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Injection of new psychoactive substance snow blow associated with recently acquired HIV infections among homeless people who inject drugs in Dublin, Ireland, 2015

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In February 2015, an outbreak of recently acquired HIV infections among people who inject drugs (PWID) was identified in Dublin, following similar outbreaks in Greece and Romania in 2011. We compared drug and risk behaviours among 15 HIV cases and 39 controls. Injecting a synthetic cathinone, snow blow, was associated with recent HIV infection (AOR: 49; p=0.003). Prevention and control efforts are underway among PWID in Dublin, but may also be needed elsewhere in Europe.

Background

In February 2015, the Department of Public Health (DPH), Health Service Executive (HSE) in Dublin, Ireland, identified an unexpected increase in cases of acute HIV infection among people who inject drugs (PWID); three cases were diagnosed p24 antigen-positive in January and February 2015, compared with two cases diagnosed during the whole year in 2014 [1]. Drug treatment clinicians had also identified increased use of a new psychoactive substance (NPS) alpha-pyrrolidinovalerophenone (α-PVP), known as snow blow, which was being used by 'chaotic' PWID, and which they suspected might be linked to the increase [2]. Clinicians defined the chaotic group as homeless PWID who, if on opioid substitution treatment (OST), required daily attendance at their treatment programme, due to continued use of a variety of other illicit substances in an intensive or chaotic way. We undertook an epidemiological investigation to identify the likely source of this increase.

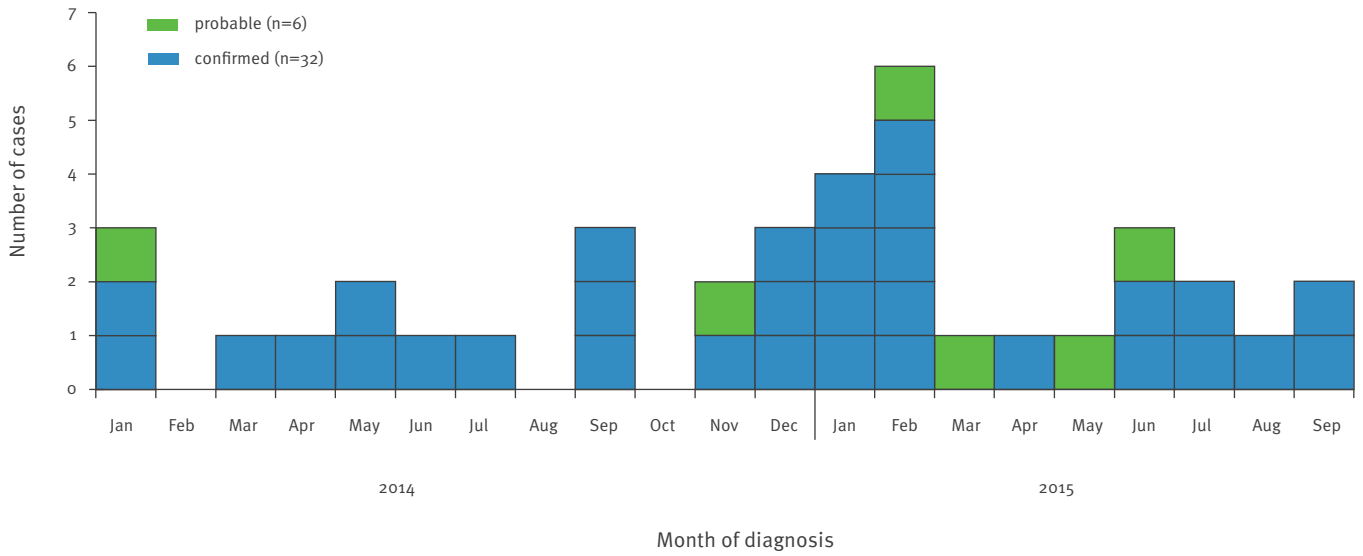
Epidemiological investigation and case definition

Clinicians in Dublin provided information on mode of transmission for new HIV diagnoses, and notified new HIV diagnoses in PWID immediately to the DPH. Clinicians asked all cases about the use of snow blow and at-risk sexual practices and whether they were homeless. Cases were crosschecked with data from the Dublin Region Homeless Executive's accommodation client database for periods of accommodation between 1 October 2013 and 30 June 2015.

We defined a case as HIV diagnosis in PWID in Dublin reporting first HIV-positive test since January 2014: a confirmed case had recent HIV infection (one or more of: p24 antigen-positive, history of negative HIV test within 12 months of first positive test, acute seroconversion illness, or recency assay test); a probable case had an HIV diagnosis of unknown duration but with an epidemiological link (sexual intercourse or drug sharing) to a confirmed case. We excluded cases with no evidence of recent infection or epidemiological links to recent HIV diagnoses, and cases previously diagnosed abroad.

Figure 1

Number of recent HIV infections or diagnoses with epidemiological link to recent infections in PWID in Dublin, by month of first diagnosis, January 2014 to September 2015[a] (n = 38)



[a] Data as at 30 September 2015.

Table 1

Distribution of demographic and social characteristics between cases (n=15) and controls (n=39), case–control study, outbreak of recent HIV infection in PWID, Dublin, Ireland, 2015

Demographic and social characteristics		Case	Control	P value
		n	n	
Sex	Female	6	6	0.05
Place of residence[a]	Home/relatives	7	14	0.36
	Homeless/hostels	7	25	
Prison		4	0	0.001
		Median (range)	Median (range)	P value
Age (years)		35 (25–51)	33 (19–51)	0.87
Age at first injection (years)		17 (11–39)	20 (11–44)	0.14
Duration of injection (years)		16 (3–26)	11 (0–39)	0.14

PWID: people who inject drugs.

[a] In the previous 12 months.

Case–control study

We conducted a case–control study among chaotic PWID, injecting within the previous 12 months, residing in Dublin and diagnosed since July 2014. We included confirmed and probable cases. A list of HIV test-negative (within three months) PWID attending the National Drug Treatment Centre (NDTC) in Dublin, registered by NDTC as homeless and requiring daily attendance due to chaotic behaviour, was generated. A random sample was taken from this list, using random figures generated from Excel. Patients on this list who attended and agreed to participate, were included. We conducted face-to-face or telephone interviews, to collect information on drug use including frequency of use, sexual and injecting at-risk practices, and living conditions. Participants were asked to report exposures for 12 months before HIV positive diagnosis (cases) or negative diagnosis (controls). To examine associations between exposures and infection, we calculated adjusted odds ratios (OR) and 95% confidence intervals (CI) using multiple logistic regression. Inclusion criteria used were $p<0.2$ for model entry.

Laboratory investigations

HIV serological diagnosis was based on the Abbott Architect HIV Ag/Ab Combo, Biomerieux VIDAS HIV Duo Ultra and P24II, and Fujirebio INNO-LIA HIV I/II SCORE assays; recency testing was performed on the Sedia Biosciences HIV-1 LAg-Avidity EIA; and HIV-1 RNA testing was performed on the Abbott Molecular m2000

Table 2

Factors positively associated with recent HIV infection in PWID in univariate analysis, case–control study, outbreak of recent HIV infection in PWID, Dublin, Ireland, 2015

Factors positively associated with recent HIV infection		Cases n (N = 15)	Controls n (N = 39)	Crude OR	(95% CI)	P value
Drugs	Administration					
Methamphetamine[a]	Injecting	7	1	33	(3.2–1535)	<0.001
Snow blow[a]	Injecting	13	7	30	(4.7–300)	<0.001
Snow blow (frequency)[a]	Injecting daily	8	1[b]	128	(10–1,595)	<0.001
	Injecting occasionally	5	6[b]	13	(2.1–85)	0.006
Amphetamines[c]	Using	3	1	9.5	(0.66–511)	0.03
Other head shop drugs[c]	Using	3	1	9.5	(0.66–511)	0.03
Benzodiazepines[c]	Using	13	23	4.5	(0.82–46)	0.05
Crack[c]	Injecting	1	1	3.6	(0.19–64)	0.39
	Other	7	13	1.9	(0.55–6.67)	0.3
Heroin[c]	Injecting	13	38	0.17	(0.003–3.7)	0.12
Cocaine[c]	Injecting	3	3	3.1	(0.54–18)	0.21
	Other	2	5	1.2	(0.21–7.4)	0.81
Opiates[c]	Injecting	0	2	NC	NC	NC
	Other	1	2	1.3	(0.10–15)	0.86
Sexual practices						
Sex with PWID [d]		10	15	3.8	(0.87–19)	0.041
Had sex [d]		10	25	1.3	(0.29–6.8)	0.7
Sex while high [d,e]		7	21	0.67	(0.08–8.9)	0.68
Unprotected sex[e]		8	22	0.55	(0.05–7.8)	0.54
At-risk injecting practices						
Use of used needles or syringes		8	2	21	(3.1–225)	<0.001
Use of used filters		6	5	4.5	(0.89–23)	0.026
Use of used containers or spoons for mixing[d]		8	10	3.7	(0.86–16)	0.038
		Median (range)	Median (range)			
Number of different drugs used		5 (1-9)	3 (1-7)	1.7[f]	(1.1–2.6)	0.011
Number of people with whom shared or reused works		1 (0-15)	0 (0-10)	1.2[f]	(0.97–1.5)	0.09

CI: confidence interval; NC: Not calculable; OR: odds ratio.

[a] Reference: not injecting in the previous 12 months or other route of administration.

[b] Five missing values.

[c] Reference: not using in the previous 12 months.

[d] One missing value.

[e] Among those who had sex.

[f] Per additional unit increase.

RealTime system. HIV subtyping has been described elsewhere [3].

Urine samples of cases attending NDTC were screened by the NDTC laboratory using an in-house Liquid Chromatography/Mass Spectrometry (LC/MS) NPS screening method. Since 2008, the laboratory of the NDTC has regularly redeveloped its methods in order to meet the challenge of testing for NPS by adding new drugs as they appear in Europe [4,5]. For the purposes of the study, the laboratory of the NDTC developed a tailored NPS LC/MS screening method targeting 26 NPS drugs and common amphetamines in urine. The method covered all relevant drugs detected in the laboratory in recent years including α-PVP which was first added to the NPS screen in 2011.

Results

Epidemiological investigation and case–control study

In 2014 and 2015, 38 confirmed and probable cases of HIV subtype B were reported (32 confirmed and six probable) (Figure). Among these, 16 were female; median age was 35 years (range: 24–51). Since January 2014, 29 of the 38 had been registered with homeless accommodation services, seven were not registered, and information was not available for two. All females and 13 of the 20 males with information available,

Table 3

Factors positively associated with recent HIV infection in multiple regression analysis, case–control study, outbreak of recent HIV infection in PWID, Dublin, Ireland, 2015

Factors positively associated with recent HIV infection	Adjusted odds ratio	95% confidence intervals	P value
Injecting snow blow	49	3.6–669	0.003
Reusing needles /syringes	13	1.01–177	0.049
Having sex with PWID	36	1.6–782	0.022
Female sex	3.5	0.27–44	0.34

were homeless. In all 18 of the 20 PWID with information available, reported injecting snow blow. At-risk practices, namely sex with PWID, or sex with an HIV-positive partner were reported by 20 of the 38 cases. Thirteen cases reported both using snow blow and sexual at-risk practices. One death was reported.

We recruited 15 cases (12 confirmed, three probable) of 24 cases and 39 controls. Cases were interviewed between 25 May and 18 August 2015 at infectious disease clinics (n=5), drug clinics (n=7), in prison (n=2) or by telephone (n=1). Controls were interviewed at NDTC. All participants but one were enrolled in methadone maintenance treatment.

