Eurosurveillance
Europe's journal on infectious disease epidemiology, prevention and control

Impact factor 5·7

Vol. 21 | Weekly issue 34 | 25 August 2016

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Cluster of human parechovirus infections as the predominant cause of sepsis in neonates and infants, Leicester, United Kingdom, 8 May to 2 August 2016

JW Tang[1,2], CW Holmes[1], FA Elsanousi[1], A Patel[1], F Adam[1], R Speight[3], S Shenoy[3], D Bronnert[3], G Stiefel[3], P Sundaram[3], S Pande[3], A Sridhar[3], V Kairamkonda[3], S Bandi[3]

1. Clinical Microbiology and Virology, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom
2. Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom
3. Leicester Childrens Hospital, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom

Correspondence: Julian Tang (julian.tang@uhl-tr.nhs.uk)

Citation style for this article:
Tang JW, Holmes CW, Elsanousi FA, Patel A, Adam F, Speight R, Shenoy S, Bronnert D, Stiefel G, Sundaram P, Pande S, Sridhar A, Kairamkonda V, Bandi S. Cluster of human parechovirus infections as the predominant cause of sepsis in neonates and infants, Leicester, United Kingdom, 8 May to 2 August 2016. Euro Surveill. 2016;21(34):pii=30326. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.34.30326

Article submitted on 11 August 2016 / accepted on 25 August 2016 / published on 25 August 2016

We report an unusually high number of cases (n = 26) of parechovirus infections in the cerebrospinal fluid (CSF) of neonates and infants admitted with sepsis in the United Kingdom during 8 May to 2 August 2016. Although such infections in neonates and infants are well-documented, parechovirus has not been routinely included in many in-house and commercial PCR assays for CSF testing. Clinicians should consider routine parechovirus testing in young children presenting with sepsis.

Parechoviruses usually causes self-limiting, mild gastroenteritis and respiratory infections, though more severe neurological and cardiovascular complications are possible. We report a sudden and unusual increase in the number of cases of human parechovirus (HPeV) infection in neonates and infants admitted to hospital with sepsis during May to August 2016, in Leicester, United Kingdom (UK).

The aim of this report is to alert other teams in Europe and elsewhere, who may not test for HPeV routinely, either in respiratory, enteric or cerebrospinal fluid (CSF) samples, in neonates and infants admitted to hospital for respiratory illness, gastroenteritis or sepsis.

Detection of a cluster of parechovirus infections

In this case series, human parechovirus PCR testing on CSF was a routine part of the septic workup for any neonate or infant admitted to hospital presenting with any combination of fever, lethargy or drowsiness, rash, poor-feeding, tachycardia and irritability.

During routine diagnostic testing of neonates and infants admitted with suspected sepsis, where CSF was taken and tested as part of the septic workup, we confirmed 26 cases (15 male, 11 female) of HPeV infection in neonates and infants aged between 8 and 197 days (median: 47).

This is in contrast to previous years: in 2015, one case was diagnosed (in July), in 2014, 10 cases were diagnosed over a four-month period (between March and July), in the same hospital using the same assay and clinical testing algorithm. The unusual aspect of this cluster was the sudden appearance of multiple cases within this short three-month period (May to August 2016) (Figure).

