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Case-control study on Puumala virus infection: smoking is a risk factor

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SUMMARY

Puumala hantavirus (PUUV) is apparently transmitted to humans by inhalation of aerosolized secretions of carrier rodents (bank voles). The means of transmission and the associated risk factors are poorly defined. An epidemiological study during the peak of an epidemic season in Finland was conducted based on 282 acute clinical PUUV infections and 204 controls without PUUV infection or immunity. The main risk factors adjusted by age, sex and living environment were cigarette smoking [odds ratio (OR) 3·6, 95% confidence interval (CI) 2·1–5·9, $P<0·0001$] and buildings with holes allowing rodents to enter (OR 3·3, 95% CI 2·0–5·6); these results were similar in two subsets. Further, use of rodent traps (OR3·5, 95% CI 2·2–5·7) and handling firewood (OR 2·7, 95% CI 1·6–4·4) were associated with a risk. The risk attributed to smoking also remained high using simulated population controls with average smoking habits. The results suggest that hantavirus transmission occurs by inhalation mainly indoors and is dependent on the condition of the respiratory tract.

Key words: Cigarette smoking, haemorrhagic fever with renal syndrome (HFRS), hantavirus, Puumala virus, rodents.

INTRODUCTION

Hantaviruses are rodent-borne, enveloped RNA viruses of the family Bunyaviridae and each hantavirus is carried by a chronically infected, specific rodent (or insectivore) host species. When transmitted to humans, Old World hantaviruses cause haemorrhagic fever with renal syndrome (HFRS), and New World hantaviruses cause hantavirus pulmonary syndrome (HPS), while many hantaviruses seem to be apathogenic to humans. In Finland, an average of 1000–1700 cases of usually mild HFRS known as nephropathia epidemica caused by Puumala virus (PUUV) have occurred annually [1]. Typical symptoms include headache, fever, nausea, vomiting, myalgia, back pain, visual disturbances and signs of renal failure. The diagnosis is based on detection of IgM antibodies to PUUV, and after infection, IgG antibodies prevail. PUUV, and its carrier rodent, the bank vole [*Myodes* (previously *Clethrionomys*) *glareolus*], are found in most of Europe excluding the Mediterranean region. The population densities of bank voles have a 3-year cycle in Finland, driven by predator–prey dynamics. The population densities are high during two consecutive autumns and winters and in parallel, human epidemics occur. Recently, the cycles have been

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synchronous over most of Finland resulting in high numbers of human cases every three calendar years, with, e.g. 2300, 774, 2603, 2526 and 3259 cases in 1999, 2000, 2002, 2005 and 2008, respectively. Approximately 5% of the Finnish population are IgG-seropositive but in endemic areas the figure rises to 20% [2].

Although the obvious source of human infection is the carrier rodent, the possible modes of transmission of hantaviruses to humans have not been thoroughly investigated and risk factors for contracting the disease beyond contact with rodents are not clear. Previous epidemiological studies of risk factors associated with hantavirus infection have shown significant odds ratios (ORs), e.g. in a Franco-Belgian study (PUUV) for exposure to dust or working with wood in a forest and entering buildings that might have rodents [3], in an American study (Sin Nombre virus) for entering closed buildings [4], and in Chinese studies (Hantaan virus) for cat ownership and sleeping in huts [5, 6].

Our aim was to study risk factors for PUUV transmission with a case-control approach in the Finnish setting during a winter epidemic peak with a large number of cases and controls, where the latter were verified to be susceptible to PUUV infection on the basis of negative serological results.

METHODS

Research material

We designed a non-matched case-control study; cases and controls were persons suspected and tested for acute PUUV infection in the diagnostic laboratory of Department of Virology, HUCH Laboratory Diagnostics (currently HUSLAB), Helsinki from 29 November 1999 to 28 February 2000. There was an epidemic peak of PUUV infection in Finland at that time. The data were collected by an extensive questionnaire (see next section) sent to all patients with a confirmed PUUV infection (cases: PUUV IgM- and IgG-positive), and to all patients suspected of PUUV infection, but who did not have markers of acute PUUV infection or of old immunity (controls: PUUV IgM- and IgG-negative). The diagnostic laboratory is an accredited (FINAS T200/M07/2008) university hospital laboratory and a WHO collaborating centre for hantavirus diagnostics and research; the PUUV μ-capture-IgM-EIA test [7] and IgG-IFA test [8, 9] have been evaluated thoroughly [10, 11] to have specificity and sensitivity of 100% and 100%, respectively, for the IgM test and 100% and 95%, respectively (compared to neutralization) for the IgG test, and are under external quality control. The questionnaire was sent together with the letter stating the result of the PUUV serology testing and was delivered to the patients (who signed a paper of agreement and informed consent) via the responsible physician. An ethical permit for the study was obtained from the coordinating ethical committee, HUCH. The research material consisted of returned questionnaires of 282 (response rate 80%) PUUV IgM- and IgG-positive (cases) and 204 (response rate 52%) PUUV IgM- and IgG-negative persons (controls).