Cases did not differ from controls in terms of age, age at first injection, duration of injection or living circumstances (Table 1). Females were more likely to become infected than males.

Compared with controls, cases were more likely to have reported injecting methamphetamine, snow blow, consuming amphetamines, other head shop drugs ('legal highs') or benzodiazepines (through various routes of administration) (Table 2).

All cases who reported injecting methamphetamine also reported injecting snow blow. Compared with those who did not inject in the previous 12 months, the odds of recent HIV infection was higher among PWIDs who reported occasional use (weekly, monthly or less than a month) and highest in those who reported injecting snow blow daily (Table 2).

Compared with controls, cases were more likely to have reused needles or syringes, to have used several types of drugs in the previous 12 months and to have had sex with PWID partners.

In the multivariable model, injecting snow blow, using used needles/syringes, and having sex with PWID were the only exposures which remained independently associated with HIV (Table 3).

Drug analysis

Urine samples from 12 cases in the case–control study were tested for NPS. Five were positive: α-PVP (n=4), α-PVP and MDPBP (n=1); seven were negative.

Discussion

This investigation among homeless chaotic PWID in Dublin is the first evidence of an association between injecting snow blow and recent HIV infection, with daily snow blow injectors being at highest risk. The epidemiological findings are supported by the detection of α-PVP in the urine of cases. Snow blow has been found to contain α-PVP, a second generation cathinone and is closely related to MDPV with similar abuse liability [2,4,6]. These findings are consistent with the known stimulant effects of synthetic cathinones and at-risk injecting practices linked to their use [6-11]. Drug treatment clinicians raised concerns that clients who injected snow blow generally exhibited more chaotic behaviours, leading to dis-inhibition, more sharing of needles and syringes, and unprotected sex.

Between 2005 and 2014, more than 81 synthetic cathinone derivatives were reported to the European Union (EU) Early Warning System [12]. Significant quantities of NPS are seized annually in Ireland [13]. In 2011, α-PVP was first detected in urine samples tested for NPS by NDTC. Urines tested for NPS in 2015 contain predominantly α-PVP, pentedrone and MDPBP [5].

Use of synthetic cathinones by homeless chaotic PWID populations has previously been reported in Ireland and in Europe [6,14]. In Dublin, there are more than 500 homeless PWID, a significant population at risk of HIV infection [15]. At the time of the study, 252 of ca 520 attendees at the NDTC (48.5%) were homeless. The general situation regarding homelessness in Dublin has also deteriorated significantly over 2014 and 2015, with a 28% increase in the number of individuals accessing emergency accommodation in Dublin in the 12 months to June 2015 [16]. The NDTC has a remit for providing OST for homeless PWID, but responding adequately to the needs of these PWID remains challenging.

In Dublin, measures taken include multi-disciplinary work to engage HIV-positive PWID in HIV care and, where appropriate, on antiretroviral therapy: offering HIV testing to PWID; awareness raising among clients, clinicians and networks of PWID about injecting head shop drugs such as snow blow, sexual at-risk behaviours and of the need to engage with methadone and treatment services. Services have been enhanced in terms of contact tracing and active case finding

and provision of greater access to needle exchange services by strengthening needle/syringe provision through outreach.

There were limitations in undertaking this investigation. Cases and controls were difficult to recruit among homeless chaotic PWID, exposed to illegal and at-risk behaviours. In the descriptive epidemiology, homeless status was determined by asking the patients if they were homeless, and by crosschecking with the homeless service database. If however homeless persons didn't register for any homeless service they might not have been identified as homeless. For the analytical study, controls identified by NDTC as homeless and chaotic were included in the study. However, we found that the homeless status of some participants had changed with a minority no longer reporting homelessness, although all remained chaotic. Cases were more likely to have been in prison. However this was subject to selection bias as we did not select controls in prisons but included cases who were in prison. Behavioural findings were based on self-reported information and some participants were under the effect of drugs while interviewed. This may have had altered the accuracy of measurement of past exposures.

Ireland is the third country reporting an outbreak of HIV among PWID within the past five years in Europe, after Greece and Romania in 2011 [14,17]. A similar increase has also recently been reported in Glasgow and Clyde in June 2015 [18]. Outbreaks of HIV among PWID have also been reported in Israel in 2012 and in Indiana, United States, in early 2015 [19,20]. With low coverage of harm reduction activities such as needle and syringe programmes and OST in most European countries, and a growth of the market in new psychoactive substances, in particular the detection of α -PVP in the 28 EU Member States, the risk of HIV among PWID, and particularly homeless chaotic PWID is likely to be increasing in Europe [2,12,21]. It may be of benefit for other countries to consider strengthening prevention programmes for PWID [21-23].

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Conflict of interest

None declared.

Authors' contributions

Design of the study: GC, ID, GZ, KE FM, WM, O'DK

Interviews of participants: GZ, GR, LF

Data analysis: GC, ID, DK

Writing of article: GC, ID, DK

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Article review: GC, ID, GZ, KE, DK, O'DK, SS, McNS, LF, WM, GR, FM, DGC, WA, EO, HC

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Genotypic anomaly in Ebola virus strains circulating in Magazine Wharf area, Freetown, Sierra Leone, 2015

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The Magazine Wharf area, Freetown, Sierra Leone was a focus of ongoing Ebola virus transmission from late June 2015. Viral genomes linked to this area contain a series of 13 T to C substitutions in a 150 base pair intergenic region downstream of viral protein 40 open reading frame, similar to the Ebolavirus/H.sapiens-wt/SLE/2014/Makona-J0169 strain (J0169) detected in the same town in November 2014. This suggests that recently circulating viruses from Freetown descend from a J0169-like virus.

In Sierra Leone, two new Ebola virus (EBOV) cases were reported from the densely populated Magazine Wharf area of Freetown in the Western Area Urban district after a period of two weeks in June 2015 with no cases in the district. The Magazine Wharf area was subsequently a focus of transmission for several weeks (http://apps.who.int/ebola/current-situation/ebola-situation-report-15-july-2015) up to 12 August 2015 (http://apps.who.int/ebola/current-situation/ebola-situation-report-12-august-2015), after which no new cases were reported from the area (http://apps.who.int/ebola/current-situation/ebola-situation-report-30-september-2015). In this study, the whole genomes of viruses from patient samples, originating from the Western Area Urban district and other districts of the country (i.e. Kenema, Kono, and Tonkolili) between January and July 2015 are sequenced. Genomes derived from samples collected from 30 June onwards in the Western Area Urban district have a particular anomaly consisting of a series of 13 T to C substitutions in a 150 bp intergenic region downstream of the viral protein 40 (VP40) open reading frame (ORF). This anomaly is also present in a viral strain, the Ebolavirus/H.sapiens-wt/SLE/2014/Makona-J0169 (J0169), which was detected in Freetown in November 2014. The finding suggests that viruses retrieved in June and July 2015 from the Western Area Urban district are direct descendants of a J0169-like virus. Near real time application of whole EBOV genome sequencing and the identification of lineage signatures can be used to monitor the ongoing outbreak and test whether newly infected patients are part of an identified transmission chain.

Ebola virus disease epidemic in West Africa

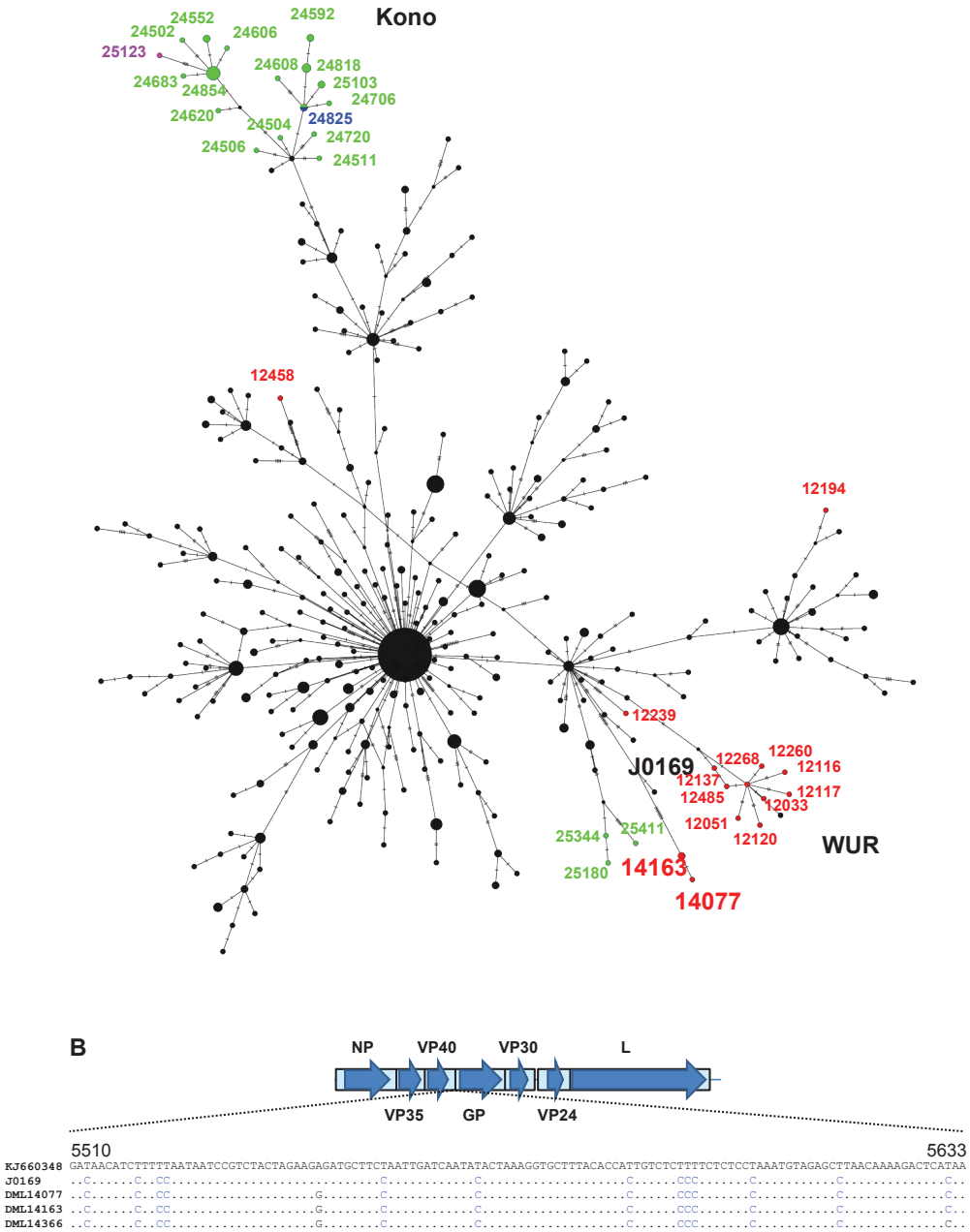
An epidemic of EBOV (a negative-sense RNA virus, family *Filoviridae*) disease has been ongoing in West Africa since December 2013 affecting mainly Guinea, Liberia and Sierra Leone [1]. As of 9 September 2015, the cumulative number of suspect, probable and confirmed cases stands at 28,183, including 11,306 deaths (http://apps.who.int/ebola/current-situation/ebola-situation-report-9-september-2015). EBOV cases continue to be detected and shifts in foci of transmission are observed. One of the pillars in the emergency response to the epidemic in the area has been the deployment of temporary (mobile) laboratories by the international community in collaboration with local authorities, among which the Dutch Mobile Laboratories (http://dutchebolalabs.nl/rapport-implementatie-dutch-mobile-labs-in-sierra-leone-en-liberia/). These laboratories provide rapid testing capacity for EBOV and malaria in support of clinical triage of (suspected) patients. In addition, the international community is currently working together in sequencing EBOV genomes in order to study EBOV evolution [2-7].