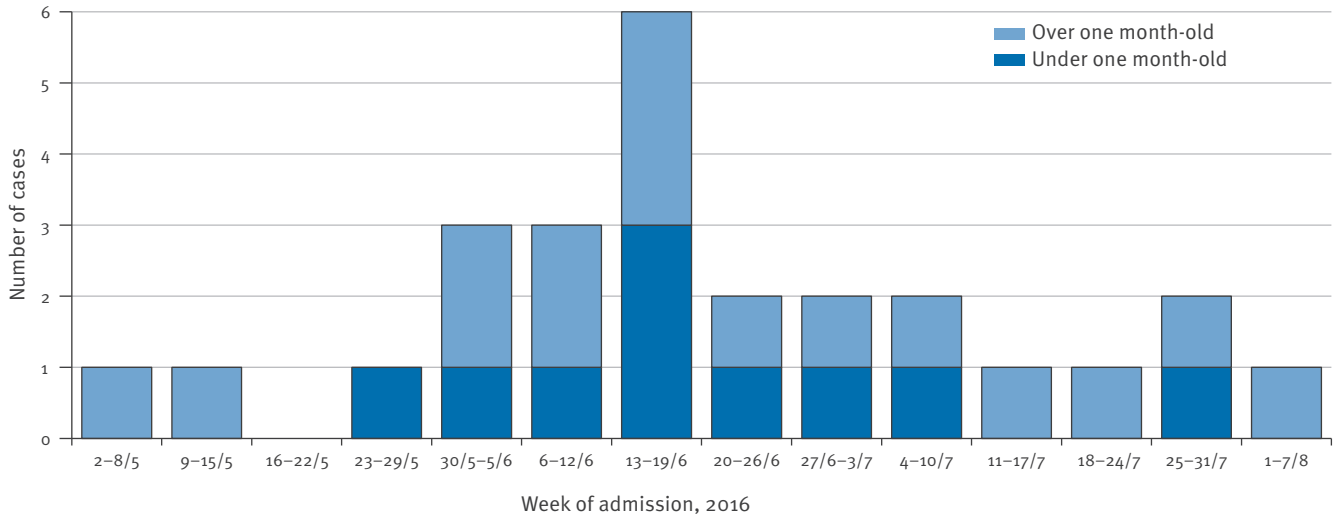
These cases were diagnosed by testing of CSF samples using a combination of multiplex PCR assays. This included a commercial polymerase chain reaction (PCR) assay for the detection of enterovirus and HPeV (FTD EPA, Fast-track diagnostics Ltd, Sliema, Malta). Although this kit is marketed specifically for respiratory and stool specimens, we have internally validated it for CSF testing also, to take advantage of the EV and HPeV components. While adenovirus is also part of this kit, this target is not routinely screened for in standard CSF panels, so this component of the kit was not used. In all of these cases, all the other targets in our CSF test panel (herpes simplex virus (HSV) 1 and 2, varicella zoster virus (VZV) and enterovirus) were screened for using an in-house assay, and were negative. The in-house HSV-1, HSV-2 and VZV PCR assays were adapted from previously published protocols [1,2].

All 26 cases presented with very similar symptoms of generalised sepsis, including high fever (up to 40 °C), lethargy or drowsiness, poor feeding, tachycardia, grunting, mottled or petechial rash and irritability, with no other viral or bacterial agent found in systemic samples (i.e. by PCR testing or blood cultures).

In most of these cases (n = 24), the CSF glucose and protein levels were within normal limits, and all but

Figure

New cases of human parechovirus infection in neonates and infants admitted with sepsis, Leicester, United Kingdom, 8 May–2 August 2016 (n = 26)



The first case was reported on 8 May, the last on 2 August 2016.

one (one sample could not be tested as it was clotted) had a total white cell count of <10 (Table). Just under half of the patients (n = 11) had moderately elevated levels for liver function tests (alanine aminotransferase or total bilirubin) (Table). In addition, in two cases, their gamma-glutamyl transferase level was elevated (80 and 200 IU/L; norm: 0–35 IU/L) and in another two, their alkaline phosphatase level was raised (424 and 529 IU/L; norm: 60–245 IU/L).

While the initial presentation was of sufficient clinical concern to lead to hospital admission, in most cases, the disease settled without further complication. Most patients (n = 18) were discharged after two to four days.

However, in one neonate, there was a more severe illness, with sepsis and encephalitis, requiring inotropic support and ventilation. A tonic seizure occurred on day two of admission, and HPeV was detected in the CSF. Further testing detected HPeV in the stool, blood and a throat swab, confirming HPeV sepsis, and intravenous immunoglobulin was given. A follow-up electroencephalogram and magnetic resonance imaging of the brain both indicated encephalitis. The neonate was discharged after 10 days with no obvious neurological sequelae.

Background

Parechovirus is a non-enveloped, single-stranded RNA virus within the family *Picornaviridae*, which also includes rhinoviruses and enteroviruses. There are at least 16 different human HPeV types, of which HPeV type 3 is the most common cause of clinical disease in humans [3]. The spectrum of disease (mainly for HPeV 3) can range from self-limiting mild gastroenteritis and respiratory infections to more severe neurological complications (acute flaccid paralysis, encephalitis) and myocarditis [4].