Questionnaires

The questionnaires (English translation downloadable at www.hi.helsinki.fi/zoonoosivirukset/questionnaire) contained 66 questions divided into eight sections: (1) basic information (name, age, gender, address, education, occupation); (2–4) clinical signs, onset of illness and previous chronic diseases; (5) potential contacts with rodents, cats, dogs, and horses; (6) habits, such as smoking, outdoor activities, visiting outbuildings (most questions referring to 1–5 weeks before onset of illness); (7) characteristics of the respondent's house and its environment; (8) information on potential summer cottage (if visited 1–5 weeks before outbreak of illness). On average, 4% and 6% of cases and controls, respectively, used 'I don't know' each question and 6% of both left a question unanswered.

Subsets

In order to confirm the relevance of the results derived from the entire dataset, we further studied two subsets of the data: (1) those who lived in the most endemic region consisting of central and eastern parts of Finland (referred to as 'Endemic area'; see Fig. 1*a*); and (2) matched pairs selected from cases and controls (matched case-control study) (Fig. 1*b*). The pairs were matched on the basis of the following criteria: maximum age difference 10 years, gender, type of housing, living environment in terms of urbanity including distance from forest or fields [evaluated also from maps available on the internet (http://020300200.com)], geographical distance $<$130 km. PUUV infection is rare in children, and the study was further restricted to persons who were at least 15 years old, resulting finally in 275 cases and 188 controls in the entire

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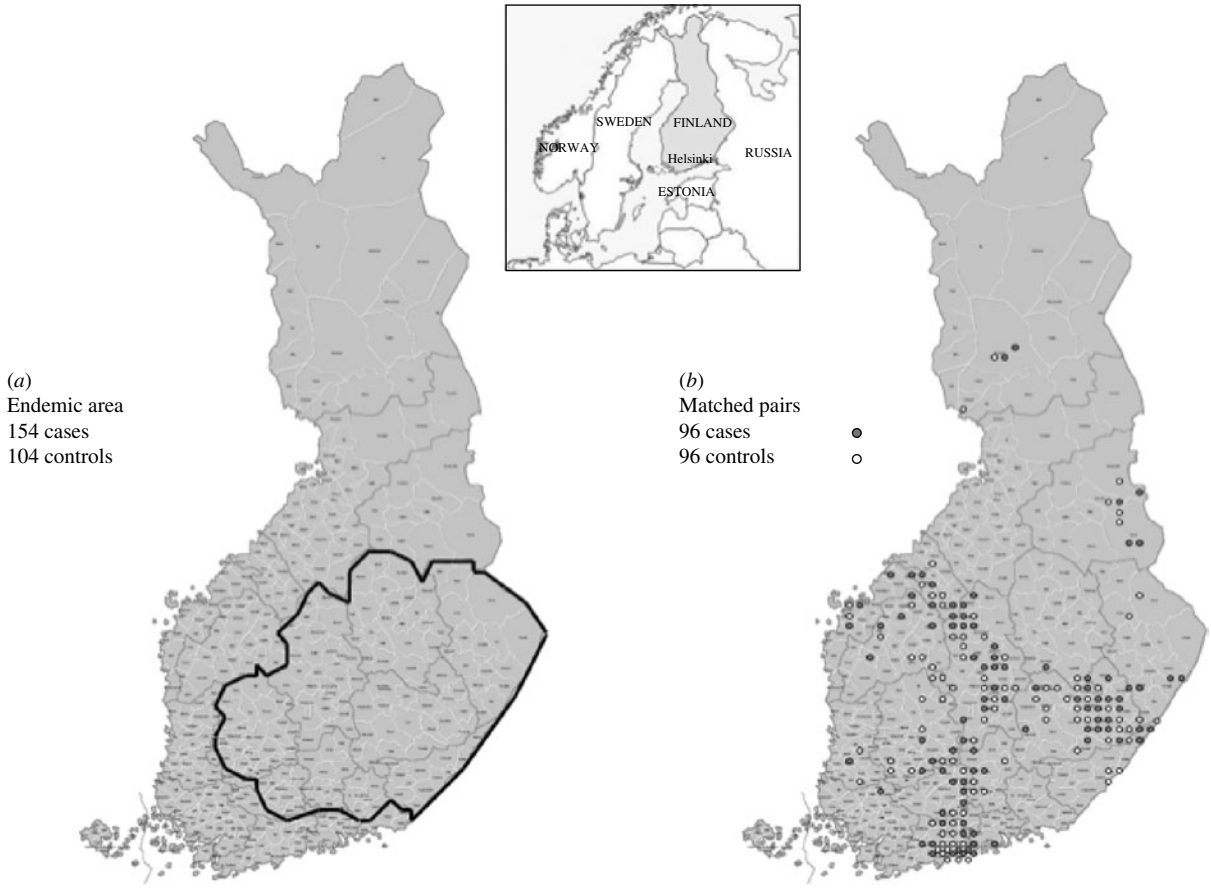