Sampling and whole genome sequencing

A total of 49 samples of EBOV positive patients who had been tested by Dutch Mobile Laboratories (Table) located in the Western Area Urban and Kono districts were included in the study. The samples were collected

Figure

A) Median-joining haplotype network constructed from an alignment of 563 Ebola virus sequences derived from clinical samples and B) alignment of a sequence region where four Ebola virus strains present a genotypical anomaly, Sierra Leone, 2015



GP: glycoprotein; L: RNA-dependent RNA polymerase L; NP: nucleoprotein; VP: virus protein; WUR: Western Area Urban district.

A: The 563 Ebola virus isolates' sequences correspond to nucleotides 148–18,629 of Zaire ebolavirus isolate H.sapiens-wt/SLE/2015/Makona-Goderich1 (GenBank accession number: KT345616). The median haplotype network was constructed using PopART version 1.7 (http://popart.otago.ac.nz). Each vertex represents a sampled viral haplotype. The size of each vertex is relative to the number of sampled isolates. Coloured vertices indicate determined viral genomes from Western Area Urban (red), Kono (green), Tonkolili (blue), and Kenema (purple) districts. The larger font numbers depicted in red represent viral genomes derived from Freetown patients' samples retrieved in June and July 2015 (two of them linked to Magazine Wharf area) with a genotypic anomaly consisting of a series of 13 T to C mutations in a 150 bp long sequence, which is located in an intergenic region downstream of the VP40 open reading frame. Hatch marks indicate single nucleotide mutations alongside each edge. The node labelled 24592 contains sequences 24592 and 24601; node 25103 contains 25083 and 25103; node 14163 contains 14163 and 14366; node 24552 contains 24552 and 24581; node 24854 contains 24854, 24853, 24605, 24669, 24677, 24604, and 24758; node 24825 contains 24825 and 24611; node 24818 contains 24818, 24553, and 24573.

B: Genotypic anomaly in four Ebola virus strains from Freetown, Western Area Urban district, consisting of a series of 13 T to C mutations in a 150 bp long sequence located in an intergenic region downstream of the VP40 open reading frame (genome positions: 5,510–5,633). Three of the strains, which are depicted as DML14077, DML14163, and DML14366 on the alignment were characterised in June and July 2015. These are respectively EBOV_DML14077_SLe_WesternUrban_20150630 (GenBank accession number: KT357860), EBOV_DML14163_SLe_WesternUrban_20150703 (GenBank accession number: KT357858), and EBOV_DML14366_SLe_WesternUrban_20150711 (GenBank accession number: KT357859). The fourth strain, which is shown as J0169 is Ebolavirus/H.sapiens-wt/SLE/2014/Makona-J0169 (GenBank accession number: KP759706) and was characterised in November 2014. The sequences of the four strains are compared with the reference Zaire ebolavirus isolate H.sapiens-wt/GIN/2014/Makona-Gueckedou-C05, complete genome (GenBank accession number: KJ660348). The Ebola virus genome organisation is shown for reference.

Table

Characteristics of Ebola virus positive specimens, which were subjected to whole genome sequencing, Sierra Leone, January–July 2015 (n=49)

Specimen ID	Specimen type	Country	District[a]	Date of specimen collection	Accession number
12033	Blood	Sierra Leone	Western_Urban	19–02–2015	KT357813
12051	Blood	Sierra Leone	Western_Urban	21–02–2015	KT357814
12116	Blood	Sierra Leone	Western_Urban	26–02–2015	KT357815
12117	Blood	Sierra Leone	Western_Urban	26–02–2015	KT357816
12120	Blood	Sierra Leone	Western_Urban	27–02–2015	KT357817
12137	Blood	Sierra Leone	Western_Urban	28–02–2015	KT357819
12194	Blood	Sierra Leone	Western_Urban	04–03–2015	KT357818
12239	Swab	Sierra Leone	Western_Urban	07–03–2015	KT357820
12260	Blood	Sierra Leone	Western_Urban	09–03–2015	KT357821
12268	Blood	Sierra Leone	Western_Urban	10–03–2015	KT357822
12458	Blood	Sierra Leone	Western_Urban	28–03–2015	KT357823
12485[b]	Swab	Sierra Leone	Western_Urban	31–03–2015	KT357824
24502[b]	Blood	Sierra Leone	Kono	13–01–2015	KT357825
24504	Blood	Sierra Leone	Kono	13–01–2015	KT357826
24506	Blood	Sierra Leone	Kono	14–01–2015	KT357827
24511	Blood	Sierra Leone	Kono	14–01–2015	KT357828
24552	Blood	Sierra Leone	Kono	17–01–2015	KT357829
24553	Blood	Sierra Leone	Kono	17–01–2015	KT357830
24573	Swab	Sierra Leone	Kono	18–01–2015	KT357831
24581	Swab	Sierra Leone	Kono	19–01–2015	KT357832
24592*	Swab	Sierra Leone	Kono	20–01–2015	KT357833
24601*	Blood	Sierra Leone	Kono	20–01–2015	KT357834
24604	Blood	Sierra Leone	Kono	20–01–2015	KT357835
24605	Blood	Sierra Leone	Kono	20–01–2015	KT357836
24606	Blood	Sierra Leone	Kono	20–01–2015	KT357837
24608	Blood	Sierra Leone	Kono	20–01–2015	KT357838
24611	Swab	Sierra Leone	Kono	21–01–2015	KT357839
24620[b]	Blood	Sierra Leone	Kono	21–01–2015	KT357840
24669	Blood	Sierra Leone	Kono	25–01–2015	KT357841
24677	Swab	Sierra Leone	Kono	25–01–2015	KT357842
24683**	Blood	Sierra Leone	Kono	26–01–2015	KT357843
24706	Blood	Sierra Leone	Kono	28–01–2015	KT357844
24708**[c]	Blood	Sierra Leone	Kono	28–01–2015	KT357845
24720[b]	Blood	Sierra Leone	Kono	29–01–2015	KT357846
24758	Blood	Sierra Leone	Kono	30–01–2015	KT357847
24818	Swab	Sierra Leone	Kono	03–02–2015	KT357848
24825	Blood	Sierra Leone	Tonkolili	04–02–2015	KT357849
24853	Blood	Sierra Leone	Kono	06–02–2015	KT357850
24854	Blood	Sierra Leone	Kono	06–02–2015	KT357851
25083***	Blood	Sierra Leone	Kono	18–02–2015	KT357852
25103***	Swab	Sierra Leone	Kono	19–02–2015	KT357853
25123[b]	Swab	Sierra Leone	Kenema	18–02–2015	KT357854
25180****	Blood	Sierra Leone	Kono	23–02–2015	KT357855
25344****	Blood	Sierra Leone	Kono	06–03–2015	KT357856
25411[b]	Blood	Sierra Leone	Kono	10–03–2015	KT357857
13828	Blood	Sierra Leone	Western_Urban	22–06–2015	Not applicable
14077	Blood	Sierra Leone	Western_Urban	30–06–2015	KT357860
14163	Blood	Sierra Leone	Western_Urban	03–07–2015	KT357858
14366	Swab	Sierra Leone	Western_Urban	11–07–2015	KT357859

ID: identity; Western_Urban: Western Area Urban district.

In the column with specimen IDs, entries with the same number of asterisks indicate specimens derived from the same patient.

[a] District of patient residence.

[b] Less reliable consensus sequence due to low coverage regions.

[c] Not shown in Figure, unreliable consensus sequence due to low coverage regions.

from patients residing in Kenema, Kono, Tonkolili and Western Area Urban districts between January and July 2015. Nucleic acids were extracted from the samples using EZ Advanced XL automated RNA extraction (Qiagen). Isolated nucleic acids were subjected to reverse transcription/polymerase chain reaction (PCR) amplification using the Ion AmpliSeq Ebola Panel Assay (Thermo Fisher Scientific) and the Ion Torrent sequencing platform at a local sequencing facility established at the Mateneh Ebola Treatment Centre in Makeni, Bombali district, Sierra Leone (European Nucleotide Archive Study: PRJEB10265). Reads were extracted from unfiltered BAM files using CLC Genomics Workbench 7.5.1 and trimmed based on quality with an ambiguous limit of 2 and quality limit of 0.05. Reads longer than 1,000 nucleotides (nt) or shorter than 15 nt were discarded. Trimmed reads were mapped to Zaire ebolavirus isolate H.sapiens-wt/GIN/2014/Makona-Gueckedou-C05, complete genome (GenBank accession number: KJ660348), using CLC Genomics Workbench 7.5.1 map reads to reference beta module, with the following parameters: no masking, mismatch cost 2, insertion and deletion open cost 7, insertion and deletion extend cost 3, length fraction 0.95, similarity fraction 0.9. Consensus sequences were extracted and regions where depth of coverage was less than 2 were called as 'N'. All generated genomes were manually inspected for accuracy, such as for the presence of intact ORFs and low coverage regions for adequate base calling, resulting in 48 near full-length EBOV genomes (Table).

Detection of evolutionary lineages and mutations

A median haplotype network was constructed in PopART version 1.7 (http://popart.otago.ac.nz) using 563 determined EBOV genomes from Sierra Leone, including those determined in this study ([3-5,7]; Figure A; Table). Accordingly, the 48 determined genome sequences appeared to belong to multiple distinct evolutionary lineages. Most viruses from the Western Area Urban district grouped together as did viruses from Kono.

Viruses EBOV_DML14077_SLe_WesternUrban_20150630, EBOV_DML14163_SLe_WesternUrban_20150703, and EBOV_DML14366_SLe_WesternUrban_20150711 (DML14077, DML14163, DML14366) isolated from patients from Freetown at the end of June and July (Table) were highly similar to each other and were clearly different from viruses isolated in Freetown between January and March 2015 (Figure A). Two of these viruses were linked to the Magazine Wharf area in Freetown, a hotspot for EBOV transmission in June and July 2015.

The DML14077, DML14163, and DML14366 viruses were most closely related to Ebolavirus/H.sapiens-wt/SLE/2014/Makona-J0169 (GenBank accession number: KP759706) isolated on 9 November 2014 in Freetown (Figure A). The J0169 virus had a striking genotypic anomaly containing a series of 13 T to C substitutions occurring in a region of 150 bp in length in an intergenic region downstream of the viral protein 40 (VP40) ORF. This anomaly was also present in DML14077, DML14163 and DML14366 (Figure B). An excessive accumulation of T to C mutations has been previously observed in a variety of virus genomes, including negative-sense RNA viruses and in EBOV in infected cells in vitro, and are a hallmark of host adenosine deaminases acting on RNA (ADARs) [8-10]. DML14077, DML14163, and DML14366 had 19, 17, and 18 additional single nt variants, respectively, compared with the J0169 virus, consistent with current estimates of evolutionary rate in the Ebola virus outbreak [5], suggesting that DML14077, DML14163 and DML14366 are direct descendants from a J0169-like virus (Figure A). The same stretch of T to C substitutions was found in a partial genome sequence from another patient at the end of June, who was also linked to the Magazine Wharf area (DML13828; Table; data not shown) and it serves as a lineage signature allowing identification and tracking of transmission chains.