Infections with HPeV in neonates and infants have been well-documented [5-10], but HPeV has only relatively recently been included as a target in our in-house and some commercial PCR assays used for testing CSF. This is most likely due to the growing recognition of HPeV as a common potential cause of sepsis and febrile seizures from various studies and outbreak investigations in recent years [11-16].

Discussion

This increase in the number of HPeV infections associated with sepsis in neonates and infants is now being confirmed elsewhere in the UK, and viral sequencing analysis is currently in progress for samples from these 26 cases and others (David Allen, Public Health England, personal communications, July 2016).

Other recent reports of HPeV activity include an outbreak of HPeV infection in 55 neonates and infants (up to the age of three months) in Queensland, Australia, between September 2015 and February 2016 [17]. The presentation of these cases was very similar to that described for the 26 UK cases reported here (i.e. high temperature, diarrhoea, abnormally rapid breathing, severe irritability or appearing to be in pain, rashes or skin discolouration and jerking movements).

A recently published Norwegian study found HPeV in 9% (30/343) respiratory samples taken from 161 preschool children and toddlers (aged 1–6.3 years), during

Table

Age, duration of hospital stay and key laboratory parameters for 26[a] cases of human parechovirus infection, Leicester, United Kingdom, 8 May–2 August 2016

Parameter	Median (range)
Age, in days	35 (8–197)
Duration of hospital stay, in days	4 (2–10)
C-reactive protein Norm: 0–10 mg/L	<5 (<5–40) mg/L
Total white cell count Norm: 6.0–17.5 × 10^9/L	6.3 (2.6–17.4) × 10^9/L
Lymphocytes Norm: 4.0–13.5 × 10^9/L	1.93 (0.91–3.59) × 10^9/L
Neutrophils Norm: 1.0–8.5 × 10^9/L	2.91 (1.20–13.92) × 10^9/L
Platelets Norm: 140–400 × 10^9/L	327 (174–661) × 10^9/L
Alanine transferase[b] Norm: 5–100 IU/L	24 (9–359) IU/L
Total bilirubin[b] Norm: 0–21 µmol/L	15 (4–217) µmol/L
Cerebrospinal fluid	
Glucose Norm: 2.5–4.4 mmol/L	3.1 (2.1–4.1) mmol/L
Protein Norm: 0.2–0.8 g/L	0.38 (0.20–1.56) g/L
Total white cell count[c] Norm: 0 × 10^6/L	1 (0–4) × 10^6/L
Red blood cells[c] Norm: 0 × 10^6/L	3 (0–5,520) × 10^6/L

CSF: cerebrospinal fluid; IU: international units.

[a] Unless otherwise indicated.

[b] For 21 of the 26 patients (not all patients were tested for all laboratory parameters, depending on the presentation of the patient).

[c] For 25 of the 26 patients, as one sample was clotted.

a two-year study in which screening was carried out for 19 respiratory virus targets. This community-based study focused on relatively mild cases of respiratory infection that did not require medical attention outside of the study [18]. This is in contrast to our case series, in which HPeV was first tested and detected in CSF in neonates and infants who were considered ill enough to be admitted to hospital for investigation. In all but one of these cases, the illness was self-limiting and no further testing was required. In the one severely ill case described above, further HPeV testing was positive in stool, blood and a throat swab, confirming disseminated infection, which may have explained the severity of the illness. Transient viraemia may well have occurred in all these sample types in the other cases, but their self-limiting illness did not justify further sampling and testing for this.

Our routine PCR panel for testing respiratory samples is not validated for and therefore does not currently include HPeV, but in light of our findings reported here, we are now considering adding this. It is possible that HPeV may contribute to febrile seizures in young children that are often preceded by a non-specific febrile respiratory illness [19-21]. For this reason also, routinely including HPeV detection in our respiratory panel is being considered.

From our experience with this ongoing case series of neonates and infants admitted for sepsis, we would recommend testing for HPeV in CSF, respiratory samples and/or stool samples, particularly for those patients presenting with unusually high fever and irritability, especially if no other pathogen can be identified.

For other infants and other young children (older than 1 year), HPeV testing may be performed on stool samples if they present with gastroenteritis (abdominal pain, diarrhoea and vomiting); or on respiratory samples such as nasopharyngeal aspirates, if they present with respiratory symptoms (e.g. bronchiolitis and croup); or on CSF if they present with febrile seizures, and other aseptic meningitis symptoms (such as photophobia, lethargy, poor feeding and poor responsiveness). Again, all of these sample types can be tested for HPeV if generalised, systemic sepsis is suspected (which could result in a combination of all of these symptoms).

