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Malaria knowledge, attitudes and practices among migrants from malaria-endemic countries in Evrotas, Laconia, Greece, 2013

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Following re-emergence of malaria in Evrotas, Laconia, in 2009–12, a malaria-control programme was implemented in 2011–12 targeting migrants from malaria-endemic countries, including house-to-house active case detection, health education and distribution of mosquito protection items. In June 2013, we surveyed migrants in Evrotas to assess their malaria knowledge, attitudes and practices to guide prevention activities. We selected participants using simple random sampling and interviewed them, using structured questionnaires. We defined mosquito protection practices (MPPs) as the use of full-length clothes/topical repellent, mosquito screens, fans or air-conditioning, and insecticides. We calculated prevalence ratios (PRs) using Poisson regression and we allowed for clustering of participants in a residence. Of 654 migrants, we invited 132 and 130 participated (all men; 120 (92%) from Pakistan). Of the 130, 56 (43%) identified fever as a malaria symptom; those who were aware of this had higher level of education (PR: 3.2; 95% confidence interval (CI): 1.2–9.0). A total of 111 (85%) used insecticide-treated bednets and 95 (73%) used more than two MPPs. Poor housing conditions (warehouses/shacks: PR: 0.8; 95% CI: 0.6–0.9), were associated with use of up to two MPPs. Despite extensive interventions in Evrotas, the level of malaria awareness among migrants remained suboptimal and poor housing conditions hindered effective mosquito protection. We recommend culturally adapted health education and improvement of housing conditions to minimise the risk of new cases and re-establishment of malaria in Greece.

Introduction

In 2012, the World Health Organization (WHO) estimated that malaria caused 207 million infections and 627,000 deaths globally [1]. In Europe, most cases are attributed to migration and international travel [2], although from 1998 to 2010, parts of southern Europe reported sporadic autochthonous cases [3]. Environmental conditions in southern Europe favour breeding of anopheline vectors of malaria, allowing for transmission of the *Plasmodium* parasites [4,5]. The carriage of *Plasmodium* by travellers and migrants from malaria-endemic areas favours the potential for local transmission under suitable ecological conditions [6].

In 1974, WHO declared Greece malaria free [7]; however, in 2009–12, 53 locally acquired and 40 imported cases of *Plasmodium vivax* malaria occurred in Evrotas, Laconia, in southern Peloponnese, a rural and historically malaria-prone area [8]. Genotyping analysis of *P. vivax* from blood specimens from autochthonous and imported cases in Evrotas in 2011 indicated that there had been multiple introductions of several south-east Asian strains of *P. vivax* and local transmission had occurred [9]. During the May to October transmission period [10] of 2013, Evrotas was host to a community of 554 to 859 male migrant farm workers from the Indian subcontinent, predominantly Pakistan. This population was mobile, with a high turnover of people and fluctuating numbers throughout the year [11]. Most of the migrants were undocumented and worked in local orchards. Many lived in crowded, poorly constructed buildings, often located close to mosquito breeding sites [10].

Since October 2011, a mobile team based in Evrotas from the Hellenic Centre for Disease Control and

Table 1

Socio-demographic and household characteristics of migrant study participants, Evrotas, Greece, June 2013 (n = 130)[a]

Characteristic	Number (%)	95% CI
Country of origin		
Pakistan	120 (92)	86–96
Afghanistan	4 (3)	1–8
Bangladesh	4 (3)	1–8
Morocco	2 (2)	0–5
Village of residence		
Skala	77 (59)	50–68
Elos	31 (24)	17–32
Leimonas	8 (6)	3–12
Vlachioti	6 (5)	2–10
Taxiarches	5 (4)	1–9
Agios Georgios	3 (2)	0–7
Age group in years		
18–24	48 (37)	29–46
25–34	62 (48)	39–57
≥35	20 (15)	10–23
Length of education in years		
0	18 (14)	8–21
1–6	52 (40)	32–49
7–12	60 (46)	37–55
Marital status		
Single	99 (76)	68–83
Profession		
Casual farm worker	128 (98)	95–100
Medical insurance		
Had insurance	4 (3)	1–8
Type of residence		
House/flat	68 (52)	43–61
Warehouse/shack	62 (48)	39–57
Mosquito screens on windows and doors		
None	59 (45)	37–54
Some	61 (47)	38–56
All	10 (8)	4–14
Other residence characteristics		
Had a fan or air conditioning	111 (85)	78–91
Had an outdoor area, e.g. yard, garden, etc.	123 (95)	89–98
Had unkept and overgrown bushes/plants (n = 123)	71 (58)	48–67
Had an outdoor area with water[b] (n = 123)	63 (51)	42–60

CI: confidence interval.

[a] Unless otherwise specified.

[b] Irrigation channels, ditches, wells, stagnant water, water containers.

Prevention (HCDCP) (from April to December 2011 and still ongoing in 2015) and the nongovernmental organisation Médecins Sans Frontières (MSF) (from April to October 2012) have carried out field work on active case detection (ACD) in the area and have implemented an integrated malaria control programme in Evrotas targeting the local and migrant communities. This included the deployment of a field team that created a regularly updated registry of all migrants from malaria-endemic and North African countries, conducted fortnightly door-to-door visits for ACD among migrants and set up a hotline for queries for the public. The vector control programme included indoor residual spraying (IRS) and distribution of mosquito coils and indoor mosquito vaporising mats and additionally, in 2013, distribution and monitoring of use of long-lasting insecticide-treated bednets (LLINs) (otherwise unavailable and not authorised for sale in Greece) to migrant residents. The team provided information on malaria and protection measures against mosquitoes to local and migrant residents, through home visits, public meetings, presentations to schools, TV and radio spots [10,12,13]. However, the impact of these health education and vector control activities had not been evaluated, and little was known about the level of awareness of malaria, mosquito protection practices (MPPs) and health-seeking behaviour among this migrant community [10]. As migrant populations are particularly vulnerable to contracting malaria, given their poor living conditions, their access to healthcare and their fragile legal status in the country, in June 2013, we surveyed migrant residents of Evrotas born in malaria-endemic countries and North Africa, to assess their knowledge, attitudes and practices in order to guide public health prevention activities and community sensitisation campaigns.

Methods

Study area and population

Evrotas is an agricultural river delta area (2011 population: 17,755) [14] with dense irrigation and drainage channels and abundant mosquito populations, including *Anopheles* spp. in low but increasing numbers in September and October [8]. The *Anopheles* mosquito in Greece is anthropophilic, nocturnal, bites both indoors and outdoors and is not considered a nuisance mosquito [15]. The predominant species in Evrotas is *An. sacharovi*, a competent malaria vector [16]. Evrotas is served by one health centre and the nearest regional hospital is in the city of Sparta, 37 km away.

The study population included the migrant residents of six villages (Skala, Elos, Vlachioti, Leimonas, Taxiarches and Agios Georgios) targeted for ACD during the May to October 2013 transmission period. We defined migrant residents as individuals over 18 years of age, born in malaria-endemic or North African countries and who had resided in one of the study villages for three months or more.

Table 2

Malaria awareness and attitudes towards malaria among migrant study participants in Evrotas, Greece, June 2013 (n = 130)[a]

Awareness or attitude	Number/Total (%)[b]	95% CI
Heard about malaria in the past	117/130 (90)	84–95
Heard about malaria from:		
ACD teams	94/117 (80)	72–87
Country of origin	59/117 (50)	41–60
Friend/family	28/117 (24)	17–33
Media[c]	5/117 (4)	1–10
Healthcare facility	4/117 (3)	1–9
Malaria present in country of origin		
Yes	100/130 (77)	69–84
No	16/130 (12)	7–19
Don't know	14/130 (11)	6–17
History of malaria		
Yes	9/130 (7)	3–13
Place where malaria symptoms appeared		
Greece	5/9 (56)	21–86
Country of origin	4/9 (44)	14–79
Other		
Awareness of malaria cases in Evrotas	115/128 (90)	83–94
Worried about becoming seriously ill (themselves or family/household member) with malaria	79/129 (61)	52–70
Worried that mosquito control insecticides are harmful to health	13/129 (10)	5–17
Satisfied with malaria information provided by authorities	129/129 (100)	97–100
Satisfied with malaria prevention methods taken by authorities	129/129 (100)	97–100

ACD: active case detection; CI: confidence interval.

[a] Unless otherwise specified.

[b] Some percentages do not add up to 100 because of multiple responses.

[c] Newspaper, radio, television, Internet, poster.

Sampling

We selected participants using simple random sampling from the May 2013 HCDCP registry of migrants in Evrotas. Assuming 50% of the migrant population had knowledge of malaria, with 8% precision, a design effect of 1, a 95% confidence level and a source population of 654 persons, we estimated a sample size of 123 participants for the survey (OpenEpi software version 3.1.). We estimated a response rate of 90%, as the field teams that were engaged in the malaria control activities had built a relationship of trust with the migrant community over the years and anticipated a relocation of 30% of migrants to outside the area at the time of the survey due to the high mobility of this population. We therefore selected 206 individuals to participate in the survey.

Data collection

We interviewed participants face-to-face using a structured questionnaire, which was available in Greek and English and was verbally translated into Urdu, the mother tongue of almost all the participants. It collected information on socio-demographic characteristics, malaria knowledge and attitudes, use of MPPs (e.g. topical insect repellent, bednets, coils) since 1 May 2013 (i.e. the month preceding the survey), healthcare-seeking behaviour and access to healthcare. All bednets distributed to the migrants were LLINs, but in the interviews, the term 'bednet' was used, for simplicity. Participants provided non-prompted multiple answers for malaria knowledge to minimise any bias from leading questions.