Discussion and conclusions

At present only a handful of Ebola virus genomes sequenced from the current outbreak contain stretches of T to C mutations, and these can be found at different locations in the genome [5,7]. All shared nt variants, such as the conserved T to C mutations observed between J0169 [7] and the three viral genomes newly characterised in this study, are unlikely the source of recurrent induced mutations in individual patients. Rather, the T to C substitutions could have been fixed in viral lineages in the past and these lineages may be capable of efficient human-to-human transmission. This would explain how a J0169-like virus with a characteristic series of T to C mutations could have further spread in the Magazine Wharf area of Freetown.

It is unlikely that the T to C mutation stretch observed in the J0169-like viruses had a fitness advantage over other spreading EBOV lineages as in the ca 6–7 months of transmission between the first identification of this virus lineage (November 2014) and the present, this EBOV lineage did not become dominant over others. The J0169-like virus lineage seemingly lingered on at sufficiently low prevalence that it was not captured in genomic surveillance until now. This is not surprising given that the amount of whole genome sequences available is only a fraction of the 28,183 confirmed, probable and suspected cases (http://apps.who.int/ebola/current-situation/ebola-situation-report-9-september-2015) and whole genome sequencing started to build momentum relatively late in the outbreak. It shows however, that near real time application of whole EBOV genome sequencing and the identification of lineage signatures can be used to monitor the ongoing outbreak and test whether newly infected patients are part of an existing known transmission chain in the area.

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Conflict of interest

None declared.

Authors' contributions

SLS, SDP, CBR, BLH and MPK were involved in data analysis, training of people operating Dutch Mobile Laboratories in Sierra Leone, and writing the manuscript. PP, CC, KD, IW, AK, and DK were involved in getting Dutch Mobile Laboratories operational and providing continuous support in gathering clinical samples. SLC, AA, LT, JL, UJ, and IG were involved in next generation sequencing in Sierra Leone and writing the manuscript.

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Outbreak of *Yersinia pseudotuberculosis* O:1 infection associated with raw milk consumption, Finland, spring 2014

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In March 2014, a *Yersinia pseudotuberculosis* (YP) outbreak was detected by a municipal authority in southern Finland. We conducted epidemiological, microbiological and traceback investigations to identify the source. We defined a case as a person with YP infection notified to the National Infectious Disease Registry between February and April 2014, or their household member, with abdominal pain and fever≥ 38 °C or erythema nodosum. Healthy household members were used as household-matched controls. We identified 43 cases and 50 controls. The illness was strongly associated with the consumption of raw milk from a single producer. The odds ratio of illness increased with the amount of raw milk consumed. Also previously healthy adults became infected by consuming raw milk. Identical YP strains were identified from cases' stool samples, raw milk sampled from a case's refrigerator and from the milk filter at the producer's farm. The producer fulfilled the legal requirements for raw milk production and voluntarily recalled the raw milk and stopped its production. We advised consumers to heat the raw milk to 72 °C for 15 s. Current legislation for raw milk producers should be reviewed and public awareness of health risks linked to raw milk consumption should be increased.

Background

Yersinia pseudotuberculosis (YP) is a pathogen transmitted to humans via the faecal-oral route through consuming contaminated water or food, especially raw and undercooked products [1]. It causes yersiniosis, a gastrointestinal disease characterised by abdominal pain and fever, which can lead to post-illness complications such as erythema nodosum and reactive arthritis [2]. The incubation period of YP is three to seven days [2] but can be up to 18 days [3].

In Finland, YP infection is a notifiable disease. Outbreaks cause large variation in the annual YP incidence, for example 4.8 per 100,000 in 2006 and 0.7 per 100,000 in 2013 [4]. Most cases are sporadic, but 10 YP outbreaks were reported between 1997 and 2008 [3]. Identified sources of previous YP outbreaks include carrots [3], iceberg lettuce [5] and vegetable juice [6].

On 27 March 2014, one local public health authority in southern Finland contacted the National Institute for Health and Welfare (THL) to notify four cases of YP infection. This was followed by a notification to the National Registry for Food and Waterborne Outbreaks. We investigated the outbreak to identify its extent and source in order to apply appropriate control measures and to prevent further cases and future outbreaks.

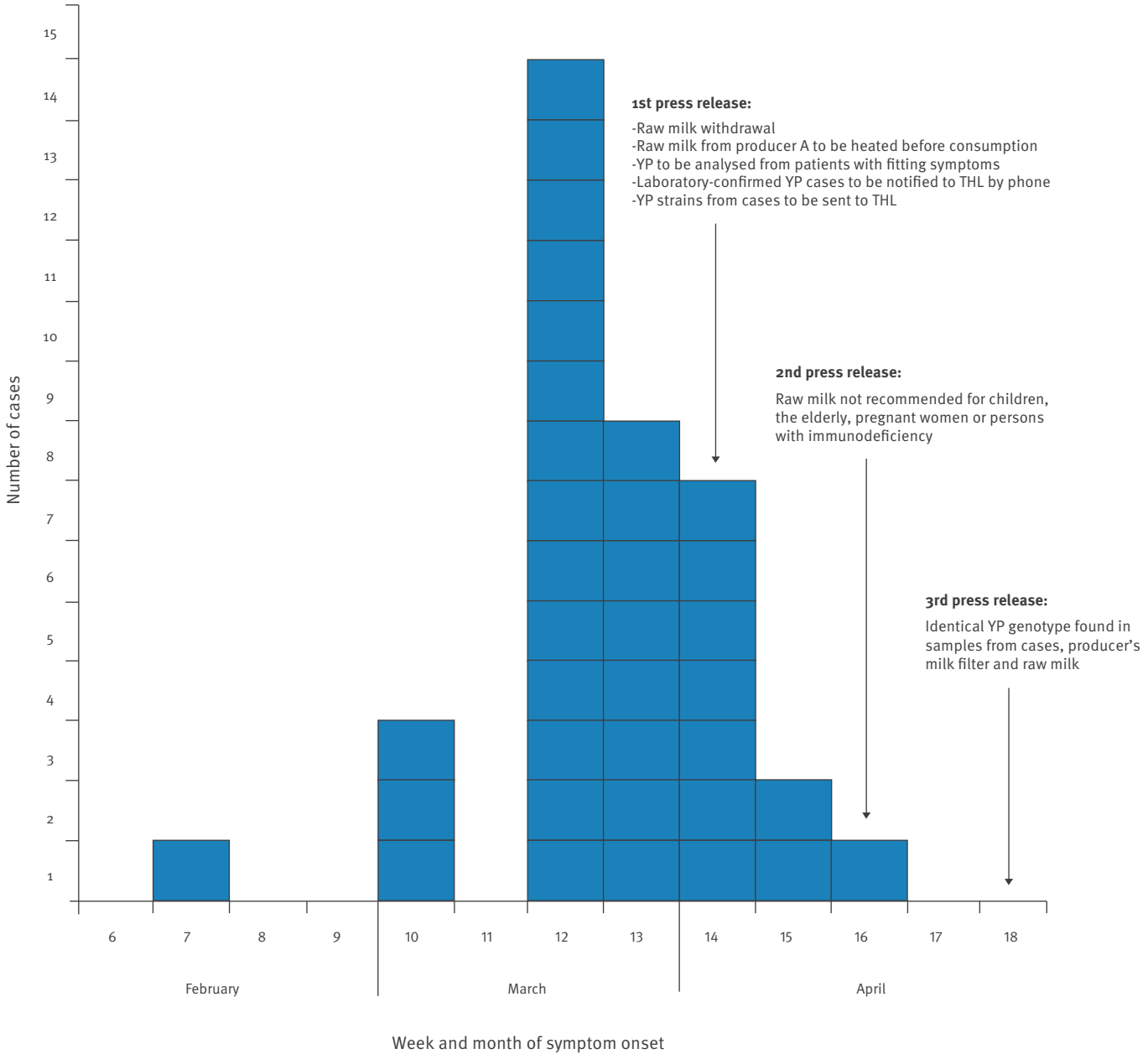
Methods

Case finding and hypothesis generation

In order to detect YP infection cases, we sent an alert to physicians and clinical laboratories all over Finland, asking them to take samples from patients with symptoms resembling yersiniosis and to report laboratory-confirmed cases to the outbreak investigation team by phone. Clinical laboratories are required by law to notify the National Infectious Disease Registry (NIDR) of any YP findings identified in stool or of antibodies in serum. We line-listed persons with YP infection notified to the NIDR from February to April 2014.

Figure 1

Number of *Yersinia pseudotuberculosis* cases by week of symptom onset and timeline of public health events, Finland, February–April 2014 (n = 36)



THL: National Institute for Health and Welfare; YP: Yersinia pseudotuberculosis.

Date of symptom onset available for 36 cases.

A public health nurse interviewed six persons from the line list, using a standard trawling questionnaire to generate hypotheses. The trawling questionnaire for gastrointestinal illnesses contained 97 questions on the clinical picture of the disease, travel history, consumed foods and drinks, cooking methods, sites for shopping and eating out, and animal contact.

Case–control study and statistical analysis

We conducted a case–control study to test the hypothesis suggested by the trawling interviews. Our study population comprised persons with YP infection notified to the NIDR and their household members. The outbreak team at THL developed a web-based questionnaire. This included questions about the demographical and clinical characteristics of the subjects, onset of the symptoms, food exposure suggested by trawling interviews and by literature as previously identified sources of YP outbreaks, and about the frequency and amount of foods consumed during the two weeks before the onset of symptoms. Respondents

Table 1

Exposure to food items among cases and controls (matched univariate analysis), *Yersinia pseudotuberculosis* outbreak, Finland, February–April 2014 (n = 93)

	Cases (n = 43)	Controls (n = 50)		
Variable	Exposed n (%)	Exposed n (%)	mOR (95% CI)	p value
Raw milk (producer A)	42 (98)	24 (48)	22.2[a] (3.6–∞)	<0.001
Raw milk (in general)	42 (98)	30 (60)	16.9 [a] (2.6–∞)	0.001
Salad	34 (79)	40 (80)	1.7 (0.1–117.8)	1.000
Raw milk (producer other than A)	1 (2)	2 (4)	1.5 [a] (0.0–58.5)	1.000
Other vegetable	26 (60)	38 (76)	0.9 [a] (0.1–∞)	1.000
Carrot	38 (88)	41 (82)	0.8 (0.0–49.1)	1.000
Vegetable juice	0 (0)	4 (8)	0.56 [a] (0.0–7.5)	0.667

CI: confidence interval; mOR: matched odds ratio.

[a] Median unbiased estimate.

Table 2

Unadjusted and adjusted dose–response relationship between consumed raw milk from producer A and *Yersinia pseudotuberculosis* infection, Finland, February–April 2014 (n = 93)

	Cases (n = 43)	Controls (n = 50)	Total (n = 93)	Univariate		Multivariable [a]	
	n (%)	n (%)	n (%)	mOR (95% CI)[b]	p value	mOR (95% CI)[b]	p value
Quantity of consumed milk							
None	2 (8)	24 (92)	26 (100)	Reference		Reference	
<1 dL/day	9 (56)	7 (44)	16 (100)	7.2 (0.71–∞)	0.098	10.7 (0.99–∞)	0.051
1–3 dL/day	19 (63)	11 (37)	30 (100)	14.5 (2.21–∞)	0.003	8.0 (1.09–∞)	0.039
>3 dL/day	12 (71)	5 (29)	17 (100)	20.0 (2.07–∞)	0.008	12.2 (1.26–∞)	0.029
Age						0.92 (0.85–0.97)	<0.001

CI: confidence interval; mOR: matched OR.