Although HPeV testing in stool is the most useful to determine the duration of HPeV shedding for hospital infection control purposes, usually, these paediatric patients are discharged home as soon as they have recovered sufficiently, clinically, to minimise any onward transmission of HPeV to other patients on the ward.

In addition, as several cases exhibited tachycardia, a routine baseline electrocardiogram should also be recorded, as HPeV has been reported to cause cardiac problems [5,22]. Each of the 26 cases is currently under longer-term outpatient follow-up to check for any late central nervous or cardiovascular sequelae from this viral infection.

While no specific therapy is available, testing for HPeV as the cause of sepsis and/or encephalitis in these young children should be routine (along with testing for enteroviruses), even if the typical laboratory markers indicating sepsis may be relatively normal. This may reduce or prevent prolonged unnecessary empirical antibiotic treatment, thereby reducing the risk of antibiotic resistance arising, as well as optimising clinical care and the use of resources.

Acknowledgments

We would like to thank other members of the paediatric team who cared for these patients: Drs A Kansara, D Britt, J Mistry, J Cusack, J Forster and D Luyt.

Conflict of interest

None declared.

Authors' contributions

Tang JW - conceived the study, wrote/edited the first draft and its various revisions into the final submitted draft. Holmes CW - compiled the Table of clinical data and the Figure, and critically reviewed the manuscript through the various revisions. Elsanousi FA - collated/compiled clinical data for the Table. Patel A, Adam F - processed the clinical samples and performed the CSF parechovirus PCR testing. Speight R - nursing sister in charge of ward caring for many of these patients; assisted in collating the parechovirus patient list from various wards. Shenoy S, Bronnert D, Stiefel G, Sundaram P, Sridhar A, Pande S, Venkatesh K, Bandi S - paediatricians caring for these patients, collated the clinical data; critically reviewed and gave final approval of the version of the manuscript to be published.