Study teams also observed the conditions of the participants' residences using a checklist that addressed the residence characteristics (e.g. house type, surrounding vegetation, water sources) and house-proofing measures such as mosquito screens. Fieldworkers and two interpreters, recruited and trained locally, interviewed participants. We piloted the questionnaire in Greek and Urdu in a village not participating in the study.

Ethics

We collected all information in an anonymous format and analysed aggregated data to ensure confidentiality. All participants in the survey provided informed verbal consent and received an HCDCP malaria information leaflet translated into their native language. The Ethics Committee of the National School of Public Health in Athens approved the survey.

Data analysis

We considered makeshift shelters, warehouses, storerooms, stables and shacks as residences not built for housing. We defined indoor insecticides as insecticide-containing mosquito vaporising mats, liquids and sprays. We considered a person's knowledge as correct for the following topics: (i) 'transmission', if at least one correct malaria transmission mode was reported; (ii) 'symptoms', if fever was stated as one of the possible malaria symptoms; and (iii) 'prevention', if more

Table 3a

Malaria knowledge and mosquito protection practices since May 2013 among migrant study participants in Evrotas, Greece, June 2013 (n = 130)[a]

Knowledge or practice		Number (%)[b]	95% CI
Knowledge			
Malaria transmission	Mosquitoes	107 (82)	75–88
	Blood transfusion	3 (2)	0–7
	Pregnant mother to child	0 (0)	0–3
	Don't know	20 (15)	10–23
	Incorrect transmission modes[c]	51 (39)	31–48
	≥ 1 correct transmission mode[d]	107 (82)	75–88
Malaria symptoms	Fever	56 (43)	34–52
	Headache	36 (28)	20–36
	Joint/body pain	25 (19)	13–27
	Chills	10 (8)	4–14
	Feeling unwell	7 (5)	2–11
	Dizziness	2 (2)	0–5
	Diarrhoea or vomiting	2 (2)	0–5
	Other (loss of appetite, cough, flu-like illness, skin rash)	6 (5)	2–10
	Don't know	66 (51)	42–60
Existence of malaria treatment	Yes	126 (97)	92–99
Malaria prevention	Bednets	53 (41)	32–50
	Full-length clothing	11 (8)	4–15
	Indoor insecticides[e]	30 (23)	16–31
	Mosquito coils	24 (18)	12–26
	Avoidance of mosquitoes	21 (16)	10–24
	Topical insect repellents	10 (8)	4–14
	IRS	4 (3)	1–8
	Cleanliness (personal and/or household)	60 (46)	37–55
	Taking malaria tablets	21 (16)	10–24
	Drainage of stagnant water	9 (7)	3–13
	Other (mosquito screens, fan, avoiding going out at night)	9 (7)	3–13
	Don't know	19 (15)	9–22
	> 1 correct malaria prevention methods[f]	43 (33)	25–42
Malaria knowledge score[g]	0	17 (13)	8–20
	1	44 (34)	26–43
	2	45 (35)	27–44
	3	24 (18)	12–26

CI: confidence interval; IRS: indoor residual spraying; MPPs: mosquito protection practices.

[a] Unless otherwise specified.

[b] Some percentages do not add up to 100 because of multiple responses.

[c] Garbage, drinking dirty water, person-to-person, sharing food with a sick person, kissing, sex, hot climate, water with mosquitoes.

[d] Mosquitoes, blood transfusion, pregnant mother to child.

[e] Mosquito vaporising mats, liquid vaporisers, spraying solutions/aerosols.

[f] Long-lasting insecticide-treated bednets, full-length clothing and/or topical insect repellents, mosquito vaporising mats/liquids and/or aerosol, mosquito coils, avoiding mosquitoes.

[g] Range of malaria knowledge score: 0–3; one point was given to each of the following: ≥ 1 transmission mode, at least fever (± ≥ 1 other symptom) and ≥ 2 malaria prevention methods.

Table 3b

Malaria knowledge and mosquito protection practices since May 2013 among migrant study participants in Evrotas, Greece, June 2013 (n = 130)[a]

Knowledge or practice		Number (%)[b]	95% CI
Practices (since 1 May 2013, i.e. the month preceding the survey)			
MPPs (used often)[h]	Bednets	111 (85)	78–91
	Fan (ceiling/standing fan) or air-conditioning in sleeping room	102 (78)	70–85
	Mosquito coils	99 (76)	68–83
	Indoor insecticides (n = 129)[e]	81 (63)	54–71
	Wore full-length clothing	65 (50)	41–59
	Mosquito screens in sleeping room	64 (49)	40–58
	Air-conditioning in sleeping room	5 (4)	1–9
	Applied topical insect repellents	4 (3)	1–8
	Avoided going out at dusk and/or night	102 (78)	70–85
	Emptied stagnant water from containers	102 (78)	70–85
	Slept outside (≥ once a week)	27 (21)	14–29
	Slept outside without a bednet (n = 27)	5 (19)	6–38
	Burned waste and/or dung	5 (4)	1–9
Personal MPPs[i]	Yes	67 (52)	43–60
Number of household MPPs[j]	> 1 MPP (n = 129)	108 (84)	76–90
Overall MPPs[k]	> 2 MPPs	95 (73)	65–80
IRS performed the previous year	No	18 (14)	8–21
	Yes	71 (55)	46–63
	Don't know	41 (32)	24–40
IRS should be performed next year	Yes	127 (98)	93–100

CI: confidence interval; IRS: indoor residual spraying; MPPs: mosquito protection practices.

[a] Unless otherwise specified.

[b] Some percentages do not add up to 100 because of multiple responses.

[c] Garbage, drinking dirty water, person-to-person, sharing food with a sick person, kissing, sex, hot climate, water with mosquitoes.

[d] Mosquitoes, blood transfusion, pregnant mother to child.

[e] Mosquito vaporising mats, liquid vaporisers, spraying solutions/aerosols.

[f] Long-lasting insecticide-treated bednets, full-length clothing and/or topical insect repellents, mosquito vaporising mats/liquids and/or aerosol, mosquito coils, avoiding mosquitoes.

[g] Range of malaria knowledge score: 0–3; one point was given to each of the following: ≥ 1 transmission mode, at least fever (± ≥ 1 other symptom) and ≥ 2 malaria prevention methods.

[h] The question had three possible answers: 'often', 'sometimes', 'rarely' (the exact frequencies were not specified in the question). We present here only the results of the 'often' response (we merged the 'sometimes' and 'rarely' responses to create a binary variable).

[i] Yes/No answer: full-length clothing or topical insect repellents.

[j] Indoor insecticides, fan or air-conditioning, mosquito screens, mosquito coils.

[k] Personal and household MPPs.

than one malaria prevention method was mentioned. The transmission, symptoms and prevention scores were combined, to create an overall malaria knowledge score (score range: 0–3). We considered MPPs as sufficient if participants used more than two MPPs. We did not include the use of bednets in the calculation of the total number of MPPs and subsequently in the regression analysis since comprehensive distribution of LLINs to all migrants took place two weeks before the survey [11]. Hence, their use did not reflect migrants' usual behaviour and could bias the results. We calculated proportions with 95% confidence intervals (CIs) for categorical variables. We estimated crude and adjusted prevalence ratios (PRs) with 95%CIs, using robust Poisson regression and allowed for clustering of participants sharing the same residency using the vce(cluster) option in STATA. We performed the analysis using STATA 12 software (StataCorp, Texas, United States, United States).

Results

We invited 206 (31%) persons of the 654 eligible individuals to participate in the survey. Of these, 74 (36%) had relocated from Evrotas and were excluded. Of the remaining 132 individuals, 130 (98%) agreed to participate. Almost all interviews (n = 128) were conducted in Urdu.

Socio-demographic characteristics

The respondents were young (median: 26 years; range: 18–55), male (100%) and predominantly from Pakistan (n = 120; 92%). They had lived in Evrotas for a median

of 3.0 years (range: 0.3–16) (Table 1). A total of 62 (48%) resided in buildings not built for housing. A median of six (range: 1–25) people lived in the same household and a median of three (range: 1–12) individuals living in a residence shared the same room.

Malaria awareness and attitudes

Of 130 participants, 117 (90%) reported having heard about malaria in the past (Table 2) while 86 (66%) (95%CI: 57–74%) said they did not need any more information about malaria. Of 129 respondents, 79 (61%) were worried that they or family/household members might become seriously ill with malaria.

Of 130 participants, 107 (82%) identified mosquitoes as the main mode of malaria transmission (Table 3). However, 51 (39%) also named at least one incorrect mode. Of these, 40 believed malaria was transmitted through garbage. Participants older than 34 years (PR: 1.3; 95% CI: 1.1–1.6), with more than seven years of education (PR: 1.4; 95% CI: 1.0–1.9) and those who would seek medical treatment for fever (› 38 °C) from ACD teams (PR: 1.3; 95% CI: 1.1–1.5) were more likely to know about malaria transmission (Table 4).

Of all 130 respondents, 56 (43%) identified fever and 36 (28%) headache as symptoms of malaria. Participants with more than seven years of education were more aware of fever as a symptom of malaria (PR: 3.2; 95% CI: 1.2–9.0). A total of 43 (33%) knew at least two malaria prevention methods; 53 (41%) mentioned bednets, 30 (23%) indoor insecticides and 4 (3%) stated IRS as a malaria control method. However, 71 (55%) reported IRS in their residence the previous year and 127 (98%) mentioned it should be sprayed next year. Participants aged 25–34 years and those coming from Pakistan knew more about prevention methods (PR: 1.8; 95% CI: 1.0–3.2 and PR: 3.9; 95% CI: 1.1–14.5, respectively). However, as the majority of participants were from Pakistan, this PR must be interpreted with caution. Of 130 respondents, 126 (97%) were aware of the existence of malaria treatment.