[a] Adjusted for age.

[b] Median unbiased estimator of OR; univariate p value for trend is <0.001; multivariable p value for trend is 0.016.

Table 3

Environmental samples tested during a *Yersinia pseudotuberculosis* outbreak, Finland, February–April 2014 (n = 21)

	Sample (n positive/n tested)				
	Milk filter	Bovine faeces	Raw milk (bulk tank)	Raw milk (case's refrigerator)	Raw milk (producer's storage)
Yersinia pseudotuberculosis	2/3[a]	2/9 [a]	5/5	1/1 [a]	3/3
YP PFGE outbreak pattern	1/1	0/2	0/0	1/1	0/0
YP MLVA outbreak profile	1/1	0/2	0/0	1/1	0/0
Campylobacter jejuni	2/3	9/9	0/5	0/1	0/3
Presumptive STEC [b]	3/3	0/0	4/5	0/0	0/0
Yersinia enterocolitica	1/3	0/0	0/5	0/1	1/3

MLVA: multilocus variable-number of tandem repeats analysis; PFGE: pulsed-field gel electrophoresis; STEC: *Shiga toxin-producing Escherichia coli*. YP: *Yersinia pseudotuberculosis*.

[a] All YP serotype O:1.

[b] Presumptive STEC is defined as real-time PCR detection of (i) the *stx* gene, (ii) *stx* and *eae* genes or (iii) *stx* and *eae* genes as well as genes associated with one of the serogroups O157, O111, O26, O103 or O145.

were invited to reply using either a web-based questionnaire or a paper form.

We defined a case as a person with YP infection notified to the NIDR or with symptoms of abdominal pain and fever of ≥ 38 °C or erythema nodosum in the period from February to April 2014. The matched controls were respondents living in the same household as the case who did not meet the case definition.

We calculated matched odds ratios to explore the associations between a single exposure and the outcome. To control for possible confounders, significant variables in the univariate analysis were included in the multivariable model. Households with only one member or no controls were excluded from the matched analysis. We coded the responses 'do not remember' or 'do not know' as missing values. We used exact conditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI) for both univariate and multivariable analyses. Where a finite conditional maximum likelihood estimate did not exist, the median unbiased estimator (MUE) of the OR was calculated [7].

To calculate the point estimates for the effects of exposure doses in the univariate analysis, we used binary variables for each milk dose in the model. In the multivariable analysis, we adjusted the model for continuous age. The significance of the dose-response trend was assessed using the ordinal level dose-response variable as continuous both in the univariate and in the multivariable analysis. For all analyses, a p value of < 0.05 was considered significant. The STATA Data Analysis and Statistical Software version 12 (Stata Corporation, College Station, United States) was used to perform the analysis.

Traceback and microbiological investigation

After raw milk was identified as a possible source of the outbreak, the municipal authority contacted the producer in order to investigate the production procedure, to identify when, where and how much of the specific product was distributed and to find out whether the producer followed the relevant regulations for food production.

On 4 April, an opened 3 L package of raw milk from a specific producer (with 'use by' date on 1 April) was sampled from a case's refrigerator. On 7 April, the local team visited the producer's farm to collect the milk filter, bulk tank milk and bovine faecal samples. Bovine faecal samples were pooled to represent ca 10 animals each. Unopened 3 L packages from the producer's storage were also collected.

Stool specimens from the patients were analysed at four clinical microbiology laboratories using faecal culture and a quantitative PCR for *Salmonella*, *Yersinia*, *Campylobacter*, *Vibrio cholera*, *Shigella*, and *Shigatoxin-producing Escherichia coli* (STEC) [8]. Milk, milk filter and bovine faecal samples were analysed at the Finnish Food Safety Authority Evira. Screening of environmental samples for YP, pathogenic *Yersinia enterocolitica* and STEC was conducted by real-time PCR [9-11]. For *Y. enterocolitica* detection, we followed the technical specification ISO/WD 10273 [12] and for STEC detection we followed ISO/TS 13136 [11], where presumptive STEC is defined as real-time PCR detection of (i) the *stx* gene, (ii) *stx* and *eae* genes or (iii) *stx* and *eae* genes as well as genes associated with one of the serogroups O157, O111, O26, O103 or O145.

For isolation of YP, samples of 10 g (faeces) 25 mL (milk) or one half of a milk filter were homogenised in phosphate-mannitol-peptone (PMP) broth [13] as described previously [3]. The growth characteristics of YP in bulk tank milk were studied by plating dilutions of the refrigerated tank milk on CIN agar on day 0, 1, 2, 5, 7, 9 and 12 after collection (up to one day after milking). Each sample was plated in duplicate and the average count of YP colonies was used. YP isolates were identified by API 20E (bioMérieux, France) and MALDI-Biotyper (Bruker Daltonics, GmbH). Isolation of *Campylobacter* spp. from milk, milk filter and bovine faecal samples was carried out according to ISO 10272–1:2006 [14] with a modification in the enrichment: an incubation time of 24 hours without a pre-incubation step was used.

Serotyping, pulsed-field gel electrophoresis (PFGE) and multilocus variable-number tandem repeat analysis (MLVA) were performed at THL to characterise and compare the isolates [5,15]. No band differences were allowed in PFGE and a single-locus difference was allowed in MLVA to designate two strains as indistinguishable. *C. jejuni* isolates were subtyped at Evira with PFGE using *Sma*I and *Kpn*I [16].

Results

Descriptive and analytical epidemiology

From February to April 2014, we identified and line-listed 55 persons with YP infection registered in the NIDR. They represented 48 households and their median age was 14 years (range: 1–67). Of those 55, 35 were men and 51 were from the Helsinki and Uusimaa hospital district in the south of Finland. In those three months, the incidence rate of YP infection cases in this hospital district was more than six times higher (incidence rate ratio (IRR): 6.4; 95% CI: 3.0–15.6) than in the same period in 2013 (13.2/100,000 vs 2.1/100,000). All six persons completing the trawling interview reported consuming raw milk from a single producer A.

In total, 93 persons from 30 households (household response rate: 63%) completed the questionnaire. The online questionnaire was used by 53 of 93. Of the respondents, 43 were identified as cases (33 based on NIDR notification, 10 based on symptoms) and 50 as controls. Respondents under the median age of 23 years were 12 times more likely to be a case than those over the age of 23 (OR = 12.1; 95% CI: 2.8–111.0;

p<0.001). Of the 43 cases, 22 were men, and the median age was 13 years (range: 1–67). One case had a campylobacter co-infection.

The first case fell ill on 10 February, and the number of cases peaked in March, week 12 (Figure). The median duration of the illness was 14 days (range: 4–27). Abdominal pain and fever were the most commonly reported symptoms (39 and 31 of the 43 cases, respectively), followed by nausea, diarrhoea and joint pain (20, 19 and 13 of the 43 cases, respectively). Three cases developed erythema nodosum, and four were hospitalised. No significant difference (p=1.000) was found between cases and controls regarding previously diagnosed chronic diseases (7/43 vs 9/50, respectively).

Eight households, comprising in total 13 cases, were excluded from the matched analysis because they had only one household member or no controls. In the univariate analysis, raw milk was the only food item significantly associated with the illness (Table 1) and an increase of one year in age decreased the odds of illness by 6% (OR=0.94; 95% CI: 0.90–0.98; p<0.001). Compared with those who did not drink raw milk from producer A, the age-adjusted odds of illness were higher than 35 for those who consumed it (MUE of OR=35.2; 95% CI: 4.07–∞; p<0.001). Of the 93 questionnaire respondents, 89 provided information on the volume of consumed raw milk. The OR of illness increased with the volume of raw milk consumed (p=0.016) (Table 2).

Traceback and microbiological investigation

The raw milk was produced on a farm that had 90 milking cows and two regular daily employees. The majority of the milk produced was delivered to a dairy for pasteurisation. In February 2014, the farm started operating a packaging facility for the raw milk once per week. The product was first distributed to retail stores on 10 February. From 10 February to 3 April 2014, 11,835 L of this raw milk were delivered to 24 shops in southern Finland. The raw milk was sold in 3 L packages, with a 'use by' date five days from the date of packing. In compliance with Finnish regulations, all packages had a warning label, stating that the milk may contain harmful microbes and should be heat-treated before serving to risk groups, i.e. children, pregnant women, the elderly or individuals with a severe primary disease [17].

In the microbiological investigation, all of the 41 YP patient isolates that had been sent to the THL reference laboratory from February to April had the serotype O:1. Isolates from eight cases were characterised by PFGE and all showed an identical profile (outbreak profile). Seven of eight isolates were also analysed with MLVA and had the indistinguishable outbreak profile 5–9-3–2-4–8-12. YP with the outbreak profile was also detected in a milk sample from the refrigerator of a case and from the milk filter (Table 3). The concentration of YP in tank milk increased steadily during storage at 4 °C. It was 2 colony-forming units (cfu)/mL when collected from the tank and 5 cfu/mL, 26 cfu/mL and 120 cfu/mL after one, two and five days of storage, respectively. After 12 days of storage, the concentration was 3,500 cfu/mL.

C. jejuni was detected by culture in environmental samples, and pathogenic *Y. enterocolitica,* presumptive STEC O103, O145 and STEC O103 were detected by PCR (Table 3). *C. jejuni* was not isolated from tank milk. Attempts at isolation did not yield any *Y. enterocolitica* or STEC isolates. A *C. jejuni* isolate from one patient was available for comparison with isolates from the milk filters (n=2) and bovine faeces (n=7). Identical PFGE profiles were identified.

Public health measures

After producer A's raw milk was identified as the suspected source of the outbreak, the producer voluntarily recalled a total of 200 L of raw milk from the market on 3 April 2014. The farm discontinued the commercial production of raw milk. The local authority, THL and Evira announced the outbreak in the media in order to inform the public about the possible contamination of the raw milk from a particular producer and to advise to heat it to 75 °C for 15 s before consumption (Figure).

Discussion

We describe the outbreak of 55 cases of YP infection that occurred between February and April 2014 in southern Finland. The YP infection was associated with the consumption of raw milk from a single producer, with 98% of the cases consuming raw milk from this source. Microbiological evidence supported the epidemiological findings. An identical YP serotype and genotype were found in isolates from the patients, the milk filter from producer's farm and raw milk from a case's fridge.

Critical selection of controls is necessary to avoid introducing bias in the study. We used a method in which any healthy responding household member was suitable for being a matched control for a case. Choosing controls among household members may result in overmatching [18]. Despite the fact that household members may share similar dietary habits, we were able to demonstrate a strong association between raw milk consumption and illness.

An increase in outbreaks related to raw milk contaminated by a variety of pathogens has been observed [19]. Pasteurisation is a well-known and effective way to eliminate pathogens and bacteria from raw milk, decreasing the risk of disease transmission [20]. THL and the Finnish Food Safety Authority Evira recommend that children, the elderly, pregnant women and persons with a severe primary disease should heat-treat raw milk before consumption. In this study, age was associated with illness: those under 23 years of age were more likely to get ill. By contrast, previously

diagnosed chronic diseases did not affect the probability of YP infection. Our results are in line with findings from a study showing that healthy adults can become infected by consuming contaminated raw milk [21], and we suggest that also adults should heat raw milk before consuming it.