Fig. 1. Map of geographical distribution of the two separately studied subsets: (*a*) the endemic area and (*b*) the matched pairs, for which the municipality where each case and control lived is indicated with grey and white dots, respectively. (Inset: location of Finland in northern Europe.)

dataset, 154 cases and 104 controls in the 'Endemic area' and 96 matched pairs. Basic characteristics of cases and controls in all three study groups are given in Table 1.

Statistical methods

Statistical analysis was carried out as described below.

(1) Potential confounding factors were considered thoroughly for the whole research material. According to published studies age, sex, living environment, type of housing and being a farmer were previously known risk factors and their role as potential confounders was checked. In addition to these, chronic diseases and visiting a summer cottage were presumed to be potential confounding factors because of uneven distribution among cases and controls and suspected association with acquiring PUUV infection. However, chronic diseases, being a farmer and visiting a summer cottage had no significant or meaningful confounding effect. This was tested by calculating ORs for all individual exposure variables with and without adjustment by these factors and modelling (by logistic regression model) with and without them. 'Type of housing' and 'living environment' had a strong correlation. Sex, age group and living environment (urban *vs.* rural/remote) remained as confounding factors. They were used in analyses for the entire dataset and the endemic area. These three confounding factors were also the criterion for matching pairs.

(2) Crude and adjusted ORs with 95% confidence intervals (CIs) for all possible individual exposure variables were calculated for the whole research material and both subgroups (Table 2).

(3) Every significant ($P<0{\cdot}05$) exposure variable (adjusted OR > 1 and lower 95% CI > 1 or OR < 1 and upper 95% CI < 1) in the entire dataset or either of the subgroups was introduced to multivariate analysis which was carried out for the whole research material and both subgroups.

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Table 1. *Basic characteristic of the Puumala virus risk-factor study material*

	Entire dataset				Endemic area				Matched pairs			
	Cases		Controls		Cases		Controls		Cases		Controls	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Sex												
Female	104	38	87	46	62	40	59	57	58	60	58	60
Male	171	62	101	54	92	60	45	43	38	40	38	40
Age												
Median	45·9		45·9		45·9		47·6		47·9		48·1	
Range	15–86		15–86		15–77		15–86		15–76		16–81	
Age group, yr												
(1) 15–36	59	21	61	32	31	20	30	29	17	18	23	24
(2) 37–59	172	63	81	43	102	66	46	44	60	63	50	52
(3) ⩾60	44	16	46	24	21	14	28	27	19	20	23	24
Type of housing												
One-family house	231	84	99	53	132	86	58	56	76	79	76	79
Other	44	16	89	47	22	14	46	44	20	21	20	21
Environment												
Rural*	180	65	58	31	102	66	37	36	55	57	47	49
Urban	85	31	125	66	47	31	64	62	36	38	44	46
Farmers												
Number	58	21	18	10	32	21	13	13	18	19	18	19
Total	275		188		154		104		96		96	

* Rural = person who reported not living in a town or suburban area; if an answer was not available, information was deduced from internet maps (http://020300200.com). Notably, the matching of pairs was not based on the reported answer, but on similar community structure and distance from forest and fields in internet maps.

Multivariate analysis was done using logistic regression modelling for the entire dataset and the endemic area, and by conditional logistic regression modelling of the matched pairs. Interactions up to the second order were taken into account.

(4) In order to compare smoking habits of cases and controls to population data on smoking habits in Finland we used the 1999 smoking statistics provided by the Finnish National Public Health institute (www.ktl.fi).

Statistical analyses were performed using SAS version 9.1 PROC LOGISTIC program (SAS Institute Inc., USA).

RESULTS

ORs for exposure variables

The most important exposure variables and their ORs with 95% CIs (adjusted for age, gender, and living environment), counted for the whole study material and both subgroups (the endemic region and matched pairs), are given in Table 2. The predisposing factors under study could be categorized into either physical living environment (properties of the building, etc.) or personal activities and habits, and if relevant, the latter were restricted in the questionnaire to include only the period of 1–5 weeks before onset of illness.