Use of mosquito protection and control measures

Of all participants, 67 (52%) used either full-length clothing or topical insect repellent; 108/129 (84%) used at least two household MPPs and 95 (73%) used more than two of five overall MPPs (median: 3; range: 0–5) (Table 3). In the month preceding the survey, 111 (85%) participants used bednets for sleeping. Six participants reported not sleeping under a bednet the previous month: the reasons given were that they had not received one (n = 3), heat (n = 1), they preferred to use an electric fan instead (n = 1) and they believed that bednets were not more beneficial than IRS (n = 1). The most common MPPs were the presence of electric fan or air-conditioning in the room used for sleeping (n = 102; 78%), mosquito coils (n = 99; 76%) and indoor insecticides (81/129; 63%); 64/130 (49%) reported the presence of mosquito screens in the room used for sleeping.

Participants who were more likely to use more than two MPPs included those who would seek fever (›38 °C) treatment from ACD teams (PR: 1.3; 95% CI: 1.0–1.6) and those worried about becoming seriously ill with malaria (PR: 1.5; 95% CI: 1.1–2.0). Residents of buildings not built for housing were less likely to use more than two MPPs (PR: 0.8; 95% CI: 0.6–0.9) (Table 4).

Treatment seeking behaviour and access to healthcare

A total of 77 (59%) participants stated that in case of fever (›38 °C) they would seek treatment from ACD teams, 28 (22%) would go to the regional hospital in Sparta and 11 (8%) would attend the local health centre; 121/125 (97%) would seek treatment within one day (Table 5).

During the year before the survey, 16 (12%) participants sought medical care in a healthcare facility, nine of whom reported facing difficulties in accessing healthcare. The reasons included long distance to travel, user fees for outpatient visits, language problems, long waiting times, unfriendly personnel or the consultation was refused because of lack of insurance or legal documentation.

Discussion

Our study of a group of migrant workers in an area of autochthonous transmission of malaria in southern Greece has shown that knowledge about malaria transmission was high. Use of mosquito control measures was higher in persons who had better housing conditions, those who had concerns about becoming ill with malaria and those who would seek treatment from ACD teams. We identified certain knowledge gaps and misconceptions about malaria among the migrants, especially recognition of malaria symptoms and prevention methods, indicating areas where health education activities might be improved. Although most of the migrants would seek fever (›38 °C) treatment within 24 hours, access to local healthcare in Evrotas was a challenge for this population.

Despite extensive fever-screening activities in Evrotas since 2011, less than half of the respondents knew that fever was the main malaria symptom. Those who were aware of this had a higher level of education, a finding that is consistent with other studies [17-19]. The early recognition of malaria symptoms is key to early healthcare-seeking behaviour. It is possible that some migrants from Pakistan may have been confused by the use of the word ‘bukhar’ for fever, as in Urdu the same word is used for malaria (‘maleria bukhar’). Interchangeable use of the same word for fever and malaria in Africa has been described and poses challenges in the interpretation of the results of malaria knowledge studies [20,21]. The level of knowledge of malaria symptoms in Evrotas was similar to that in a

Table 4

Factors associated with malaria knowledge and mosquito protective methods among migrant study participants in Evrotas, Greece, June 2013 (n = 130)[a]

Knowledge and mosquito protective methods		Number/Total (%)	aPR[b]	95% CI
Knowledge of transmission: ≥ 1 mode[c]				
Age group in years	18–24	38/48 (79)	ref	–
	25–34	49/62 (79)	1.0	0.9–1.2
	≥ 35	20/20 (100)	1.3	1.1–1.6
Length of education in years	0	11/18 (61)	ref	–
	1–6	42/52 (81)	1.3	0.9–1.9
	7–12	54/60 (90)	1.4	1.0–2.0
Seeking fever (>38 °C) treatment from ACD teams	No	37/53 (70)	ref	–
	Yes	70/77 (91)	1.3	1.1–1.5
Knowledge of symptoms: fever				
Length of education in years	0	3/18 (17)	ref	–
	1–6	20/52 (38)	2.3	0.8–6.5
	7–12	33/60 (55)	3.2	1.2–9.0
Malaria in country of origin	Yes/Don't know	45/114 (39)	ref	–
	No	11/16 (69)	1.7	1.1–2.5
Knowledge of malaria prevention: > 1 method[d]				
Age group in years	18–24	10/48 (21)	ref	–
	25–34	24/62 (39)	1.8	1.0–3.2
	≥ 35	9/20 (45)	1.5	0.7–3.1
Country of origin	Other[e]	1/10 (10)	ref	–
	Pakistan	42/120 (35)	3.9	1.1–14.5
Length of education in years	0	5/18 (28)	ref	–
	1–6	10/52 (19)	0.8	0.3–2.0
	7–12	28/60 (47)	1.7	0.8–3.7
Time lived in Evrotas in years	< 1	10/25 (40)	ref	–
	1–2	7/33 (21)	0.6	0.3–1.3
	3–5	15/56 (27)	0.6	0.3–1.0
	> 5	11/16 (69)	1.3	0.8–2.2
Mosquito protection practices: > 2 methods[f] (n = 129)				
Type of residence	House/flat	55/68 (81)	ref	–
	Warehouse/shack	40/62 (65)	0.8	0.6–0.9
Worried about becoming seriously ill (themselves or family/household member) with malaria	No	27/50 (54)	ref	–
	Yes	68/79 (86)	1.5	1.1–2.0
Seeking fever (>38 °C) treatment in ACD teams	No	31/53 (58)	ref	–
	Yes	64/77 (83)	1.3	1.0–1.6
Marital status	Single	70/99 (71)	ref	–
	Ever married	25/31 (81)	1.2	1.0–1.4

ACD: active case detection; aPR: adjusted prevalence ratio; CI: confidence interval; ref: reference value.

[a] Unless otherwise specified.

[b] Calculated using the vce(cluster) option in STATA to allow for clustering of participants sharing the same residence.

[c] Mosquitoes, blood transfusion, pregnant mother to child.

[d] Long-lasting insecticide-treated bednets, full-length clothing or topical insect repellents, mosquito vaporising mats/liquids and/or aerosol, mosquito coils, avoiding mosquitoes.

[e] Afghanistan, Bangladesh, Morocco.

[f] Full-length clothing or topical insect repellents, indoor insecticides (mosquito vaporising mats, liquid vaporisers, spraying solutions/aerosols), fan or air-conditioning, mosquito screens, mosquito coils.

Table 5

Treatment-seeking behaviour and access to healthcare among migrant study participants in Evrotas, Greece, June 2013 (n = 130)[a]

Treatment-seeking behaviour and healthcare access	Number (%)	95% CI
Type of healthcare facility sought following onset of fever (>38 °C)		
ACD teams	77 (59)	50–68
Hospital	28 (22)	15–30
Health centre	11 (8)	4–15
Pharmacy	9 (7)	3–13
Other	5 (4)	1–9
Time to presentation at healthcare facility following onset of fever (>38 °C) (n = 125)		
1 day	121 (97)	92–99
>1 day	4 (3)	1–8
Usual mode of transportation to healthcare facility		
Private car[b]	54 (42)	33–51
Taxi	47 (36)	28–45
Public transport	17 (13)	8–20
On foot	8 (6)	3–12
Other	4 (3)	1–8
Sought medical care in the previous year (for any reason)		
Yes	16 (12)	7–19
Any previous perceived difficulty while seeking medical care (n = 16)		
Yes	9 (56)	30–80

ACD: active case detection; CI: confidence interval.

[a] Unless otherwise specified.

[b] Of a friend, colleague, employer.

survey conducted in 2009 among Cambodian migrants who had recently entered Thailand [22]. In contrast, higher awareness of the classical symptoms of malaria has been reported in populations in malaria-endemic areas, such as Bangladesh and Ethiopia [23,24]. The above suggests that continuous and long-term health education is required to raise the community's awareness about the clinical manifestations of the disease.

As in other studies in populations in malaria-prone areas [17,18,23,25], knowledge that mosquitoes transmit malaria was high among the participants, especially among those who were educated. In addition, awareness about malaria transmission was associated with older age and seeking treatment from the ACD teams, possibly reflecting better access to health education activities provided by those teams. However, participants also had misconceptions about malaria transmission and prevention, including mentioning garbage or presence of dirty water. Knowledge of both correct and incorrect modes of malaria transmission has been widely reported in rural communities [18,23,24] and may influence the prevention activities that individuals choose, including non-beneficial practices, although our study findings cannot confirm this. Improving the community's understanding of malaria transmission can greatly contribute to targeted prevention and control efforts [17]. In Evrotas, the level of awareness of malaria prevention methods was associated with the country of origin. In these countries, vector control programmes may differ in type and coverage and may also target other vector-borne diseases, such as dengue [1,26]. Such differences may create confusion about diseases, vectors and protection methods in the migrant population. This may explain the low recognition of IRS as a malaria prevention method in our study, despite its high acceptance among this community.

Most participants were aware of the existence of malaria treatment. In addition, the most commonly mentioned sources of treatment were the ACD teams. This timely health-seeking behaviour may be attributed to the continuous door-to-door ACD activities and the use of a dedicated telephone line for queries, providing easier access to information and treatment. However, in Evrotas, only a small proportion would seek care at the public local health centre, which might suggest difficulties in accessing this particular healthcare facility. Given the difficult economic situation in Greece since 2010, the consequent budget cuts in healthcare and chronic insufficiencies in the system [27], this finding might also reflect limited coverage or provision of services in the centre, quality issues or general health inequities between the local and migrant population [28]. Strengthening services and improving accessibility for migrant workers to the only public local healthcare facility in the area may play an important role in early diagnosis and treatment.