This outbreak was identified because a cluster of YP cases visited the same hospital. In Finland, *Yersinia* and *Campylobacter* infection notifications are not monitored in real time, and strains isolated from these cases do not need to be sent to the national reference laboratory for typing. Therefore, detection of geographically widespread *Yersinia* or *Campylobacter* outbreaks and routine comparison of YP isolates would be impossible. No YP profile identical to the current outbreak strain was identified among 61 Finnish YP strains tested in a previous study [15]. In this outbreak, identification of a cluster of cases allowed a timely response and prompt control measures. The producer voluntarily discontinued raw milk production and withdrew the product from the market immediately after the trawling interviews were completed. Simultaneously, the national authorities informed the public and healthcare professionals about health risks linked to raw milk consumption. No new YP cases were detected after the recall of the raw milk.

Despite the early detection and rapid control measures, this outbreak was larger than previously reported outbreaks linked to raw milk consumption [22]. Moreover, there were probably more cases than recorded. Additional cases on the basis of symptoms were detected by us in one in four of the responding households. Persons with severe symptoms are more likely to seek medical care and thus more likely to be sampled. It has been estimated that only ca 20% of persons contracting gastroenteritis consult a physician [23].

In this outbreak, the milk was contaminated by several organisms. The most probable route for contamination was through bovine faeces during milking. YP with an identical profile was detected in patients and bovine faeces. In addition, an identical *C. jejuni* profile was identified in one patient, bovine faeces and the milk filter. Raw milk may also become contaminated via cleaning water, cow mastitis or cross-contamination through humans or rodents [24]. Rodents and other small mammals are the main reservoirs for YP [2]. In previous YP outbreaks in southern Finland, rodents on farms were suggested as a possible source of YP transmission [3,25]. Further studies are needed to clarify the transmission dynamics of YP on farms.

In this study, the case from whose refrigerator raw milk had been sampled reported consumption of less than 1 dL of milk three days after the packaging date, and the amount of YP in tank milk was estimated at 26–120 cfu/mL at the same time point. While the infectious dose of YP is believed to be 10^8 or more bacteria if ingested orally [26], ingestion of fewer than 10^4 cfu of YP could have sufficed for infection in this outbreak. YP may reach infectious concentrations in products stored for at least several days at refrigerator temperature, since it can multiply at 4 °C [27].

YP is one of the pathogens included in the European Food Safety Authority's shortlist of microbiological hazards, indicating that it can be transmitted to humans through the milk [28]. It is, however, seldom studied and little information is available about its prevalence in unpasteurised milk [18,29,30]. No YP was found in raw cow milk samples from bulk tanks collected from 183 dairy farms in Finland in 2011 [31]. Raw cow milk producers in Finland are required to test their milk for *Listeria monocytogenes, Campylobacter* and STEC O26, O103, O111, O145 and O157 but not for YP [17]. In this outbreak, the producer's raw milk fulfilled the requirements of the law. Five persons who had consumed this producer's raw milk had fallen ill with campylobacteriosis in March 2014, before the YP outbreak notification. At that time, their illness could not be associated with the raw milk because *Campylobacter* had not been found in the producer's raw milk. The current testing regime for *Campylobacter* may be insufficient, because the concentration of the pathogen may be below the threshold of what is detectable in milk. In our investigations in April 2014, an identical *C. jejuni* genotype was detected in samples of one YP patient, bovine faeces and the producer's milk filter. Examination of milk filters instead of bulk tank milk samples seems to increase the probability of detection of pathogens. In the filter, the concentration of pathogens is higher than in bulk tank milk because large volumes of raw milk flow through it. The milk filter could therefore be used as the standard for testing.

Current control measures for raw milk production are not sufficient to prevent illness in those drinking this product. In this outbreak, contamination occurred although the raw milk met the microbiological criteria. The producer also fulfilled other legal requirements: raw milk was labelled with information on the possible microbial health hazards, risk groups and appropriate storage conditions in order to protect the consumer [17]. Before starting production, the producer is required to prove that the dairy cattle are free from *Salmonella* and STEC O157. Specific requirements are in place for the farm buildings and operations on the farm to avoid contamination of milk through contact with manure. The milk has to be cooled immediately after milking and stored at 6 °C or cooler [17]. The producer also met these requirements. It is relevant to review the legislation and to increase the awareness of the general public and policymakers concerning the potential hazards of raw milk consumption.

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Conflict of interest

None declared.

Authors' contributions

TP contributed to the collection and analysis of data and drafted the manuscript as the lead writer. SHa and MH were responsible for laboratory analysis of food and environmental samples and contributed to the comparison of human, food and environmental samples. SS was responsible for the microbiological investigation of the human samples and contributed to the comparison of human, food and environmental samples. AP contributed to the trace-back investigation. SHe was in charge of the local outbreak investigation. HTN contributed to the local outbreak investigation and was in charge of patient management. JO contributed to the case–control study design and statistical analysis of data. SHu contributed to the questionnaire design and data collection. RRF was responsible for the epidemiological investigations and contributed to the case–control study design, questionnaire design and analysis of the data. SS, SHa, AP, MH, SHe, JO and RRF contributed to manuscript writing. All co-authors critically reviewed the draft of the paper and approved the final version.

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Tick-borne encephalitis in north-east Italy: a 14-year retrospective study, January 2000 to December 2013

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Italy is considered at low incidence of tick-borne encephalitis (TBE), and the occurrence of human cases of TBE appears to be geographically restricted to the north east of the country. However, most information to date derives from case series, with no systematic data collection. To estimate incidence rates (IR) and spatial distribution of TBE cases, we conducted a retrospective study in north-eastern Italy. Data were collected through the infectious disease units and public health districts of three regions (Friuli Venezia Giulia, Trentino Alto Adige and Veneto) between 2000 and 2013. Overall, 367 cases were identified (IR: 0.38/100,000). The cases' median age was 56 years and 257 (70%) were male. Central nervous system involvement was reported in 307 cases (84%). Annual fluctuations in case numbers occurred, with peaks in 2006 and in 2013, when 44 and 42 cases were respectively observed. A strong seasonality effect was noted, with the highest number of cases in July. In terms of geographical location, three main endemic foci with high TBE IR (> 10/100,000) were identified in three provinces, namely Belluno (Veneto region), Udine (Friuli Venezia Giulia) and Trento (Trentino Alto-Adige). When investigating the whole study area in terms of altitude, the IR between 400 and 600 m was greater (2.41/100,000) than at other altitudes ($p < 0.01$). In conclusion, the incidence of TBE in Italy is relatively low, even considering only the three known affected regions. However, three endemic foci at high risk were identified. In these areas, where the risk of TBEV infection is likely high, more active offer of TBE vaccination could be considered.

Introduction

Tick-borne encephalitis (TBE) is considered a disease of the central nervous system (CNS) caused by infection with the TBE virus (TBEV), a flavivirus discovered in 1937, during an expedition in Far-East Russia [1].

There are three TBEV subtypes: the European, the Siberian, and the Far Eastern subtype. The main hosts and reservoirs of TBEV are small rodent species, whereas ticks (*Ixodes persulcatus* and *I. ricinus*) act as the vector [2]. The principal vector of the European TBEV subtype is *I. ricinus*.

The clinical manifestations of TBE usually start with a febrile illness, but ca 20 to 30% of the patients subsequently develop CNS disease, such as meningitis or encephalitis [2-4]. Thus, the neurological manifestation defining the syndromic picture of TBE underrepresent the overall clinical burden of disease related to TBEV infection.

The natural habitat of TBEV is represented by the forests of Europe and Asia. In the European continent, TBEV is distributed in an endemic pattern of so-called natural foci over central and north-eastern Europe, with the highest incidence (>10 cases/100,000) in Baltic countries, the Czech Republic, Russia, and Slovenia [5]. In

Table 1

Descriptive characteristics of persons diagnosed with tick-borne encephalitis in 'Triveneto', north-eastern Italy, 2000–2013 (n=367)

Characteristics		N (%)[a]
Nationality	Italians	355 (96.7)
	Others	12 (3.3)
Area of residence	Living in north-eastern Italy	364 (99.2)
	Living in other areas	3 (0.8)
Sex	Female	110 (30.0)
	Male	257 (70.0)
Age	Median (IQR)	56 (42–67)
Month of onset symptoms	January	2 (0.5)
	February	1 (0.3)
	March	3 (0.8)
	April	25 (6.8)
	May	50 (13.6)
	June	64 (17.4)
	July	79 (21.5)
	August	44 (12.0)
	September	32 (8.7)
	October	53 (14.4)
	November	11 (3.0)
	December	3 (0.8)
Clinical syndrome	Encephalitis	175 (47.7)
	Meningoencephalitis	94 (25.6)
	Febrile illness only	60 (16.4)
	Aseptic meningitis	25 (6.8)
	Meningoencephalomyelitis	13 (3.5)
Vaccinated	–	3 (0.8)
Sequelae	–	60 (16.3)
Deaths	–	2 (0.5)

IQR: interquartile range.

[a] Unless otherwise specified.

Austria, which is the only country with a significantly decreasing trend of TBE cases, due to mass vaccination programmes, the incidence in the unvaccinated population is ca five per 100,000 [5,6].

Italy is considered a country with geographically restricted, low incidence of TBE [6]. The disease is not notifiable in the country but individuals with TBE can be reported at the regional level, where they are classified as 'viral meningitis and encephalitis' (notifiable diseases, class 2). Although the extent of affected areas in Italy is not well defined, the regions of the north east appear to be those where cases have been documented, as suggested by case series of TBE published in the last decade [7-10]. However, to the best of our knowledge, systematic information on the occurrence of persons with TBE is lacking even in these affected areas of Italy, and both the incidence rates (IR) and the geographical distribution of persons with the disease have been mostly derived from international literature or reports of aggregated cases [6].

In the European Union (EU), TBE was added to the list of notifiable diseases in 2012 with a harmonised case definition focused on neuroinvasive illness with laboratory confirmation [11], highlighting the need of detailed epidemiological data.

To fill the gap in knowledge about TBE distribution in Italy, we conducted a retrospective study in three affected regions (Friuli Venezia Giulia, Trentino Alto Adige and Veneto) in the north-east of the country. The study was aimed at estimating the incidence of TBE (including febrile illness and/or CNS disease) in these regions from 2000 to 2013. We also sought to identify at risk areas within the affected regions, and to describe demographic parameters of TBE cases. Some environmental factors were also investigated.