Significant risk factors according to adjusted ORs in the whole study material and both subgroups were smoking, use of rodent traps for control, presence of holes in the building through which rodents may enter, and seeing rodents (Table 2).

Multivariate logistic regression models

We attempted to build the model in various ways and combinations of risk factors, e.g. adding or removing individual factors one by one or doing backwards or forwards modelling for selected combinations. When we included smoking, holes in the building, use of rodent traps, handling firewood and confounding factors in the model, the remaining factors had no

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Table 2. *Odds ratios of relevant exposure variables for PUUV infection*

	Cases		Controls		Entire dataset				Endemic area			
					Crude		Adjusted*		Adjusted*		Matched pairs	
Variable	*N*	%	*N*	%	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Smoking	116	42	36	19	**3·1**	2·0–4·8	**3·6**	2·1–5·9	**3·9**	1·9–7·7	**4·6**	2·0–10·4
Has used rodent traps	181	66	56	30	**5·4**	3·5–8·3	**3·5**	2·2–5·7	**2·7**	1·4–5·2	**4·3**	1·8–10·5
Holes in the building	143	52	37	20	**4·6**	2·9–7·3	**3·3**	2·0–5·6	**3·3**	1·6–6·6	**4·7**	1·9–11·3
Has visited summer cottage	59	21	32	17	1·3	0·8–2·2	**2·9**	1·6–5·2	**2·3**	1·1–5·1	1·7	0·7–3·8
Has seen rodents	164	60	47	25	**4·6**	3·0–7·0	**2·9**	1·8–4·7	**2·8**	1·5–5·3	**5·2**	2·0–13·5
Has handled firewood	224	81	102	54	**3·8**	2·4–5·9	**2·7**	1·6–4·4	**2·7**	1·4–5·4	2·0	0·9–4·3
Has seen rodent droppings	157	57	53	28	**4·1**	2·7–6·3	**2·6**	1·6–4·2	1·6	0·9–3·0	**2·3**	1·1–4·8
Lives in a wooden house	202	73	73	39	**4·5**	3·0–6·7	**2·6**	1·6–4·2	**2·1**	1·1–4·1	2·3	0·7–7·3
Hunting	44	16	12	6	**3·0**	1·5–5·9	2·0	0·9–4·3	1·4	0·5–3·6	1·6	0·5–4·9
Has handled dead rodents	94	34	25	13	**3·3**	2·0–5·5	**2·0**	1·2–3·4	1·3	0·7–2·6	**2·0**	1·0–3·9
House built >40 years ago	128	47	46	24	**2·8**	1·9–4·2	**2·0**	1·2–3·2	**1·8**	1·0–3·3	1·6	0·8–3·2
Lives <100 m from field	191	69	65	35	**4·1**	2·7–6·2	**1·9**	1·1–3·3	1·8	0·9–3·8	**2·5**	1·1–5·7
Well in the yard	131	48	38	20	**3·6**	2·4–5·6	**1·9**	1·1–3·3	**2·2**	1·1–4·7	1·6	0·8–3·5
House with fireplace	212	77	94	50	**3·6**	2·4–5·5	**1·9**	1·2–3·0	**2·2**	1·0–4·6	1·1	0·4–2·9
Has used rodent poison	71	26	23	12	**2·7**	1·6–4·6	**1·9**	1·0–3·4	1·3	0·6–2·9	1·3	0·6–3·0
Outdoor toilet	50	18	12	6	**3·4**	1·7–6·5	1·8	0·9–3·9	1·9	0·8–4·8	**3·2**	1·2–8·7
Forest work	67	24	23	12	**2·5**	1·5–4·2	1·7	0·9–3·3	1·1	0·5–2·4	1·1	0·5–2·6
Dog ownership	136	49	60	32	**2·0**	1·3–3·0	**1·7**	1·1–2·7	**2·7**	1·4–5·0	1·4	0·7–2·7
Has entered empty buildings	114	41	50	27	**2·1**	1·4–3·1	**1·6**	1·0–2·6	1·1	0·6–1·9	1·2	0·6–2·2
House heated with wood	172	63	68	36	**3·0**	2·0–4·4	**1·6**	1·0–2·6	**2·2**	1·2–4·1	1·4	0·6–3·2
Cat ownership	90	33	40	21	**1·8**	1·2–2·9	1·3	0·8–2·2	1·3	0·7–2·4	1·1	0·5–2·2
Lives <100 m from forest	192	70	106	56	**1·7**	1·1–2·5	1·3	0·8–2·0	1·3	0·7–2·4	1·5	0·7–3·0
House with attic	150	55	90	48	1·4	0·9–2·0	1·1	0·7–1·7	1·2	0·7–2·2	1·1	0·6–1·9
Farmer	58	21	18	10	**2·5**	1·4–4·4	1·0	0·5–2·0	1·0	0·4–2·3	1·0	0·5–2·2
Visits forest ⩾2 week	97	35	49	26	1·1	0·7–1·8	1·0	0·6–1·7	1·1	0·6–2·2	0·6	0·2–1·5
Has worked with hay	71	26	32	17	**1·8**	1·1–2·9	1·0	0·6–1·7	1·0	0·5–1·9	0·7	0·3–1·4
Has a composter?	113	41	69	37	1·2	0·8–1·8	0·9	0·6–1·5	0·8	0·4–1·4	0·8	0·4–1·4
House with cellar	182	66	119	63	1·2	0·8–1·7	0·9	0·5–1·3	0·7	0·4–1·3	1·0	0·5–1·7
Has handled animal feed	59	21	30	16	1·6	1·0–2·5	0·7	0·4–1·3	0·9	0·4–1·8	0·7	0·3–1·5