Most participants reported adopting multiple mosquito protection measures, mainly bednets, coils and indoor insecticides. However, only a few reported that these measures can prevent malaria. This suggests that participants may have associated these measures with mosquito biting nuisance rather than malaria prevention and control. In addition, knowledge of malaria was not associated with MPPs in this study. This pattern was also reported in Bangladesh in 2011 [25]. In our study, ACD teams provided the bednets (LLINs), coils and indoor vaporising insecticides free of charge and monitored their use. This may explain the high acceptance and reported use of these measures in our study, although the use of bednets was later shown to decline over time, mainly due to heat and the use of electric fans in the room used for sleeping [11]. In addition, the distribution of free-of-charge LLINs by the ACD teams was an essential measure, as LLINs are not licenced

and therefore are not commercially available in Greece. Greek authorities need to make LLINs easily available, given that their use is one of the most effective measures against malaria [1,29] and is usually associated with a high financial burden [30,31] which hinders their use, especially among individuals of low socio-economic status [18,23].

Poor and inappropriate housing conditions were associated with insufficient MPPs in our study. Most residences were overcrowded, not designed for human housing and lacked window and door mosquito screens. Other studies have shown that in areas with low to moderate transmission, improved house design, including mosquito screens, decreases malaria vector and other mosquito densities and reduces malaria transmission, while crowding results in the opposite [32,33]. Moreover, mosquito screens for houses are a sustainable, long-lasting, relatively inexpensive and acceptable protection measure used by communities elsewhere, but also traditionally in Greece, although mainly to decrease mosquito nuisance [15,34,35].

Our study has a number of limitations. It focused only on the migrant community and did not assess malaria knowledge and prevention practices among the Greek residents, among whom locally acquired malaria cases occurred [12]. We considered the migrant community more vulnerable to malaria, because of their low socioeconomic status, poor living conditions and limited access to healthcare. In addition, the study included only men: there were no women and children in the migrant population in Evrotas. Data quality may have been affected by some variability in verbal translation of the questionnaire or cross-cultural variability in the respondents' comprehension of the survey questions. Over-reporting of desirable MPPs may have occurred, as it was not possible to verify the practices through direct observation. Our study did not have enough power to detect reasons for the difficulty in accessing healthcare. Although we took into account clustering of participants sharing the same residence in the analysis, we could not capture the frequent movement of people between residences and therefore the variation in their MPP use and habits.

Despite extensive public health interventions, following the re-emergence of *P. vivax* malaria in Evrotas in 2009 [36], the level of malaria awareness among migrants in our study was suboptimal, access to the main local healthcare facility was limited and poor housing conditions hindered effective mosquito protection. We consider that the public health authorities need to continue and reinforce ACD, distribution of mosquito protection items and health education activities, tailored to the education level and culture of migrants, to achieve better awareness of malaria and protection against mosquitoes among this population. Furthermore, health authorities need to identify and overcome barriers to access to the local healthcare facility, to ensure early diagnosis and treatment of malaria.

To achieve effective mosquito protection, the housing conditions of migrants need to improve. Activities to improve housing of migrants, such as availability of residencies built for housing or improvement of existing ones and installation of mosquito screens, with the involvement of Greek house-owners, could be incorporated into a comprehensive vector control programme in the area. Finally, a similar survey among the local Greek population may contribute to the design of evidence-based public health interventions and the expansion of effective malaria control activities to this population, leading to a comprehensive malaria control programme, in order to decrease the risk of emergence of new cases and the re-establishment of malaria in Greece.

The findings of our study might also be useful for other European national public health authorities due to the increased influx of migrants and refugees from malaria-endemic countries into Europe [37], where favourable ecological conditions for malaria transmission [4,5] and potential malaria vectors are present [38,39].

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Conflict of interest

IE participated in the field teams in 2012. MT was the coordinator of the HCDCP interventions. The other members of the study team declare no conflict of interest.

Authors' contributions

IE performed the data analysis and drafted the manuscript. IE and KD designed the study. IE, KD, AL and TP developed the questionnaire; IE and YT administered the questionnaires; IE and MT organised the field activities. All authors contributed to the development of the manuscript and approved the final version.

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Incidence and seroprevalence of tularaemia in Finland, 1995 to 2013: regional epidemics with cyclic pattern

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We studied the incidence of reported tularaemia by year and region and the prevalence of antibodies against *Francisella tularensis* in the adult general population in Finland. Moreover, we assessed the correlation between vole population cycles and human tularaemia outbreaks. The seroprevalence study made use of serum samples from a nationwide population-based health survey (Health 2000). The samples of 1,045 randomly selected persons, representative for the Finnish population in each region, were screened with an enzyme-linked immunosorbent assay (ELISA) for the presence of IgG antibodies against *F. tularensis*, and positive results were further confirmed by immunoblotting. A serological response to *F. tularensis* was found in 2% (95% confidence interval: 1.1–3.5) of the population. Incidence and seroprevalence were highest in the same areas, and vole population peaks clearly preceded tularaemia outbreaks one year later.

Introduction

Tularaemia is a zoonotic disease caused by the intracellular bacterium *Francisella tularensis* [1,2]. The disease is caused primarily by two of four subspecies: the highly virulent type A strain *F. tularensis* subsp. *tularensis* which is almost completely restricted to North America, and the less virulent type B strain *F. tularensis* subsp. *holarctica*, which occurs in many regions of the northern hemisphere, including Finland [1,2].

Recurrent outbreaks with hundreds of cases are reported in Finland and Sweden [3,4]; in other European countries, the disease is rare, but many countries report sporadic outbreaks [5-7]. Geographically, the disease shows a focal distribution [8]. The pathogen is most likely to persist in the local environment but seems to cause epidemics only when the ecological conditions are favourable for an active infectious cycle [9]. Thus, appropriate reservoirs, amplifiers, vectors and suitable climatic conditions are needed for an outbreak of human tularaemia [9]. Tularaemia is typically associated with outdoor activities, and farmers and hunters are at particular risk for infection [3-7,10]. In Finland and Sweden, the disease is typically mosquito-transmitted and most cases are reported during August and September in connection with the occurrence of late summer mosquito species [3,4], whereas in Norway, the disease occurs in autumn and winter and the most common source is drinking water contaminated by rodents [11]. Outbreaks in Norway and Kosovo* have been linked to high rodent densities [11-14].

Airborne outbreaks, mainly associated with activities that can generate aerosols, such as farming, gardening or hunting, occur occasionally in all endemic countries and are very local [5-7,15].

Human tularaemia typically starts with non-specific influenza-like symptoms [16]. Other clinical manifestations depend mainly on the route of transmission, and the disease severity depends on the infecting subspecies and strain [1,16]. After infection, antibodies against *F. tularensis* rise slowly but are detectable for several years [17]. Here we report a study conducted in Finland, aiming to determine the incidence of tularaemia and the prevalence of *F. tularensis* antibodies in the population in 2000 and 2001, to compare the seroprevalence rates with the number of reported cases for the period 1995 to 2013, and to assess for the same period the role of vole population cycles in the temporal and spatial pattern of human tularaemia outbreaks.

Methods

National laboratory-based surveillance

The Finnish national healthcare system is organised in 20 geographically and administratively distinct

healthcare districts. Laboratory-confirmed tularaemia has been a notifiable disease by the diagnosing laboratory since 1995, and clinical microbiology laboratories report cases directly to the National Infectious Disease Register (NIDR) which is maintained by the National Institute for Health and Welfare (THL). Diagnostic criteria for reporting include (i) isolation of *F. tularensis* in a clinical specimen, (ii) a more than four-fold rise in serum antibody titre or a single antibody titre of ›160 when using an agglutination assay or (iii) the presence of specific IgM and IgG antibodies in the serum when an enzyme-linked immunosorbent assay (ELISA) is used. With each notification, the following information is given: date and type of specimen, date of birth, sex, place of treatment and place of residence.

Human serum samples and background health information

Serum samples were collected in a multidisciplinary epidemiological health survey, the Health 2000 Study, carried out in the years 2000–01 in Finland [18]. Detailed study methods have been described elsewhere [18]. Sera from adults were collected in 80 different areas covering most of the country. For the current study, serum samples from 1,045 randomly selected persons were included. For geographical coverage, all 20 healthcare districts were represented with 50 samples, except for Central Ostrobothnia where only 45 samples were available and the capital district of Helsinki and Uusimaa, which was represented with 100 samples, reflecting a union of two formerly separate districts. An extensive health interview and health examination had been done for all participants in the original study. In this study, we especially focused our analyses on common symptoms and medical conditions, living environment, occupation, leisure activities, physical condition, smoking and alcohol use and demographic factors. The Health 2000 Survey was approved by the Ethical Committee for Research in Epidemiology and Public Health at the Hospital District of Helsinki and Uusimaa.

Sera from seven patients who had laboratory-confirmed tularaemia (diagnosed at the Department of Medical Microbiology and Immunology, University of Turku) one to 16 years before serum collection, were used as positive control sera.

Serological testing

ELISA

All serum samples were screened for *F. tularensis* antibodies by ELISA as previously described [17], with whole bacterium lysate prepared from *F. tularensis* live vaccine strain (LVS) as the antigen. Serum samples were tested at a dilution of 1:100. Absorbances (OD405) were measured with BEP III apparatus (Siemens Healthcare Diagnostics Products GmbH, Erlangen, Germany). Results were expressed as arbitrary enzyme-immunosorbent units (EIU) on the scale from zero to 100 units. Conventional receiver operating characteristic ROC) analysis to determine the cut-off of the ELISA could not be performed because the true tularaemia antibody status (defined by a gold standard) of our samples or for the ELISA test in general was not known. This is why a confirmatory Western blot was employed.

Western blot

Western blotting was conducted as earlier described [19]. Positive sera showed a typical lipopolysaccharide (LPS) band pattern at a dilution of 1:100. Samples were screened from the highest EIU (83.7) in ELISA down to EIU 26.8 (including 55 serosurvey samples and seven positive control samples). The lowest ELISA EIU value that was still positive in Western Blot was 28.5. The following 10 samples were WB negative and screening was stopped due to low EIU values of the remaining samples. Only samples positive in both EIA and WB were considered positive.