Methods

Study area

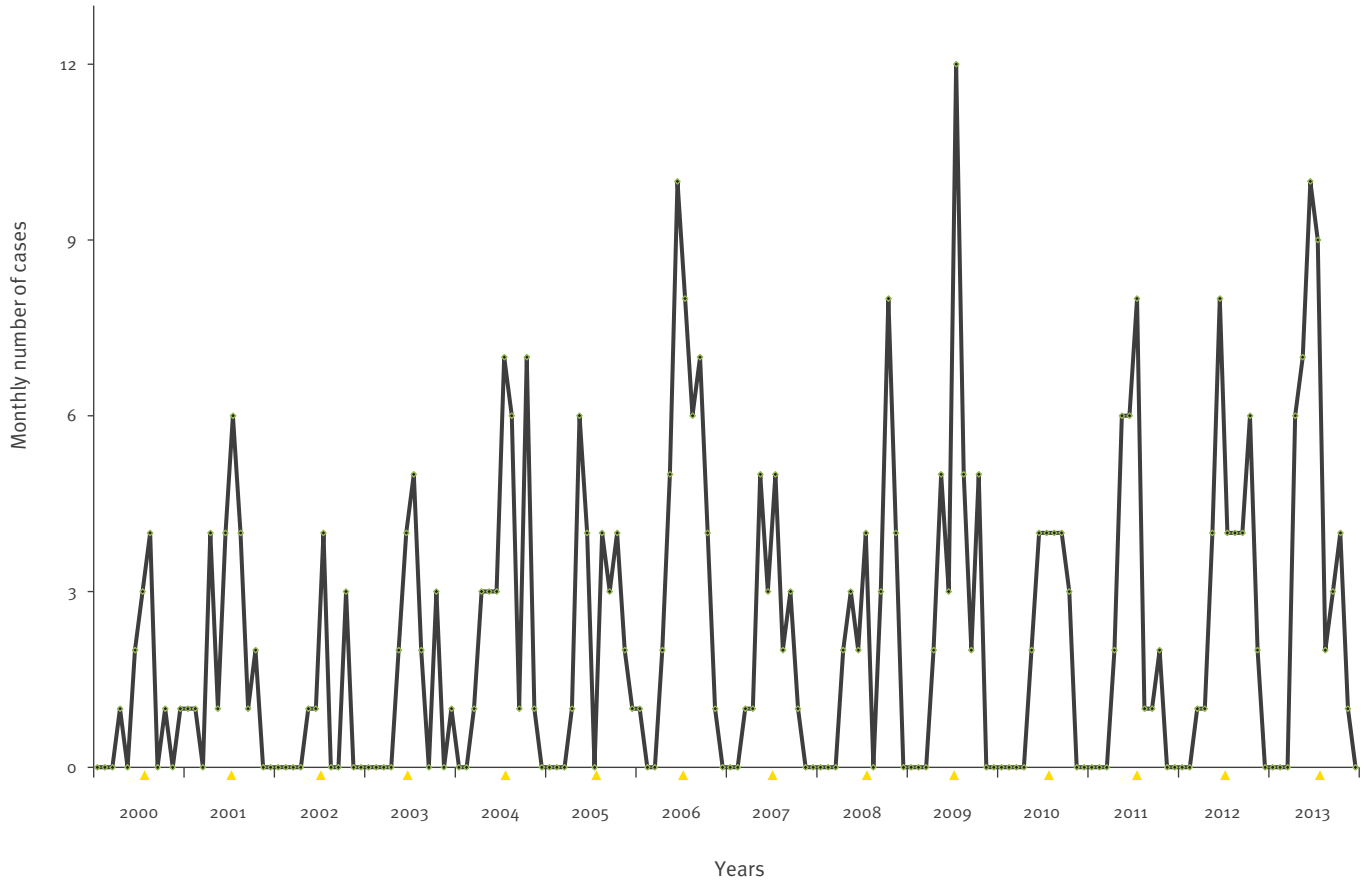
The study was conducted in three north-eastern Italian regions: Friuli Venezia Giulia, Trentino-Alto Adige (i.e. the autonomous provinces of Trento and Bolzano), and Veneto. Together, these regions, which cover 39,728 km^2, and at the beginning of 2013 comprised around 7.1 million inhabitants, are commonly referred to as 'Triveneto'. The northern and eastern parts of the 'Triveneto' area border with Austria and Slovenia respectively and have a mountainous landscape (the Alps), while the southern part comprises the eastern part of the Po valley. The area is administratively divided in around 1,130 municipalities and their altitude shows a strong gradient increasing from south to north. The study area was identified as that of major interest for TBE through a literature review (i.e. PubMed search using 'tick-borne encephalitis' or 'TBE' or 'TBEV' and 'Italy' as keywords, in addition to revision of grey literature, such as national and EU reports), and consultations with experts in the field. Entomological data (i.e. the distribution of TBEV infected ticks) was also revised using a similar approach (PubMed searches, examination of grey literature as well as reliance on expert opinion). On the basis of this information, the expected area of interest for TBE was only a fraction of the three regions; however, we included contiguous areas in the study, to exclude the occurrence of autochthonous cases, and to identify possible cases in individuals travelling to, and/or spending their vacations in the affected areas. Public health authorities and clinicians of other regions of northern Italy (i.e. those bordering regions or countries reporting TBE cases) were also contacted during the study to exclude the presence of known persons affected by TBE.

Data collection

To gather information on cases of TBE diagnosed between January 2000 and December 2013, all the infectious diseases units and public health districts in the study area were contacted. Study visits were planned to support clinicians in standardised data collection. Clinical registers were accurately checked;

Figure 1

Monthly distribution of tick-borne encephalitis cases in 'Triveneto', north-eastern Italy, 2000–2013 (n=367 cases)



The yellow marks (triangles) indicate the month of July of each year, as during this month a higher number of cases was frequently observed.

when needed, laboratory registries were revised by trained staff. Collection of information on TBE affected persons was guided by a questionnaire relating to demographic data (age, sex, place of residence, and nationality), clinical information (i.e. febrile illness, meningitis, encephalitis, meningoencephalitis, other neurological symptoms), and presumed place of exposure to tick bite. When available from the clinical register, data on environmental factors or behaviour, which could potentially have led to tick exposure were also gathered.

Case definition

To the purpose of this study, we adopted a case definition based on clinical and epidemiological criteria, similar to some of those used in other EU Member States [11].

A case of TBE was defined by the presence of a febrile illness, with/without encephalitis, meningitis, meningoencephalitis or meningoencephalomyelitis, and laboratory criteria (i.e. specific IgM and IgG response to TBEV, or seroconversion, or fourfold increase in IgG, or detection of TBEV nucleic acid by polymerase chain reaction).

Statistical analysis

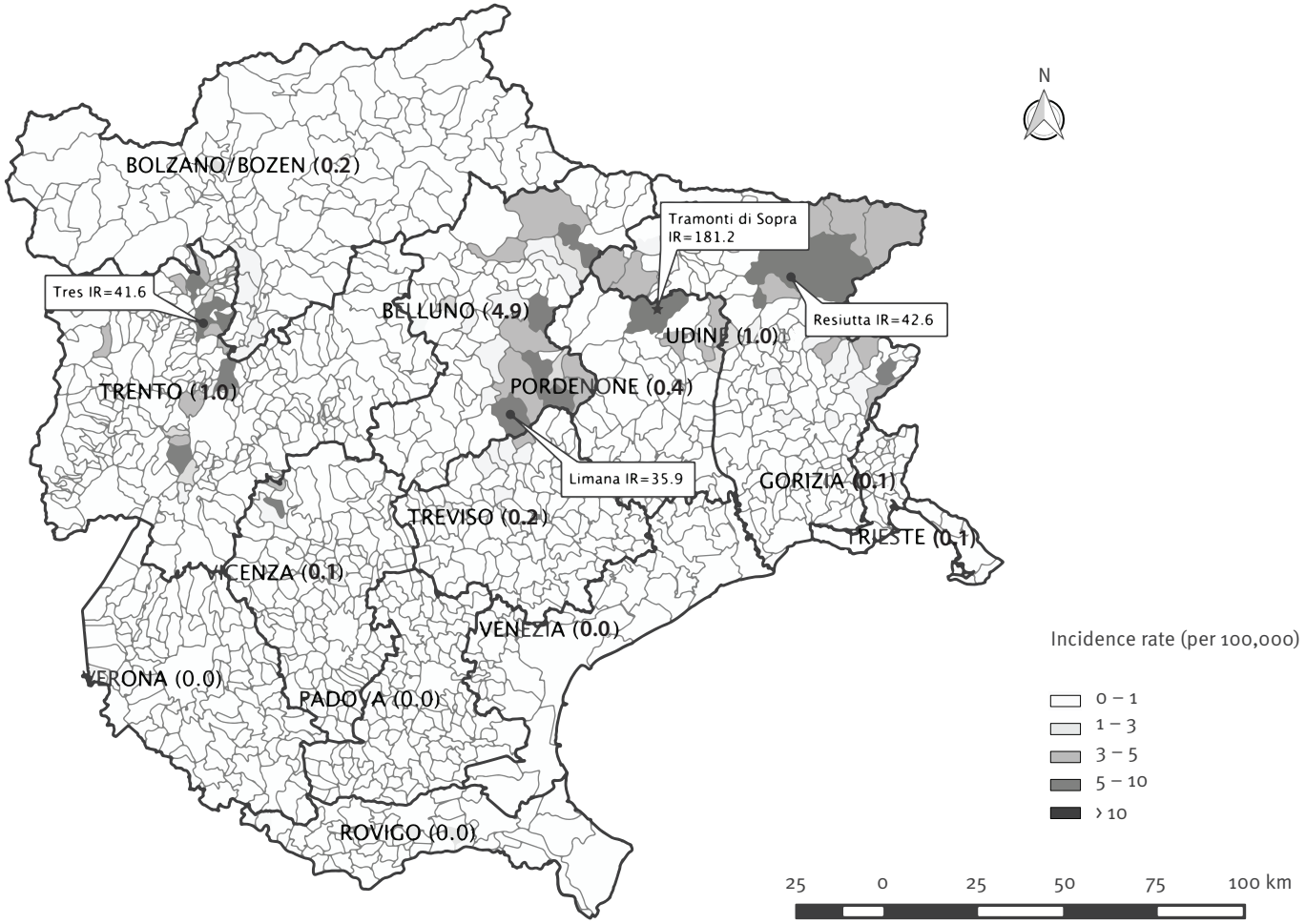
Descriptive techniques, such as frequency distributions, percentages, medians, and interquartile ranges, were used to summarise the data. As measure of TBE occurrence, IRs were then calculated as the number of cases per 100,000 individuals of population at risk. This population was considered that reported by the Italian National Bureau of Census (www.demo.istat.it) as individuals living at the beginning of each year in the study regions. IRs were also stratified by province and municipality of residence. The temporal trend was evaluated using Poisson regression; incidence rate ratios (IRR) per one calendar year were then calculated. IRRs by Poisson regression were also calculated to evaluate the association with the altitude of the municipality. IR per municipality of residence were graphically represented as geographical maps.

Results

During the period from 2000 to 2013, 367 cases (0.38/100,000 inhabitants) were diagnosed in the study area. Table 1 shows the main characteristics of the cases. Almost all of them (n=355) were Italian nationals and a majority of cases (n=364) was also residing in the study area; cases were mainly male (n=257; 70%), and around 70% (n=255) were between 30 and

Figure 2

Annual incidence rates (per 100,000 inhabitants) of tick-borne encephalitis cases by municipalities of residence in north-eastern Italy, 2000–2013



IR: incidence rate.

Numbers in parentheses are the IRs per province of residence. For some municipalities with high IRs, the names of the municipalities and the corresponding IR values are shown.

70 years-old. Encephalitis was the most common clinical presentation (n=175 cases), while febrile syndrome without CNS involvement (n=60 cases) accounted for less than a sixth of the cases. Symptom onset occurred mainly between April and October (n=347 cases; 94.5%). Almost all cases (n=364) were not vaccinated and whether full vaccination was achieved among those who were is not known. Sequelae were reported in 60 (16.3%) individuals. Of them, 23 developed tiredness, 19 paresis, 11 tremor, seven headache, six memory and/or concentration disturbances, three pain in the extremities; insomnia, anxiety, or nausea were reported by two patients, respectively, whereas hearing disturbance, dizziness, or electroencephalography anomalies only by one (data not shown in table). Two cases died (case fatality rate: 0.5%).

Modality of tick bite exposure was available for 156 of the 367 cases. Recreational activities were mentioned by 111 cases; of them, some specified trekking (n=25), mushrooming (n=16), whereas only five hunting. Living in a high risk area was reported by 32 individuals (for 6 of them it was a summer house). Nine cases were agricultural workers. Finally, four cases reported probable tick-bites while abroad (2 in Austria and 2 in Slovenia, respectively).