OR, Odds ratio; CI, confidence interval. The ORs that are statistically significant ($P>0·05$ when 95% CI lower limit >1) are in bold. The exposure variables are listed in (descending) order of adjusted ORs for the whole research material.
* Adjusted by age, gender and living environment (rural *vs.* suburban/urban).

significant effect. Apparently, this was because of considerable correlations between the above-mentioned and other factors. Only smoking, holes in the building, use of rodent traps, handling firewood were therefore selected in the model. However, 'holes in the building' reduced markedly the effect of the use of rodent traps and handling firewood (due to correlations), but not the effect of smoking. We finally built the model in two ways: either including all subjects in the study or excluding from the model those who had reported 'holes in the building' (models 1 and 2, respectively, in Table 3). This was done for all three groups (entire dataset, endemic area, matched pairs). The best Hosmer and Lemeshow goodness-of-fit test value was obtained in model 2 for the entire dataset ($\chi^2=1·542$, $P=0·98$)

Smoking remained a significant risk factor independent of the model. In Model 1, 'holes in the building' was another clear risk factor in all the three groups. The significance of the two other factors, 'handling firewood' and 'using rodent traps' varied.

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Table 3. *Multivariate analysis of significant risk factors for PUUV*

	Entire dataset		Endemic area		
	Model 1	Model 2 Restricted to those who had no holes in the building	Model 1	Model 2 Restricted to those who had no holes in the building	Matched pairs* Model 1
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Confounding factors					
Gender (male)	1·1 (0·6–2·1)	1·1 (0·4–2·8)	1·1 (0·5–2·3)	1·3 (0·4–4·7)	—
Age (group 2 *vs.* 3)†	2·7 (1·3–5·7)	3·8 (1·3–11·4)	2·0 (0·8–5·4)	2·8 (0·6–12·3)	—
Age (group 1 *vs.* 3)†	1·0 (0·4–2·4)	0·8 (0·2–3·3)	0·5 (0·1–1·5)	0·1 (0·0–1·2)	—
Living enviroment (rural)	4·4 (1·9–10·0)‡	2·4 (0·9–6·1)	1·8 (0·8–4·2)	1·7 (0·4–6·3)	—
Risk factors					
Smoking	4·2 (2·1–8·7)	6·9 (2·3–20·7)	6·1 (2·4–15·8)	48·1 (4·4–523·0)	5·9 (1·5–22·3)
Using rodent traps	2·6 (1·3–5·1)‡	3·7 (1·5–8·9)	1·7 (0·7–4·0)	3·3 (0·9–12·5)	1·7 (0·5–6·4)
Handling firewood	2·1 (1·0–4·5)‡	5·3 (1·9–14·9)	2·0 (0·8–5·0)	14·8(1·6–135·8)	0·4 (0·1–1·8)
Holes in the building	1·9 (1·0–3·7)	—	3·5 (1·4–8·4)	—	6·6 (1·6–28·3)
Living environment × using rodent traps	0·4 (0·2–0·7)	—	—	—	—
Living environment × handling firewood	0·4 (0·2–0·7)	—	—	—	—
Number of cases/controls	192/111	69/79	107/66	34/46	53/53

OR, Odds ratio; CI, confidence interval.

* Model 2 was not applicable to matched pairs because, e.g. for smoking none of the remaining 16 controls living in houses with 'no holes in the building' were smokers (compared to 7/16 of cases).

† See Figure 1 for definitions of age groups.