References

1. Namvar L, Olofsson S, Bergström T, Lindh M. Detection and typing of Herpes Simplex virus (HSV) in mucocutaneous samples by TaqMan PCR targeting a gB segment homologous for HSV types 1 and 2.J Clin Microbiol. 2005;43(5):2058-64. DOI: 10.1128/JCM.43.5.2058-2064.2005 PMID: 15872222
2. Weidmann M, Meyer-König U, Hufert FT. Rapid detection of herpes simplex virus and varicella-zoster virus infections by real-time PCR.J Clin Microbiol. 2003;41(4):1565-8. DOI: 10.1128/JCM.41.4.1565-1568.2003 PMID: 12682146
3. de Crom SC, Rossen JW, van Furth AM, Obihara CC. Enterovirus and parechovirus infection in children: a brief overview.Eur J Pediatr. 2016;175(8):1023-9. DOI: 10.1007/s00431-016-2725-7 PMID: 27156106
4. Sun G, Wang Y, Tao G, Shen Q, Cao W, Chang X, et al. Complete genome sequence of a novel type of human parechovirus strain reveals natural recombination events. J Virol. 2012;86(16):8892-3. DOI: 10.1128/JVI.01241-12 PMID: 22843855
5. Verboon-Maciolek MA, Krediet TG, Gerards LJ, de Vries LS, Groenendaal F, van Loon AM. Severe neonatal parechovirus infection and similarity with enterovirus infection. Pediatr Infect Dis J. 2008;27(3):241-5. DOI: 10.1097/INF.0b013e31815c1b07 PMID: 18277927
6. Verboon-Maciolek MA, Groenendaal F, Hahn CD, Hellmann J, van Loon AM, Boivin G, et al. Human parechovirus causes encephalitis with white matter injury in neonates. Ann Neurol. 2008;64(3):266-73. DOI: 10.1002/ana.21445 PMID: 18825694
7. Wolthers KC, Benschop KS, Schinkel J, Molenkamp R, Bergevoet RM, Spijkerman IJ, et al. Human parechoviruses as an important viral cause of sepsislike illness and meningitis in young children. Clin Infect Dis. 2008;47(3):358-63. DOI: 10.1086/589752 PMID: 18558876
8. Sedmak G, Nix WA, Jentzen J, Haupt TE, Davis JP, Bhattacharyya S, et al. Infant deaths associated with human parechovirus infection in Wisconsin. Clin Infect Dis. 2010;50(3):357-61. DOI: 10.1086/649863 PMID: 20047496
9. Eis-Hübinger AM, Eckerle I, Helmer A, Reber U, Dresbach T, Buderus S, et al. Two cases of sepsis-like illness in infants caused by human parechovirus traced back to elder siblings with mild gastroenteritis and respiratory symptoms. J Clin Microbiol. 2013;51(2):715-8. DOI: 10.1128/JCM.02731-12 PMID: 23241372
10. Fischer TK, Midgley S, Dalgaard C, Nielsen AY. Human parechovirus infection, Denmark.Emerg Infect Dis. 2014;20(1):83-7. DOI: 10.3201/eid2001.130569 PMID: 24377661
11. Esposito S, Rahamat-Langendoen J, Ascolese B, Senatore L, Castellazzi L, Niesters HG. Pediatric parechovirus infections.J Clin Virol. 2014;60(2):84-9. DOI: 10.1016/j.jcv.2014.03.003 PMID: 24690382
12. Jeziorski E, Schuffenecker I, Bohrer S, Pain JB, Segondy M, Foulongne V. Relevance of human parechovirus detection in cerebrospinal fluid samples from young infants with sepsis-like illness.J Clin Lab Anal. 2015;29(2):112-5. DOI: 10.1002/jcla.21737 PMID: 24687608
13. Cabrerizo M, Trallero G, Pena MJ, Cilla A, Megias G, Muñoz-Almagro C, et al. Comparison of epidemiology and clinical characteristics of infections by human parechovirus vs. those by enterovirus during the first month of life. Eur J Pediatr. 2015;174(11):1511-6. DOI: 10.1007/s00431-015-2566-9 PMID: 25982340
14. Cumming G, Khatami A, McMullan BJ, Musto J, Leung K, Nguyen O, et al. Parechovirus Genotype 3 Outbreak among Infants, New South Wales, Australia, 2013-2014. Emerg Infect Dis. 2015;21(7):1144-52. DOI: 10.3201/eid2107.141149 PMID: 26082289
15. Vollbach S, Müller A, Drexler JF, Simon A, Drosten C, Eis-Hübinger AM, et al. Prevalence, type and concentration of human enterovirus and parechovirus in cerebrospinal fluid samples of pediatric patients over a 10-year period: a retrospective study. Virol J. 2015;12(1):199. DOI: 10.1186/s12985-015-0427-9 PMID: 26607060
16. Britton PN, Dale RC, Elliott E, Festa M, Macartney K, Booy R, et al. Pilot surveillance for childhood encephalitis in Australia using the Paediatric Active Enhanced Disease Surveillance (PAEDS) network. Epidemiol Infect. 2016;144(10):2117-27. DOI: 10.1017/S0950268816000340 PMID: 26916674
17. ProMedMail. Parechovirus infection – Australia: (Queensland) Children. Archive Number: 20160219.4035899. 19 Feb 2016. Available from: http://promedmail.org/post/4035899
18. Moe N, Pedersen B, Nordbø SA, Skanke LH, Krokstad S, Smyrnaios A, et al. Respiratory Virus Detection and Clinical Diagnosis in Children Attending Day Care. PLoS One. 2016;11(7):e0159196. DOI: 10.1371/journal.pone.0159196 PMID: 27433803
19. Teran CG, Medows M, Wong SH, Rodriguez L, Varghese R. Febrile seizures: current role of the laboratory investigation and source of the fever in the diagnostic approach. Pediatr Emerg Care. 2012;28(6):493-7. DOI: 10.1097/PEC.0b013e3182586f90 PMID: 22653461
20. Tang J, Yan W, Li Y, Zhang B, Gu Q. Relationship between common viral upper respiratory tract infections and febrile seizures in children from Suzhou, China.J Child Neurol. 2014;29(10):1327-32. DOI: 10.1177/0883073813515074 PMID: 24453149
21. Sharawat IK, Singh J, Dawman L, Singh A. Evaluation of Risk Factors Associated with First Episode Febrile Seizure.J Clin Diagn Res. 2016;10(5):SC10-3. DOI: 10.7860/JCDR/2016/18635.7853 PMID: 27437319
22. Eisenhut M. Features of Myocarditis in Infants With Human Parechovirus Infection.Clin Infect Dis. 2015;61(1):139. DOI: 10.1093/cid/civ237 PMID: 25810286

