would implicate about 14000 seropositive individuals. Such a high number of seropositive individuals would indicate a large proportion of subclinical and/or atypical NE cases. However, the rate of clinical to subclinical PUV infection can only be revealed in a prospective population study. A cohort prospective study in China demonstrated seroconversion to HFRS virus in 2·3% (30/1325 subjects) during the one year observation period. The ratio of apparent to inapparent infection was 1:14 and the infection rate was only slightly higher among men than women [16].

The results of the present study show that farmers and forestry workers have an increased risk of NE (odds ratio 3·8, $P = 0{\cdot}002$). They are more frequently exposed to rodents and their excretions, and farmers commonly live in rural areas. Similar increased occupational risks have previously been reported for clinical NE [17]. A HFRS virus seroprevalence study of telephone engineers in Korea showed a higher seropositivity rate when compared to the local population [18]. In China higher incidence rates for HFRS infection were reported in those engaged in heavy farm work, and in those with a history of sleeping in straw huts or on the ground [19]. In the present study, health care workers had similar or even lower seroprevalence rates than the whole group, indicating no increased risk of nosocomial PUV transmission. NE has a considerable morbidity during the acute phase and severe complications, including death, may occur. Vaccines against the

Asian forms of HFRS are already commercially available [20, 21]. No vaccine is available for NE.

It has been suggested that NE is associated with an increased long-term morbidity. However, the data in this study indicate that individuals with past PUV infection do not have an increased risk for cardiovascular disease, hypertension, myocardial infarction or diabetes mellitus. This is in contrast to recent reports suggesting that previous hantavirus infection is associated with hypertension and chronic renal disease [22–24]. Prospective longitudinal studies are needed to establish a definite causal relationship.

This study has showed an overall PUV seroprevalence of 5·4% in a large and highly endemic region for NE in Northern Sweden. Persons, notably farmers and forestry workers, living in rural parts of this region, have a significantly increased risk of NE and therefore a future vaccine should be considered for these groups.

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