‡ OR values counted without interactions. When counted with interactions the ORs and CIs were for living environment (rural) (OR 0·5, 95% CI 0·2–1·5), using rodent traps (OR 0·9, 95% CI 0·4–2·2) and handling firewood (OR 0·7, 95% CI 0·3–2·1). Interactions did not affect other variables or other models.

Smoking

Smoking was a clear risk factor according to adjusted ORs (Table 2) and in the multivariate logistic regression models (Table 3) in all groups studied (in different models the significance of smoking varied from $P<0{\cdot}0001$ to $P=0{\cdot}0015$). Unlike for most other exposure variables shown in Table 2, adjusting did not reduce the significance of the ORs for smoking, but actually increased it. To confirm that smoking was a true risk factor, we further compared the smoking habits of the controls and cases with those of the Finnish general population based on statistics available for 1999 (Fig. 2). In each age group, the cases smoked considerably more often than either controls or the general population, e.g. 11/14 cases in males in the youngest age group (15–24 years old) were smokers (Fig. 2*a*); the findings were similar for females (Fig. 2*b*). We also evaluated smoking by counting the OR of smoking using simulated controls (same number as in our study) whose smoking habits were replaced with the national 'average smoking' data. Especially for females, the smoking habits of our controls were very similar to those of the general population (Fig. 2*b*). The OR for smoking adjusted by age for the comparable age groups was with our 'real' controls 2·8 (95% CI 1·7–4·6) and with our 'simulated' controls 2·5 (95% CI 1·6–4·0).

DISCUSSION

PUUV infection is a common disease in Finland with 5% seroprevalence and an average 1700 reported cases annually (www.thl.fi). Although the excreta of the chronically infected specific host rodent are the source of human hantavirus infection, it has remained unclear how the infection is actually acquired and which risk factors are involved.

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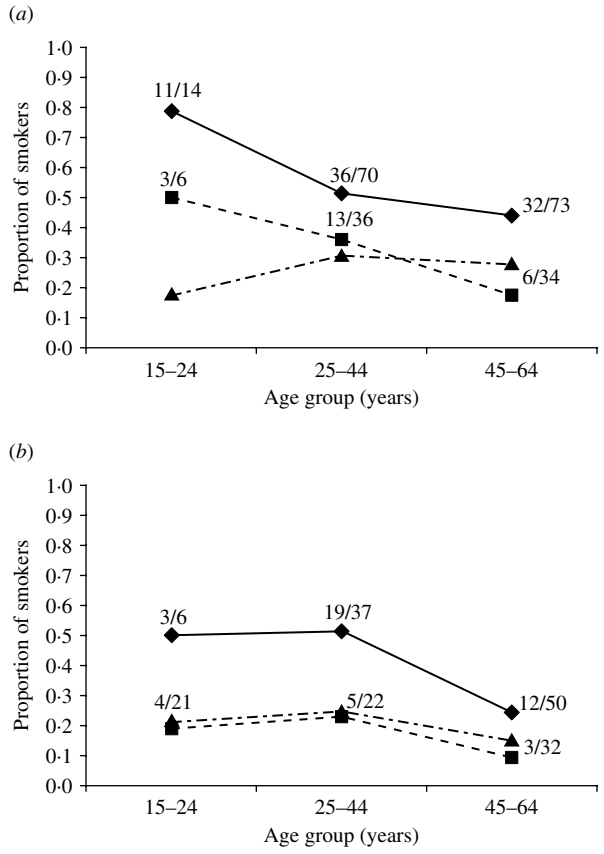

Fig. 2. Age-group-specific proportion of smokers in (*a*) male and (*b*) female acute Puumala virus infection cases (–◆–, PUUV IgM- and IgG-positive), controls (- -■- -, PUUV IgM- and IgG-negative), and in the general population (· -▲- ·) in 1999 in Finland. The fraction numbers refer to total number of smokers per number of cases or controls in each age group.

The approach was to recruit as many PUUV cases [PUUV IgM(+)] and susceptible [PUUV IgG(–)] controls studied by our diagnostic laboratory as possible for a questionnaire study during the peak of an epidemic season (from December to February). The epidemic peak under study turned out to be exceptionally high resulting in a high case:control ratio with 275 cases and 188 controls, aged at least 15 years, accepted in the study. Because the possibility of contracting the infection is strictly temporally linked to the presence of virus in carrier rodents and their population densities, recruiting more controls afterwards was not possible, because they would no longer have had the opportunity to have been exposed to the infection. To control for possible geographical differences in rodent population densities of living environments of cases and controls, in addition to the entire dataset we studied separately a highly endemic region with 154 cases and 104 controls, and 96 matched case-control pairs. Exclusion of people with pre-existing PUUV immunity was especially important in the endemic area since, e.g. in the commune of Ilomantsi in Northern Karelia >50% of males aged >60 years are PUUV-seropositive compared to an average of 5% of Finns in general [1].