License and copyright

Increase in reptile-associated human salmonellosis and shift toward adulthood in the age groups at risk, the Netherlands, 1985 to 2014

L Mughini-Gras[1], M Heck[1], W van Pelt[1]
1. National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Bilthoven, the Netherlands

Correspondence: Lapo Mughini-Gras (lapo.mughini.gras@rivm.nl)

Citation style for this article:
Mughini-Gras L, Heck M, van Pelt W. Increase in reptile-associated human salmonellosis and shift toward adulthood in the age groups at risk, the Netherlands, 1985 to 2014. Euro Surveill. 2016;21(34):pii=30324. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.34.30324

Article submitted on 14 October 2015 / accepted on 29 March 2016 / published on 25 August 2016

While the contribution of the main food-related sources to human salmonellosis is well documented, knowledge on the contribution of reptiles is limited. We quantified and examined trends in reptile-associated salmonellosis in the Netherlands during a 30-year period, from 1985 to 2014. Using source attribution analysis, we estimated that 2% (95% confidence interval: 1.3–2.8) of all sporadic/domestic human salmonellosis cases reported in the Netherlands during the study period (n = 63,718) originated from reptiles. The estimated annual fraction of reptile-associated salmonellosis cases ranged from a minimum of 0.3% (corresponding to 11 cases) in 1988 to a maximum of 9.3% (93 cases) in 2013. There was a significant increasing trend in reptile-associated salmonellosis cases (+19% annually) and a shift towards adulthood in the age groups at highest risk, while the proportion of reptile-associated salmonellosis cases among those up to four years-old decreased by 4% annually and the proportion of cases aged 45 to 74 years increased by 20% annually. We hypothesise that these findings may be the effect of the increased number and variety of reptiles that are kept as pets, calling for further attention to the issue of safe reptile–human interaction and for reinforced hygiene recommendations for reptile owners.

Introduction

Salmonella is a natural inhabitant of the reptile gut microflora, detected in ca 50% of reptile pets [1]. As pet reptiles have become increasingly popular, so have reptile-associated *Salmonella* infections in humans [2]. Most *Salmonella* isolates from reptiles belong to the *Salmonella enterica* subspecies II (*salamae*), IIIa (*arizonae*), IIIb (*diarizonae*), VI (*houtenae*), and a few to *S. bongori* (formerly subspecies V) and VI (*indica*). However, also the subspecies I (*enterica*), mainly associated with warm-blooded organisms, is often found in reptiles [3], as it can be present in the reptiles' meals (e.g. rodents, birds or raw vegetables). Accordingly, exposure to reptiles is associated with a four- and twofold increased risk for infection with typical and atypical reptile-associated *Salmonella*, respectively [2]. Moreover, reptile-associated salmonellosis mainly affects young children and results in a higher incidence of hospitalisation and invasive disease than other *Salmonella* infections [2].

The contribution of the main food-related sources to human salmonellosis is well documented [4-7]. In contrast, the knowledge on the contribution of reptiles is limited [3]. In order to address this knowledge gap, we quantified and examined trends in reptile-associated salmonellosis in the Netherlands during a 30-year period from 1985 to 2014.

Methods

We performed source attribution of human salmonellosis cases using the modified Dutch model, which has been presented in detail previously [4,5,8]. Briefly, the model infers probabilistically the most likely sources of human cases by comparing their *Salmonella* subtype distribution with that of the sources, weighted by the *Salmonella* prevalence in these sources and the human exposure to them, i.e. the per capita food consumption and likelihood of consuming raw/undercooked food or the per capita ownership of reptiles in the general population. Model parameters are summarised in Table 1.

We used national surveillance data for all 73,124 laboratory-confirmed human salmonellosis cases reported in the Netherlands during the period from January 1985 to December 2014. Non-typhoid salmonellosis is not a notifiable disease in the Netherlands. However, a national passive surveillance system for *Salmonella* has been in place since 1984, with an estimated 62% coverage of the general population based on a network of diagnostic laboratories that submit *Salmonella* isolates (with accompanying metadata) to the RIVM for further typing [9].