Vole data

Data on small mammals were collected biannually in ca 30 locations around the country by the Natural Resources Institute, as described by previously [20]. The trapped mammals were predominantly voles, which dominate the rodent and other small mammal fauna in Finland [20,21]. Only vole populations show cyclic fluctuations. In addition, three wild murine (i.e. non-vole rodent) species inhabit Finland, but they are restricted to the southern half of the country, and usually occur at low densities with seasonal, but not cyclic dynamics. As such, the national monitoring programme of vole populations was planned to produce comprehensive data on the spatiotemporal dynamics of vole population fluctuations around the whole country. By and large, vole populations in Finland fluctuate pronouncedly in cycles of three to four years [20]. For the purposes of this study, the vole population data (density indices; voles per 100 trap nights) were qualitatively classified into three distinct cyclic phases, each corresponding to a year: population peak years, population decline years and population increase years.

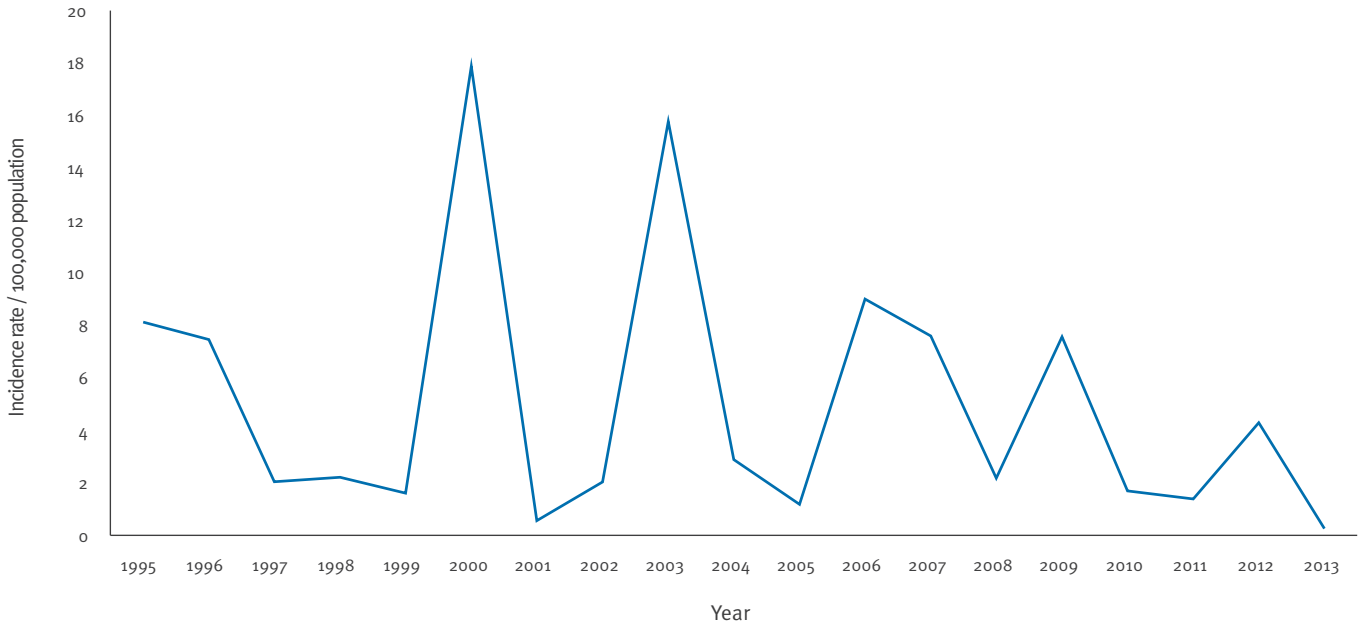
Statistical analyses

Serological survey

Statistical analyses were performed using IBM SPSS Statistics version 22 and the programme R maptools package was used to construct the maps. Our dataset was a subsample selected from the Health 2000 survey main study by stratified simple sampling of size 50 samples per hospital district, and the original sampling weights were reweighted to match the population size of a given hospital district at year 2000. Of the very comprehensive background information, we especially focused our analyses on common symptoms and medical conditions, living environment, occupation, leisure activities, physical condition, smoking and alcohol use and demographic factors. Univariate chi-square tests were computed taking into account the sampling design, its strata and clusters and the sampling weights for the test of independence of the categorical

Figure 1

Incidence rates of laboratory-confirmed tularaemia infections, Finland, 1995–2013



variables. The population sizes and prevalences were estimated accordingly, taking into account sampling design. Logistic regression was used to model the relationship between seroprevalence odds and explanatory variables. The incidence ratio of notified/total tularaemia cases was calculated assuming that the population at risk and the prevalence pool were stationary. Applying a formula for a time stationary situation, the (sero)prevalence odds of tularaemia $p / (1 - p)$, the mean total incidence I and the mean duration of the seroprevalence D are related as $p / (1 - p) = I \times D$, with D equal to the estimated average residual life time after the mean age of acquiring tularaemia.

Vole and surveillance data analysis

The association between tularaemia outbreaks and phase of vole cycle was analysed with Poisson regression. Poisson model calculations were done by hospital district. The effect of vole cyclic phase factor was assumed independent of the year. Possible overdispersion was corrected by Pearson chi-squared scale parameter method, and possible autocorrelations of person residuals from the model were checked by autocorrelation plots (ACF plots).

Results

National laboratory-based surveillance

From 1995 to 2013, 5,086 notifications of laboratory-confirmed tularaemia cases were reported to the NIDR. The annual number of notified cases ranged from 14 to 926. The average annual incidence was 5.1/100,000 population and the highest incidence (18/100,000 population) was recorded during the year of the major epidemic in 2000 (Figure 1).

Rates were typically highest in the health districts of Northern and Southern Ostrobothnia and Central Finland. The mean age of the cases was 45 years (range: 0–93 years) and 55% were male. The annual variation in reported cases in the three healthcare districts with particularly high incidence is shown in Figure 2. Typically, epidemics occurred in different districts every third or fourth year.

Epidemics were strongly seasonal, with the majority of cases diagnosed during summer and early autumn (Figure 3).

Figure 4 shows the incidence rates by healthcare district in 1995–2000, five to six years before sampling for the seroprevalence study.

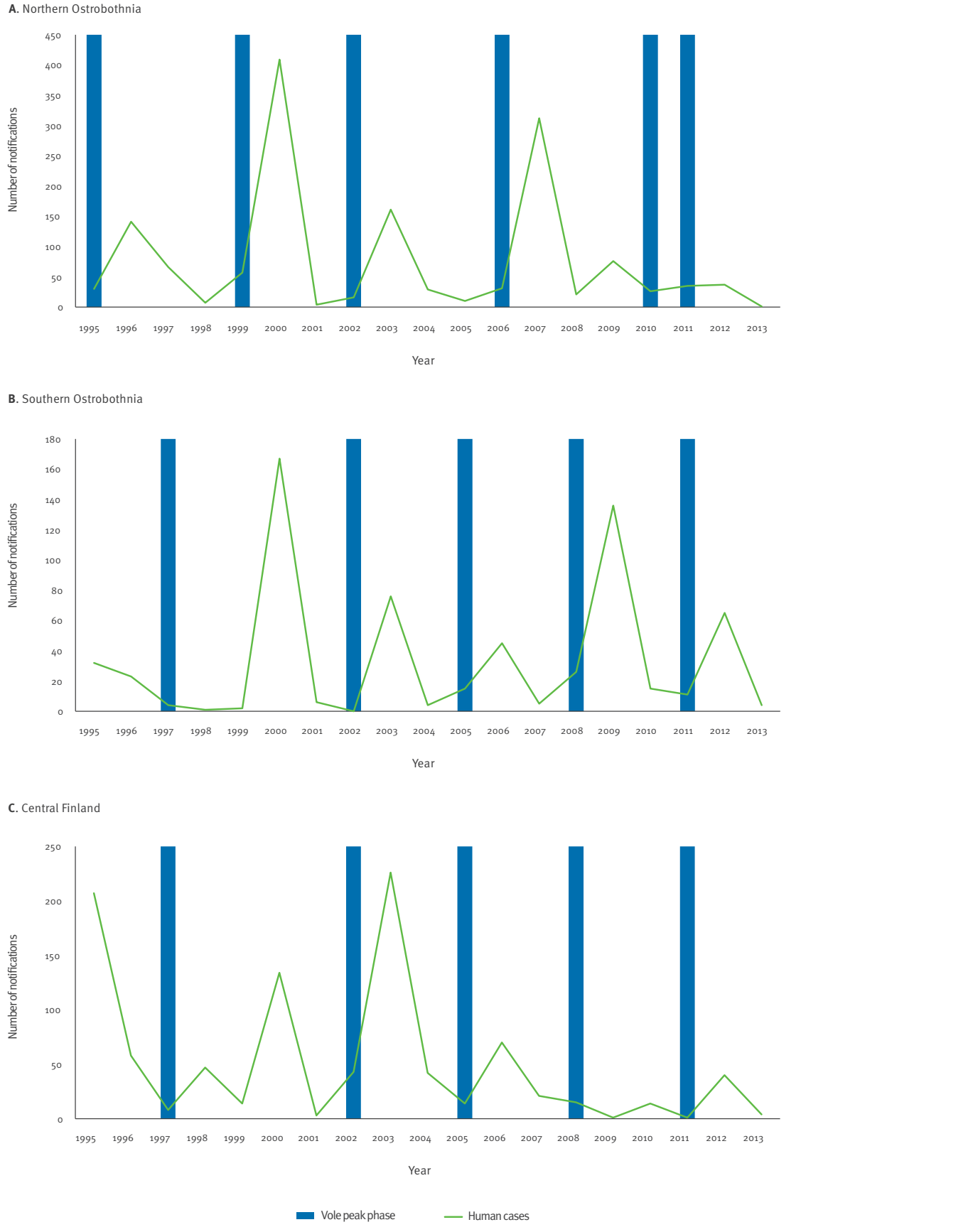
Human seroprevalence study

Our study sampling of 1,045 persons comprised 46% men and 54% women. Their mean age was 53 years (range: 30–92 years) and for geographical coverage, they represented all 20 healthcare districts. We found 16 positive samples (1.5%), which after adjustment with survey weights gave an estimated overall *F. tularensis* antibody prevalence of 2.0% (95% confidence interval: 1.1–3.5) on population level. The distribution of EIU values in our study and control samples is shown in Figure 5; the positive control sera showed EIU values between 64.3 and 93.0.