The most striking and clear finding was that smoking was an obvious risk factor for acquiring hantavirus infection. The relationship was stronger after adjusting for confounding factors and in the models if the data were restricted to those who did not have 'holes in the building'; the variable had no significant interactions with other risk factors or confounding factors. Moreover, comparison with population-based control data showed that our cases were more often smokers in all age groups (Fig. 2). This finding is consistent with hantaviruses being primarily transmitted to man by inhalation of aerosols; it could be hypothesized that the condition of airways, e.g. the ciliary movement, influences whether the infectious aerosol enters the alveoli and remains there long enough for the infection to occur. One possibility is that the infection could occur by ingestion of hantavirus particles by macrophages or immature dendritic cells in the alveoli; the latter were recently shown to be induced to mature by hantavirus infection [12] which could lead them to be recruited to lymph nodes for further systemic spread of the infection. Interestingly, cigarette smoke extract has been shown to cause immunomodulatory effects in dendritic cell function [13]. Moreover, chronic inflammation could contribute to the initial spread of the infection. It has been shown that smoking is also a risk factor for some other, at least partially aerosol-transmitted or airway pathogen diseases such as common cold, influenza, and invasive pneumococcal pneumonia [14]. Our result could further help to explain why children rarely acquire PUUV infection, although they are probably exposed to the virus: both PUUV antibody positivity and the disease are almost non-existent in children aged <10 years [1]. On the other hand, as an alternative explanation smoking may lead to more frequent contact between hand and mouth. In any event, our results suggest that cigarette smoking can be a risk factor, not only for infections arising from the flora of the respiratory tract, but also for microbial infections acquired from the environment via the respiratory route. More detailed studies on the impact of history and quantity of smoking are still needed.

Another clear risk factor was the presence of holes in the building through which rodents could enter. Together with cigarette smoking, this risk further suggests that transmission occurs by inhalation indoors; the possibility of contracting high amounts of infectious aerosols for inhalation is evidently higher when rodents can enter the building.

The association with rodent control was interesting, because instead of being protective, using rodent traps seemed to be associated with some risk (Tables 2 and 3) whereas in the two subsets, using rodent poison for control was not a risk factor at all. It is likely that when traps are used, one needs to remove the dead rodent and therefore be exposed to their excreta. On the contrary, when poison is used, rodents usually leave human dwellings before they die, without further human contact. For example, in our dataset, 50% *vs.* 5% of the diseased using *vs.* not using traps had handled a dead rodent, respectively.

Handling firewood is believed to be a risk factor for acquiring PUUV infection and Swedish patients have reported that this was their most likely exposure [15]. Our study findings support this view, although the ORs were not uniformly significant (Tables 2 and 3). Peridomestic woodsheds are a probable contact place for rodents and humans.

Other potential risk factors were also found but they were not uniformly detected in all subsets of the data. Unlike for Hantaan virus in China [6] we could not find compelling evidence for cats being a vehicle of transmission nor to have a protective effect, whereas some risk could be attributed to dog ownership. Observation of rodents was a risk factor, as in previously published studies, indicating that our case and control groups were adequate. In this study we analysed the risk factors beyond this phenomenon (observation of rodents), which is dependent on the rodent densities, and it did not remain as an independent risk factor in the regression analysis.

Previous epidemiological studies on PUUV infection have shown occupational risk attributed to farming in Finland [16] and Sweden [17]. In a case-control study conducted in Belgium and France, the only statistically significant risks were working in the forest and entering potentially rodent-infested buildings; e.g. rodent control had a non-significant protective effect [3]. A Belgian study suggested that woodcutting, reopening of a non-aerated room, and strenuous physical effort were risk activities [18]. Studies in China have suggested that, e.g. camping or living in huts in the fields, living in a house on the periphery of a village, and cat ownership are risk factors [5, 6, 19]. In a study of American soldiers in Korea, sleeping in tents was a risk [20]. The risk of acquiring Sin Nombre virus infection has been attributed, in addition to high rodent contact [21], to entering peridomestic buildings that have been unused for long periods [4].

CONCLUSIONS

In conclusion, our study has shown that cigarette smoking and living in buildings which have holes allowing rodents to enter were the most important risk factors for PUUV infection. These findings are compatible with hantavirus infection being an aerosol-transmitted disease dependent on the condition of airways and infectious virus concentration in the aerosol, which is highest indoors. In addition, considerable risk may be attributed to use of rodent traps (as opposed to poison) and handling firewood, but this finding was not as uniformly clear as for the two main risk factors. The findings could have direct public health impact on recommendations for prevention of the disease, emphasizing the role of blocking the entry of rodents to human dwellings and avoiding or minimizing inhalation of potentially contaminated dust indoors, suggesting that poison could be better than traps for rodent control and adding yet another health threat attributable to cigarette smoking.