Figure 1 shows the distribution of the number of cases per month, during the study period. A strong seasonality could be observed, with the number of cases usually peaking in July, and in large part occurring between April and October (see also Table 1). Considering the annual IR, a significant temporal increase was observed during the study period, with annual IR increasing from 0.18 in the year 2000 to 0.59 per 100,000 in 2013 (IRR=1.05 per one calendar year increase; 95% confidence interval (CI): 1.02–1.08, $p<0.01$) (data not shown in Figure 1); however, the overall increase was mainly

Table 2

Incidence rates (IR) and incidence rate ratios (IRR) of tick-borne encephalitis (TBE) in 'Triveneto' according to the altitude of the municipalities of residence, north-eastern Italy, 2000–2013

Municipality altitude (metres)	Number of municipalities	Municipalities with at least one case N (%)	Total TBE cases	IR (per 100,000)	IRR (95% CI)	P-value[a]
0–200	589	44 (7.5)	78	0.10	1.00 (–)	<0.01
200–400	146	40 (27.4)	162	1.54	15.16 (11.57–19.86)	<0.01
400–600	101	22 (21.8)	70	2.41	23.81 (17.24–32.87)	–
600–800	103	15 (14.6)	28	1.27	12.52 (8.13–19.27)	<0.01
>800	196	17 (8.7)	26	0.55	5.42 (3.48- 8.45)	<0.01

[a] P-values refer to comparisons between IR in municipalities with a mean altitude between 400 and 600 m with that of the municipalities with another mean altitude.

due to the change in incidence that occurred between 2003 (IR: 0.25/100,000) and 2004 (IR: 0.48/100,000). In fact, after excluding the years before 2004, the estimated IRR was 1.01 (95%CI: 0.97–1.05, p = 0.64). Similar results were obtained when excluding from the analysis cases with only febrile illness (data not shown).

Figure 2 shows the map of the mean annual IR by municipality of residence. Noteworthy, the municipality with the highest IR in the entire area was Tramonti di Sopra (181.2/100,000) in the province of Pordenone. At the province level, the map clearly identifies three provinces with the highest IR: Belluno province (Veneto region), of which the municipality of Limana presented the highest IR (35.9/100,000), Udine province (Friuli Venezia Giulia region), with the municipality of Resiutta having the highest IR (42.6), and the Trento province (Trentino Alto-Adige region), with the highest IR observed in the municipality of Tres (41.6). The highest number of cases was also reported in these three provinces; in particular, the most affected municipalities were Belluno (n=49 cases), Limana (n=24), and Ponte nelle Alpi (n=17), all located in the province of Belluno (data not shown).

Table 2 summarises the number of cases, the estimated IR, and the IRR, after stratifying the municipalities of residence by altitude. Overall, there was a significant association in terms of altitude, with the lowest IR value in municipalities at an altitude ≤ 200 m and the highest in municipalities between 400 and 600 m. For altitudes above 600 m, IR decreased with further municipality altitude increase; however, the IR for municipalities with a mean altitude > 800 m remained five times significantly higher than for municipalities with a mean altitude of ≤ 200 m.

Discussion

Italy is considered a country at low risk for TBE, which appears to be restricted to a relatively small fraction of its territory. However to date, accurate information on the occurrence of TBE and/or TBEV infection in Italy is not available, and the major source of data has originated from case reports or case series [6-10], all from the north-eastern regions, and especially from areas bordering high incidence countries such as Austria and Slovenia. Thus, our data allow, for the first time, to accurately calculate incidence rates and to describe the geographical distribution of TBE cases in the affected Italian areas.

The total number and IR of cases found in our study appear to be much higher than that reported in the past, presumably from the same geographical area. For example, according to an article summarising data from different European countries, only 102 cases were observed in Italy in the period from 1975 to 2001; of these, 14 occurred between 1975 and 1991, and 88 from 1992 to 2001 [12]. However, a direct comparison with these data is not possible, since their source and the study area were not well defined.

We found an IR of ca 0.4 per 100,000 in the overall study area. However, at the level of the most affected provinces of Trento, Belluno, and Udine, the IR ranged between 1 and 5 per 100,000 which appears to be lower than that reported in neighbouring countries such as Austria (before mass vaccination) and Slovenia [6]. In particular, incidence rates of 17.9 per 100,000 (ranging from 1.2 to 42.8/100,000 over the last four decades) and eight per 100,000 have been reported respectively in the unvaccinated population of Carinthia and Tirol, two Austrian regions bordering 'Triveneto' [13].

The annual number of cases observed in 'Triveneto' tended to vary, showing two peaks respectively in 2006 and 2013, whereas the lowest number of cases was registered in 2002. Yearly trend fluctuations were observed also in other countries such as Switzerland, where a study from 2005 to 2011 found the lowest and the highest number of cases in 2002 and 2006, respectively [14].

Most cases occurred between April and October, with peaks in June and July. This is rather consistent with the seasonality of tick activity, with slight differences compared with central Europe, where seasonal peaks

of feeding activity of ticks tend to occur between May and June, and between September and October [1].

The demographic characteristics of our patients, showing male predominance and rather advanced median age, were similar to those observed in neighbouring countries such as Switzerland, where TBE incidence peaked in 60–69 year-olds, and male predominance was observed in all age groups [14].

Most cases were concentrated in three main foci, one in the autonomous province of Trento, one in the pre-alpine Belluno province of Veneto, and one at the extreme north-east of Friuli Venezia Giulia. In this respect, it should be underlined that TBEV epidemiology is closely associated with ticks' ecology, and that ticks inhabit specific foci in the forests with enhanced moist vegetation and wild animals supplying blood meals for them [1]. The special combination of climatic conditions, including temperature, moisture, and vegetation, occurring only within specific geographical zones, provides an heterogeneous tick distribution, generating as many as 20,000 or even 30,000 estimated natural foci of TBEV infections across the northern hemisphere [1].

The heterogeneous geographical distribution of human cases in our study appears to be consistent with that of competent vectors, as suggested by entomological studies conducted in the same area. To this regard, TBEV was detected in 2.1% of 193 ticks collected in 14 sites of north-eastern Italy between April and June in the years 2006 to 2008 [15]. Further studies showed that the density distribution of competent ticks and the prevalence of TBEV infection is not homogeneously distributed within the affected regions. In fact, 0.21% of 2,361 of *I. ricinus* ticks collected in the alpine area of the extreme north-eastern Italy (Friuli Venezia Giulia) between 2005 and 2006 were positive for TBEV, but all of them were found in three sites where the highest tick density was found [16].

Recreational activities were found to be the most common possible exposure to tick bite in our study; however, almost all the cases were reported among residents. This might be due to the fact that, though the Alps represent a touristic area, tourists tend to spend their vacations at higher altitudes than those found to be at highest risk. To this regard, a case–control study conducted in Poland found that, in endemic areas, the highest TBE risk was associated with spending at least 10 hours a week in mixed forests and harvesting forest foods, being unemployed, or employed as a forester [17].

TBEV infection may be prevented not only adopting behavioural precautions, but also by using effective vaccines. In our study, only three individuals reported having been vaccinated against TBE; however, whether they had completed the full vaccination schedule is unknown. In Austria, data collected in the period between 1972 and 2011 showed that mass vaccination reduced incidence to ca 16% of that of the pre-vaccination era: average incidence declined from 5.7 per 100,000 in the first ten years to 0.9 per 100,000 in the last decade of the study, while TBE vaccination, which started in 1972, reached 88% in 2005 [18]. TBE vaccination is recommended to high risk population groups (foresters, scouts, persons with hobbies or leisure activities potentially leading to tick exposure) in Veneto and in Trentino Alto Adige. In Friuli Venezia Giulia, vacccination has been offered free of charge to all the population of the region since 2013; however, whether vaccination had any impact on the disease burden in north-eastern Italy remains undefined.

Before drawing conclusions, some limits and possible biases of our study should be mentioned. Firstly, our case-definition included also febrile illness without CNS involvement. This may supply a more comprehensive figure of TBE cases and their distribution, but it makes the data less reliable since a large proportion of febrile cases could have not been diagnosed; moreover, the proportion of undetected cases may vary unpredictably in time and space. Secondly, we cannot rule out completely the presence of TBE foci in other Italian regions. To this purpose, it should be reminded that in the 1970s, a few TBE cases and infected ticks were found in Tuscany [19,20]. However, recent studies on the aetiology of meningoencephalitis have not confirmed these findings (data not shown). Moreover, studies of forestry rangers showed a seropositivity of 0.6% for TBE in the north-eastern region of Friuli Venezia Giulia [21], while none of those investigated in Tuscany or Lazio (both regions are located in central Italy) had TBE antibodies [22,23]. Finally, we cannot exclude completely the occurrence of a few cases in other regions of northern Italy, such as Lombardy, bordering Switzerland, another country at relatively high incidence of TBE. However, as reported above, active surveillance failed to identify human cases of TBE. These findings highly suggest that TBEV infection is restricted to the north-eastern area of the country, and it does not seem for the moment to expand its range to other Italian areas. The absence of cases away from the Alps area, even in the surveyed provinces of the three affected regions of north-eastern Italy, strengthens this argument.

In conclusion, seasonal occurrence of human cases of TBE in three regions of north-eastern Italy is confirmed. The incidence is relatively low, when compared with other countries in central and northern Europe. However, three endemic foci where risk of TBEV infection is likely high, were identified. Moreover the highest IRs were between 400 and 600 m of altitude. Most cases were detected among residents performing recreational activities. More active offer of anti-TBE vaccine to people living in high risk areas might be considered.

TBE Virology Group

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Conflict of interest

None declared.

Authors' contribution

GR had the original idea and wrote the first draft; FF, MC, and ALP supervised data collection, and FF also organised the study and managed the database; PP performed statistical analysis and revised the manuscript; all the other authors contributed to patient diagnosis and data collection at the local level.

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European Commission launches consultation on preliminary opinion on access to health services

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The European Commission together with the 'Expert Panel on effective ways of investing in health' have launched a public consultation on the preliminary opinion on the 'Access to health services in the European Union' (EU).

The preliminary opinion seeks to highlight barriers to accessing healthcare and discusses possible policy responses to address them.

It focuses on the needs of underserved groups as Roma, undocumented migrants and people with mental health problems.

Access to healthcare was improved in the EU between 2005 and 2009 and the number of people who reported that their medical needs were not being met fell from 24 million to 15 million over that period of time. However, the economic crisis in Europe reversed this positive trend and 18 million EU citizens reported needs which had not been met by healthcare, in 2013.

Those interested in submitting contributions should do so by 6 November.

The preliminary opinion is available here: http://ec.europa.eu/health/expert_panel/opinions/docs/010_access_healthcare_en.pdf .

To read more about the consultation and to contribute, see here: http://ec.europa.eu/health/expert_panel/consultations/access_healthcare_en.htm

EU project provides training materials for health professionals to improve delivery of services for ethnic minorities and migrants

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Article 08 October 2015

On 2 October, the European Commission presented European Union-funded training packages for health professionals aimed at improving health services for ethnic minorities and migrants in Europe, at a workshop in Brussels.

Developed by academic institutions from Denmark, Italy, the Netherlands and Spain the training packages have been tailored for front-line healthcare professionals and includes modules specifically developed for the Roma population. The final training package is expected to be available in January 2016.

To read more about the initiative, see here: http://ec.europa.eu/dgs/health_food-safety/dyna/enews/enews.cfm?al_id=1624