Five participants had an EIU value › 50 in addition to a typical LPS band pattern in the Western Blot (Figure 6). One of them reported being hospitalised because of tularaemia during the period from 1995 to 2000 (precise time and duration of hospitalisation not available).

Figure 2

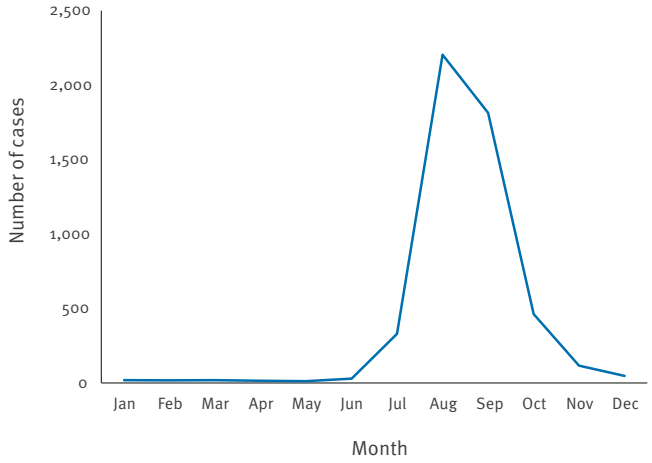
Number of laboratory-confirmed tularaemia cases and timing of vole population peaks, by district, Finland, 1995–2013 ((n = 3,011)



B and C: The year 1999 was not a typical wide-spread peak year, but showed patchy local peaks. For details see text.

Figure 3

Cumulative number of laboratory-confirmed tularaemia cases by month, Finland, 1995–2013 (n = 5,086)



Geographically, the seroprevalence was highest in Northern Ostrobothnia (Figure 7).

No significant differences in age or sex distribution were found between the seropositive and seronegative group. The mean age of seropositive persons in our study was 55 years and 50% of them were males. No single risk factor was significantly associated with seropositivity. *F. tularensis* seropositivity in general was higher in persons with lower educational level (likelihood ratio: 0.048), but no single occupation was related to seropositivity. The ratio of notified vs total (estimated based on the observed seroprevalence) *F. tularensis* infections was 1/10.5.

Vole cycles and their association with human tularaemia

The temporal occurrence of vole peak years clearly predicted human tularaemia outbreaks. Outbreaks mostly occurred during the years immediately after the vole peak years, i.e. in decline years (Figure 2). The tularaemia incidence in vole decline years was on average ca six times higher than in rodent increase years (Table). In 10 of the 20 hospital districts, the vole decline years had a significantly higher tularaemia incidence (incidence rate ratio›1) than the non-decline years. In the remaining districts, tularaemia is rare and the impact of vole cycles could not be observed (the regression model could not be fitted to those particular hospital districts).

Discussion

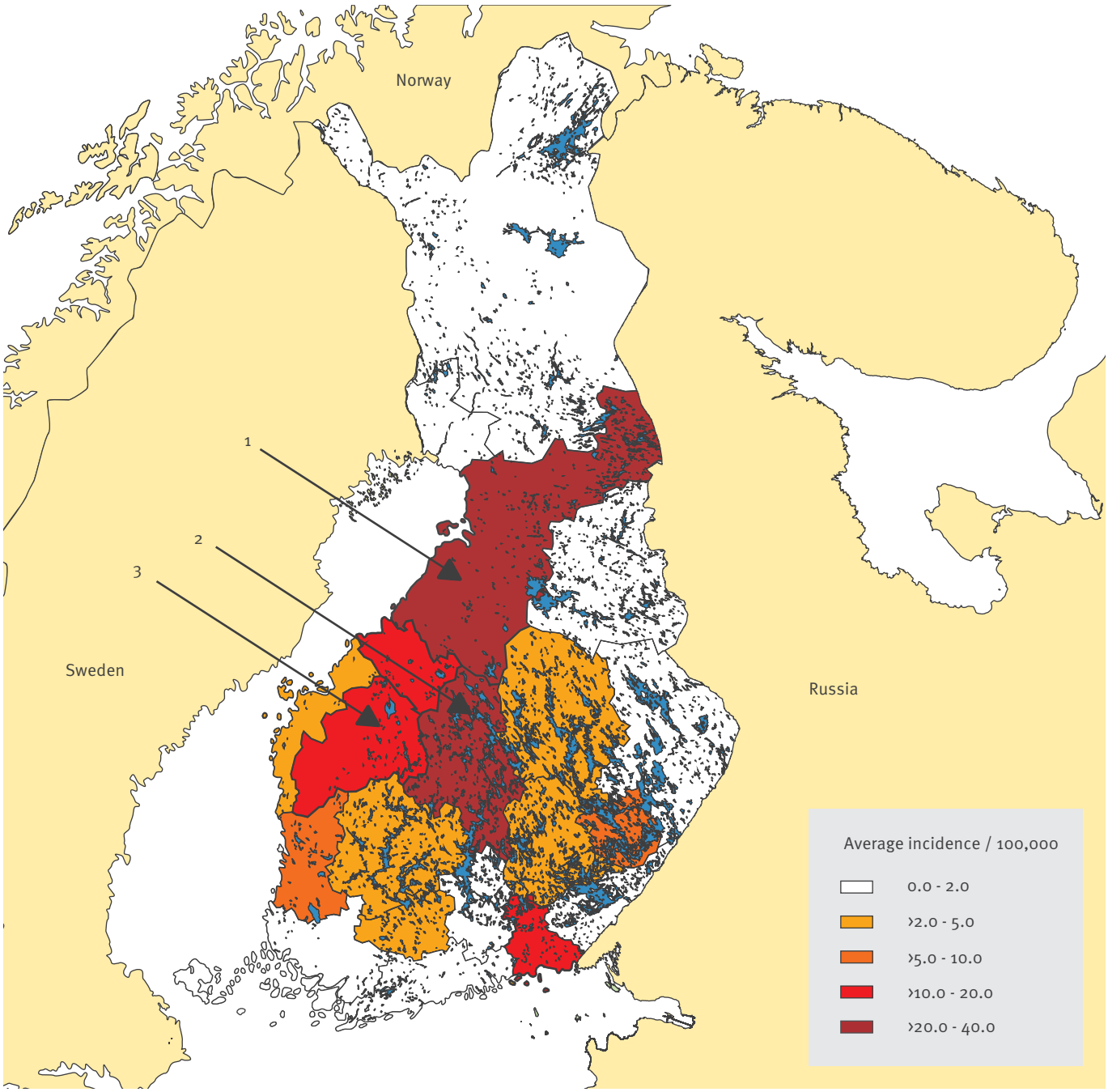
The epidemiology of tularaemia in Finland is characterised by recurrent regional and seasonal outbreaks occurring in short cycles of typically three to four years [3,21]. In the long term, the incidence is highest in Northern Ostrobothnia as shown also in the current study. Outbreaks involving hundreds of cases occur every three to five years in some areas such as Northern and Southern Ostrobothnia and Central Finland [3]. In other areas, outbreaks of this magnitude are rare. The majority of cases are reported in August and September, during or right after the late summer mosquito season. It has been shown that the *F. tularensis* carriage rate in mosquitoes in Sweden increases with declining mosquito populations in late summer and early autumn [22], which may explain the high number of notifications in August and September. Also other important mosquito-transmitted diseases in Finland (such as Pogosta disease caused by Sindbis virus) are transmitted particularly by the late summer mosquito species [23].

On the other hand, there is generally a time lag of several weeks between onset of symptoms and laboratory confirmation of tularaemia [3]. Presumably, most cases notified in August and September acquired the infection some weeks earlier. In our serological survey, we found an overall *F. tularensis* antibody prevalence of 2% on population level after adjustment with survey weights (1.5% among our study participants). Seroprevalence was highest in Northern Ostrobothnia, which is in line with the number of notifications to the NIDR. The observed seroprevalence was comparable to results from Germany where seroprevalences up to 2.3% have been found [24,25]. In Martha's Vineyard, Massachusetts, where only landscapers were tested, 9.1% of the studied population was seropositive for *F. tularensis* [10]. In rural Azerbaijan [26] and Iran [27], seroprevalences significantly higher than in our study were found. In those studies, rodent exposure [26] and hunting [27] were shown to clearly increase the risk of tularaemia. These are well known risk factors for tularaemia.