Our study represents one of the largest epidemiological studies of risk factors for acquiring hantavirus infection. While more studies are needed and these results cannot perhaps be fully extrapolated outside the endemic regions for PUUV infection in wintertime, the findings should be helpful in designing future studies and in planning for preventive measures for hantavirus infections.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Brummer-Korvenkontio M, *et al.*** Nephropathia epidemica in Finland 1989–1996: an epidemiological study of 6701 cases. *Scandinavian Journal of Infectious Diseases* 1999; **31**: 427–435.
2. **Vapalahti O, *et al.*** Hantaviruses in Europe. *Lancet Infectious Diseases* 2003; **3**: 653–661.
3. **Crowcroft NS, *et al.*** Risk factors for human hantavirus infection: Franco-Belgian collaborative case-control study during 1995–6 epidemic. *British Medical Journal* 1999; **318**: 1737–1738.
4. **Zeitz PS, *et al.*** A case-control study of hantavirus pulmonary syndrome during an outbreak in the southwestern United States. *Journal of Infectious Diseases* 1995; **171**: 864–870.
5. **Xu ZY, *et al.*** Epidemiological studies of hemorrhagic fever with renal syndrome: analysis of risk factors and mode of transmission. *Journal of Infectious Diseases* 1985; **152**: 137–144.
6. **Xu ZY, *et al.*** Cats-source of protection or infection? A case-control study of hemorrhagic fever with renal syndrome. *American Journal of Epidemiology* 1987; **126**: 942–948.
7. **Vapalahti O, *et al.*** Antigenic properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. *Journal of Clinical Microbiology* 1996; **34**: 119–125.
8. **Hedman K, Vaheri A, Brummer-Korvenkontio M.** Rapid diagnosis of hantavirus disease with an IgG-avidity assay. *Lancet* 1991; **338**: 1353–1356.
9. **Vapalahti O, *et al.*** Human B-cell epitopes of Puumala virus nucleocapsid protein, the major antigen in early serological response. *Journal of Medical Virology* 1995; **46**: 293–303.
10. **Sjolander KB, *et al.*** Evaluation of serological methods for diagnosis of Puumala hantavirus infection (nephropathia epidemica). *Journal of Clinical Microbiology* 1997; **35**: 3264–3268.
11. **Kallio-Kokko H, *et al.*** Evaluation of Puumala virus IgG and IgM enzyme immunoassays based on recombinant baculovirus-expressed nucleocapsid protein for early nephropathia epidemica diagnosis. *Clinical and Diagnostic Virology* 1998; **10**: 83–90.
12. **Raftery MJ, *et al.*** Hantavirus infection of dendritic cells. *Journal of Virology* 2002; **76**: 10724–10733.
13. **Vassallo R, *et al.*** Cigarette smoke extract suppresses human dendritic cell function leading to preferential induction of Th-2 priming. *Journal of Immunology* 2005; **175**: 2684–2691.
14. **Nuorti JP, *et al.*** Cigarette smoking and invasive pneumococcal disease. *New England Journal of Medicine* 2000; **342**: 732–734.
15. **Olsson GE, *et al.*** Human hantavirus infections, Sweden. *Emerging Infectious Diseases* 2003; **9**: 1395–1401.
16. **Vapalahti K, *et al.*** Puumala virus infections in Finland: increased risk for farmers. *American Journal of Epidemiology* 1999; **149**: 1142–1151.
17. **Ahlm C, *et al.*** Prevalence of antibodies specific to Puumala virus among farmers in Sweden. *Scandinavian Journal of Work Environment & Health* 1998; **24**: 104–108.
18. **Van Loock F, *et al.*** A case-control study after a hantavirus infection outbreak in the south of Belgium: who is at risk? *Clinical Infectious Diseases* 1999; **28**: 834–839.
19. **Ruo SL, *et al.*** Retrospective and prospective studies of hemorrhagic fever with renal syndrome in rural China. *Journal of Infectious Diseases* 1994; **170**: 527–534.
20. **Pon E, *et al.*** Outbreak of hemorrhagic fever with renal syndrome among U.S. Marines in Korea. *American Journal of Tropical Medicine and Hygiene* 1990; **42**: 612–619.
21. **Childs JE, *et al.*** A household-based, case-control study of environmental factors associated with hantavirus pulmonary syndrome in the southwestern United States. *American Journal of Tropical Medicine and Hygiene* 1995; **52**: 393–397.