It was surprising that the *F. tularensis* antibody prevalence was so low in Finland the European Union Member State with the highest reported tularaemia incidence [21,24]. On the other hand, low incidence but relatively high seroprevalence in other countries probably indicates underdiagnosing. This could be explained by the different clinical picture: in Finland, tularaemia is mainly mosquito-borne and manifests as the ulceroglandular form which is easy to diagnose based on the typical symptoms. In central and southern Europe however, the most common is the typhoidal form which is very difficult to diagnose because similar symptoms can have other causes and because the awareness among clinicians is low. Also in Finland, a certain proportion of infections are not notified, which is not surprising, especially when taking into account the challenging laboratory diagnosis of tularaemia. General practitioners in endemic areas probably often treat the disease based on a clinical diagnosis only and diagnostic laboratory tests are not requested. Some patients may also recover after an influenza-like febrile illness without seeking medical attention [10]. On the other hand, the low prevalence of antibodies against *F. tularensis* indicates that tularaemia infection is not very common and is in most cases associated with distinct acute clinical symptoms [2,16]. Based on the

Figure 4

Incidence of laboratory-confirmed *Francisella tularensis* infections reported to the National Infectious Disease Register, by healthcare district, Finland 1995–2000



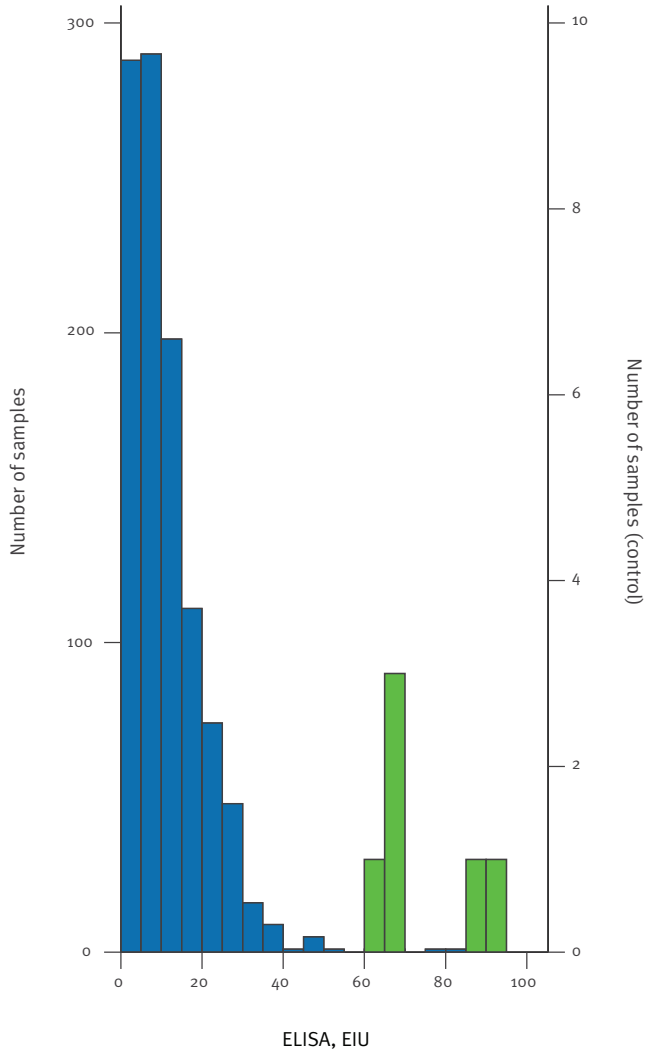
Incidences per 100,000 population.
Healthcare districts: 1. Northern Ostrobothnia; 2. Central Finland; 3. Southern Ostrobothnia.

notifications and serocoprevalence, we estimate that ca one in 10 (9.5%) *F. tularensis* infections are notified.

We have shown a correlation between vole population dynamics and human tularaemia outbreaks. Human tularaemia outbreaks typically occur during the vole decline phase a year after the vole population peak. In 2000, human tularaemia outbreaks occurred throughout Finland, including Southern Ostrobothnia and Central Finland, although characteristics of a widespread vole peak in the preceding year were only met in the northern part of the country. Vole peaks occurred in a patchy fashion in 1999, and particularly in Southern Ostrobothnia, our long-term monitoring sites representing the large hospital districts may not have coincided with the localised vole peak areas [28]. We did not include 1999 as a peak year in our statistics, but

Figure 5

Distribution of ELISA results, tularaemia seroprevalence study, Finland, 2000–01 (n = 1,045)

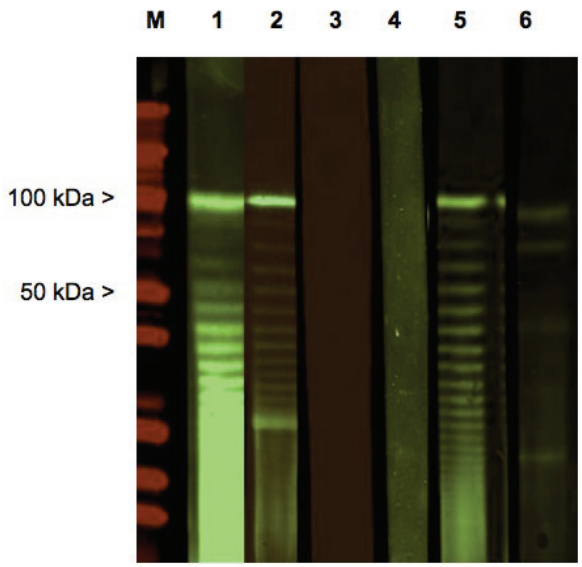


ELISA: enzyme-linked immunosorbent assay; EIU: enzyme-immunosorbent units.

Blue bars: study samples; green bars: positive control samples.

Figure 6

Western blot of representative positive and negative sera, tularaemia seroprevalence study, Finland, 2000–01



Lanes 1, 2 and 5: samples considered *Fransicella tularensis* antibody-positive according to a typical ladder pattern; lanes 3 and 4: negative samples from the serosurvey panel; lane 6: a sample considered as an unspecific reaction. M: size marker.

the association between the cyclic phase and human tularaemia was nevertheless significant.

We have previously shown that voles can serve as amplification hosts for *F. tularensis* [29]. Large vole populations allow the bacteria to replicate intensively. During a peak phase, live rodents can shed the bacteria into the environment, and in the decline phase, infected dead rodents release large amounts of *F. tularensis* into the environment, including breeding sites of mosquitoes [29]. Mosquito larvae can take up the bacteria [22] and once they become adults, spread the bacteria to susceptible hosts that act as local amplifiers. This provides a likely explanation for the suggested association between vole cycles and human tularaemia incidence [29]. However, the variation in the magnitude and the locality of human tularaemia outbreaks warrant further analyses. High vole density is probably one, but not the only prerequisite for tularaemia outbreaks. The local ecological factors crucial for disease outbreaks are still not known very well. It has been shown that the presence of certain aquatic amoebae enhances the multiplication of *F. tularensis* and that its infection process in amoebae resembles that in macrophages [30]. Possibly vole carcasses contaminate natural waters, amoebae support the local persistence of *F. tularensis* in these waters, mosquito larvae feed on these protozoa and thus get infected. Weather conditions influence the amount of mosquitoes, which impacts on the transmission to humans and thus the amplitude of the outbreak [31].

Conclusion

In summary, human tularaemia in Finland is focal, and most of the cases occur in a few districts. This warrants landscape ecological analyses [8,32]. The answer may not be simple because the endemic provinces differ considerably in topography. The seasonality of tularaemia occurrence strongly indicates a major role of mosquitoes in disease spread. The multiannual cyclic pattern of the epidemics is associated with vole density cycles, with vole peak years preceding epidemic years. The interactions between voles, mosquitoes and *F. tularensis* need still further studies, as well as the discrepancy between the relatively low seroprevalence and considerable incidence of tularaemia.

Table

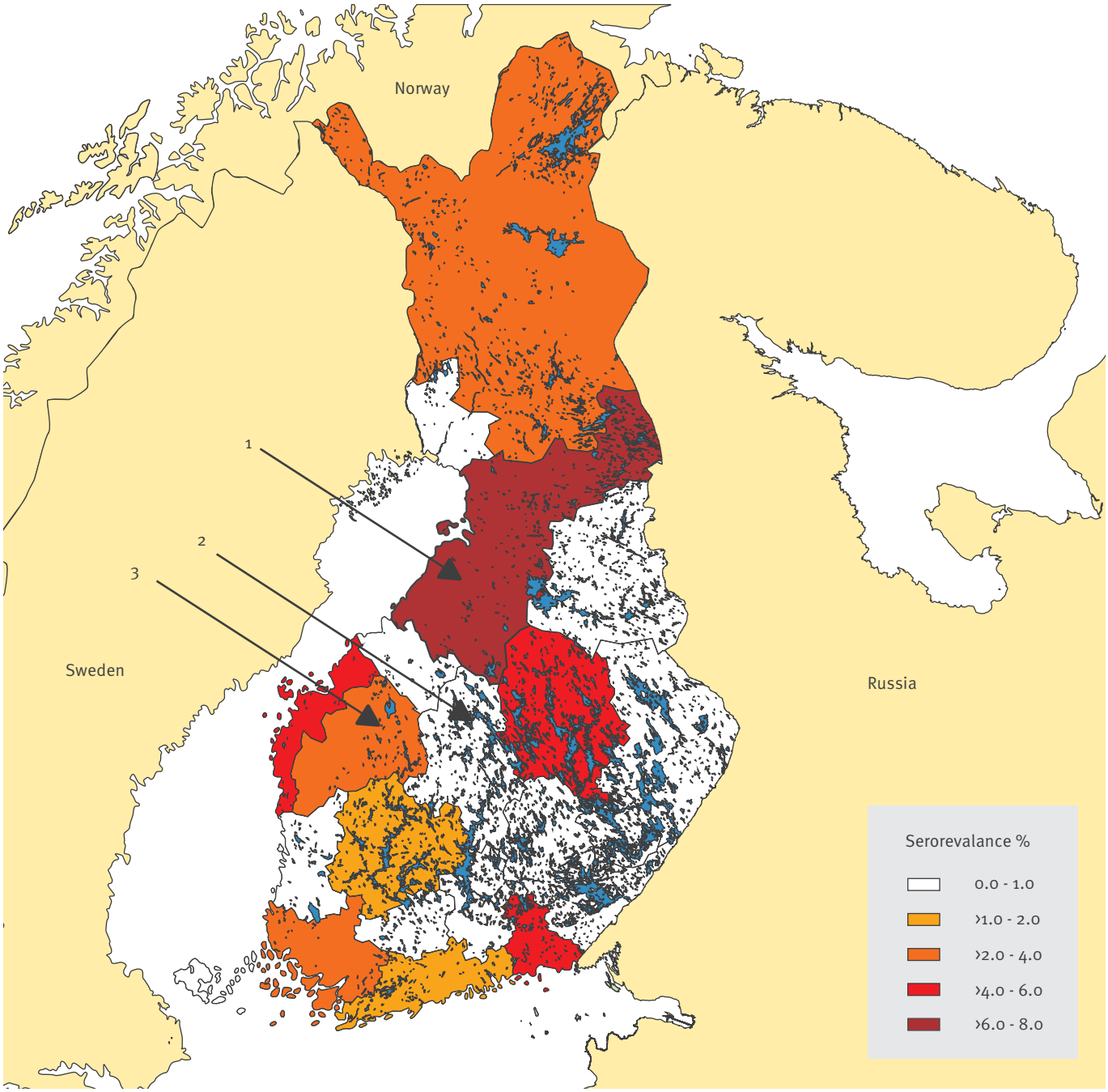
Incidence of tularaemia in different hospital districts and its relation to rodent cycles, Finland, 1995–2013

Hospital district	Vole cycle phase	Cases (n)	Population	Incidence	IRR	95% Wald CI for IRR		p value
Southern Karelia	non decline years	6	1,417,302	0.42	3.14	0.98	10.01	0.05
	decline years	12	902,885	1.33				
Southern Ostrobothnia	non decline years	111	2,164,737	5.13	7.37	2.83	19.22	<0.001
	decline years	522	1,381,053	37.80				
Southern Savo	non decline years	15	1,149,203	1.31	1.46	0.47	4.53	0.51
	decline years	14	732,287	1.91				
Helsinki and Uusimaa	non decline years	100	15,789,947	0.63	4.29	2.15	8.55	<0.001
	decline years	272	10,020,284	2.71				
Eastern Savo	non decline years	2	702,300	0.28	18.08	0.61	536.11	0.09
	decline years	23	446,602	5.15				
Kainuu	non decline years	3	1,245,547	0.24	3.28	0.63	17.01	0.16
	decline years	2	253,206	0.79				
Kanta-Häme	non decline years	9	1,856,789	0.48	5.07	1.90	13.49	<0.001
	decline years	29	1,181,058	2.46				
Central Ostrobothnia	non decline years	204	931,902	21.89	1.22	0.16	9.36	0.85
	decline years	124	462,955	26.78				
Central Finland	non decline years	233	2,934,836	7.94	4.89	1.95	12.26	<0.001
	decline years	725	1,865,762	38.86				
Kymenlaakso	non decline years	78	1,991,154	3.92	2.58	0.86	7.69	0.09
	decline years	128	1,267,302	10.10				
Lapland	non decline years	15	2,074,033	0.72	2.34	0.41	13.32	0.34
	decline years	2	118,189	1.69				
Western Ostrobothnia	non decline years	20	1,149,854	1.74	0.00	0.00	0.00	<0.001
	decline years	0	64,655	0.00				
Päijänne Tavastia	non decline years	17	2,293,263	0.74	2.03	0.48	8.63	0.34
	decline years	22	1,459,719	1.51				
Pirkanmaa	non decline years	104	5,098,236	2.04	3.60	0.88	14.84	0.08
	decline years	238	3,237,195	7.35				
Northern Karelia	non decline years	11	1,885,503	0.58	0.71	0.19	2.70	0.62
	decline years	5	1,203,895	0.42				
Northern Ostrobothnia	non decline years	408	4,931,836	8.27	6.75	3.31	13.75	<0.001
	decline years	1,060	1,898,901	55.82				
Northern Savo	non decline years	20	2,764,993	0.72	4.95	2.04	12.00	<0.001
	decline years	63	1,760,755	3.58				
Satakunta	non decline years	54	2,519,350	2.14	10.77	2.75	42.20	<0.001
	decline years	371	1,607,604	23.08				
Vaasa	non decline years	29	1,996,883	1.45	7.58	2.74	20.99	<0.001
	decline years	110	999,243	11.01				
South-west Finland	non decline years	11	5,038,620	0.22	6.87	2.63	17.95	<0.001
	decline years	48	3,201,242	1.50				
Whole country	non decline years	1,450	59,936,288	2.42	4.57	4.31	4.86	<0.001
	decline years	3,770	34,064,792	11.07				

CI: confidence interval; IRR: incidence rate ratio.

Figure 7

Estimated seroprevalence (prevalence of Western blot positive samples), by healthcare district, tularaemia seroprevalence study, Finland, 2000–01 (n = 1,045)



Healthcare districts: 1. Northern Ostrobothnia; 2. Central Finland; 3. Southern Ostrobothnia.

*Note

This designation is without prejudice to positions on status, and is in line with United Nations Security Council Resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence.

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Conflict of interest

None declared.

Authors' contributions

Conceived and designed the experiments: HRo, MK, OV. Performed the experiments: HRo, HRi, OH. Analysed the data: HRo, JO, JH, MK, HH, OV. Contributed reagents/materials/analysis tools: OV, JH, HH. Contributed to the writing of the manuscript: HRo, JH, JO, MK, OV.

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Letter to the editor: There is a need to consider all respiratory viruses in suspected mumps cases

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To the editor: The recent paper by Thompson et al. [1] highlighted the detection of influenza A (H3N2) virus in oral fluids from children with a clinical diagnosis of mumps. They concluded that influenza A(H3N2) virus should be considered as part of the differential diagnosis for mumps-like illness, particularly during influenza outbreaks caused by drifted strains. We report here similar findings in our Scottish cohort of patients during the 2014–15 winter season.

Between October 2014 and April 2015, the West of Scotland Specialist Virology Centre in Glasgow received 610 respiratory samples, submitted specifically for mumps virus testing. Mumps outbreaks were known to be occurring within our sample catchment area during that time [2]. The samples submitted consisted of throat swabs (n = 319), buccal/mouth swabs (n=143), gargle samples (n = 132), saliva (n = 11), unspecified swabs (n = 4) and nasopharngeal aspirate (n = 1). Of these 610 samples, 250 (41%) were real-time PCR positive for mumps [3]. The small hydrophobic gene of the mumps virus was sequenced from samples from 19 positive cases and all were genotype G, a prominent genotype in Europe [4].

We re-tested a random subset (n = 137) of the mumps-negative samples for influenza A virus, influenza B virus, parainfluenza 1 to 4 viruses, human metapneumovirus, rhinovirus, adenovirus, coronaviruses (229E, NL63, OC43), respiratory syncitial virus (RSV) and mycoplasma by an in-house real-time PCR [5].

The clinical details for the patients from whom the 137 analysed samples were obtained were as follows: 102 (74%) were described as having clinical mumps or parotitis, 26 (19%) had enlarged glands and swollen face/neck, while the remaining nine had symptoms including an erythematous rash (n=1), orchitis (n = 1), pyrexia (n = 1) and sore throat (n = 3) or were defined by the requesting clinician as a contact of a known mumps case (n = 3). The median age for the group tested was 23 years (range: 4 months to 80 years). Vaccination history was unavailable for all patients tested.

Overall, 29 (21%) of the 137 samples tested were positive for respiratory viruses. A total of 10 samples (7%) were positive for influenza A virus. Nine were subtyped as A(H3N2) one was subtyped as A(H1N1). Eight of the nine A(H3N2) viruses were sequenced: five were found be subclade 3C.2a (A/Hong Kong/3579/2014) and the remaining three were clade 3C.3 (A/ Victoria/208/2009). The five patients with 3C.2a viruses and two of the patients with 3C.3 had symptoms described as parotitis or clinical mumps by the requesting clinician. The other patient with a 3C.3 virus was a contact of a mumps case for whom no clinical details were available. It should be noted that we were unable to sequence one A(H3N2) virus (from a patient with a clinical diagnosis of mumps) and the A(H1N1) virus (from a patient whose symptoms were parotid swelling) due to low viral load. Two samples were positive for influenza B virus (one patient had enlarged glands and the other had a sore throat) and were found to be B/Yamagata lineage by real-time PCR [6].

Six patients were positive for parainfluenza 3 and all were reported to have had symptoms of parotitis or mumps. Six of eight coronavirus-positive patients also reported symptoms of parotitis or gland swelling. We detected coronavirus 229E (n = 3), NL63 (n = 1) and OC43 (n = 1); the remaining patient had a mixed infection of NL63 and RSV. Another patient with coronavirus 229E had query mumps and one with pyrexia was found to have NL63 infection. One of two patients positive for rhinovirus had parotitis while the other had testicular swelling. One patient was found to have adenovirus: the clinical presentation was described as enlarged glands.

Our work confirms the findings of Thompson et al., showing that A(H3N2) 3C.2a (A/Hong Kong/3579/2014) virus may be found in patients with mumps-like illness. We also highlight that other influenza and non-influenza viruses may also be found in patients with this clinical presentation. This suggests that laboratories and clinicians should consider testing patients who present with mumps-like illness for mumps virus and

other respiratory pathogens, as relying on clinical diagnosis alone may lead to skewed data for public health teams and surveillance.

Conflict of interest

None declared.

Authors' contributions

The study idea was conceived by RNG and CA. Work was performed by SJS and ARM. The letter was written by SJS, ARM and RNG

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