Serotyping of all these isolates and further phage typing of the *S.* Enteritidis and *S.* Typhimurium isolates was performed by the national reference laboratory for *Salmonella* at the Dutch National Institute for Public Health and the Environment (RIVM) as described elsewhere [10]. We also used all available *Salmonella* isolates from five putative sources, i.e. pigs (n = 14,395), cattle (n = 11,189), broiler chickens (n = 51,492), table eggs/table egg-laying hens (n = 7,412) and reptiles (n = 2,281) that had been collected during the same period by the Dutch veterinary services (food-producing animals) and private clinics (reptile pets) as part of their routine diagnostic activities and monitoring/surveillance programmes on animals and animal-derived foods at the levels of farm, slaughterhouse and retail (Table 2). Also these isolates were typed at the RIVM within the framework of the national surveillance system for *Salmonella* using the same methods as for the human isolates.

Of the 73,124 human cases, 5,579 (7.6%) and 1,683 (2.3%) were excluded from the source attribution analysis because they were travel- and outbreak-related, respectively. Another 2,144 cases (2.9%) were excluded because their sero/phage types were not found in any of the considered sources; these cases were then assigned to an unknown source. The model attributed the remaining 63,718 sporadic/domestic cases to the five animal sources. To avoid issues related to sparse data, each year of human cases was attributed based on the subtypes of three years of data for pigs, broilers and layers/eggs (i.e. the same year and the years before and after) and based on all years of reptile data. Interannual trends in the fraction of cases attributed to reptiles were assessed using the Cochran-Armitage test.

Results

Most reptile isolates (59%) belonged to *S. enterica* subspecies other than subspecies I, particularly to

Table 1

Parameters of the modified Dutch model for source attribution

Parameter	Description/estimation	Reference
λ_{ij}	Estimated number of human infections caused by subtype *i* from source *j*, given by $\frac{p_{ij} \times m_j \times c_j}{\sum_j p_{ij} \times m_j \times c_j} \times e_i$	[4,5,8]
p_{ij}	Prevalence of subtype *i* from source *j*, given by $\pi j \times rij$	[1,3,4,16]; this study
π_j	Overall prevalence of *Salmonella* spp. in source *j*	[1,3,4,16,17]
r_{ij}	Relative frequency of serotype *i* in source *j*	This study
m_j	Amount of source *j* per person per year available on the market (kg for food-animals or number for reptiles)	[4,18,19]
c_j	Probability for foods from source *j* to be eaten raw/undercooked by the population (not applicable for reptiles)	[5,20]
e_i	Frequency of human salmonellosis cases of subtype *i*	Data

Table 2

***Salmonella* subspecies and serotypes in humans (n = 63,718) and animal sources (n = 86,769), the Netherlands, 1985–2014**

Subspecies	Serotype	Humans [a]		Reptiles		Pigs		Cattle		Layers/eggs		Broilers	
		n	%	n	%	n	%	n	%	n	%	n	%
S. enterica (I)	Typhimurium and its monophasic variant	27,709	43.49	63	2.76	8,984	62.41	4,620	41.29	359	4.84	8,832	17.15
	Enteritidis	18,913	29.68	22	0.96	78	0.54	113	1.01	3,279	44.24	5,200	10.10
	Typhi	402	0.63	0		0		0		0		0	
	Paratyphi A/B/C	487	0.76	1	0.04	14	0.10	4	0.04	82	1.11	4,409	8.56
	Others	16,107	25.28	849	37.22	5,316	36.93	6,450	57.65	3,688	49.80	33,032	64.15
S. salamae (II)		24	0.04	276	12.10	1	0.01	0		2	0.03	9	0.02
S. arizonae (IIIa)		13	0.02	194	8.51	0		0		1	0.01	2	0.004
S. diarizonae (IIIb)		41	0.06	580	25.43	1	0.01	2	0.02	1	0.01	6	0.01
S. houtenae (IV)		21	0.03	293	12.85	1	0.01	0		0		2	0.004
S. bongori/indica (V/VI)		1	0.00	3	0.13	0		0		0		0	
Total		**63,718**		**2,281**		**14,395**		**11,189**		**7,412**		**51,492**	

[a] Includes only isolates from sporadic, domestic cases.

Figure 1

Annual number of reported human salmonellosis cases attributed to different animal sources in the Netherlands, 1985–2014 (n = 73,124)

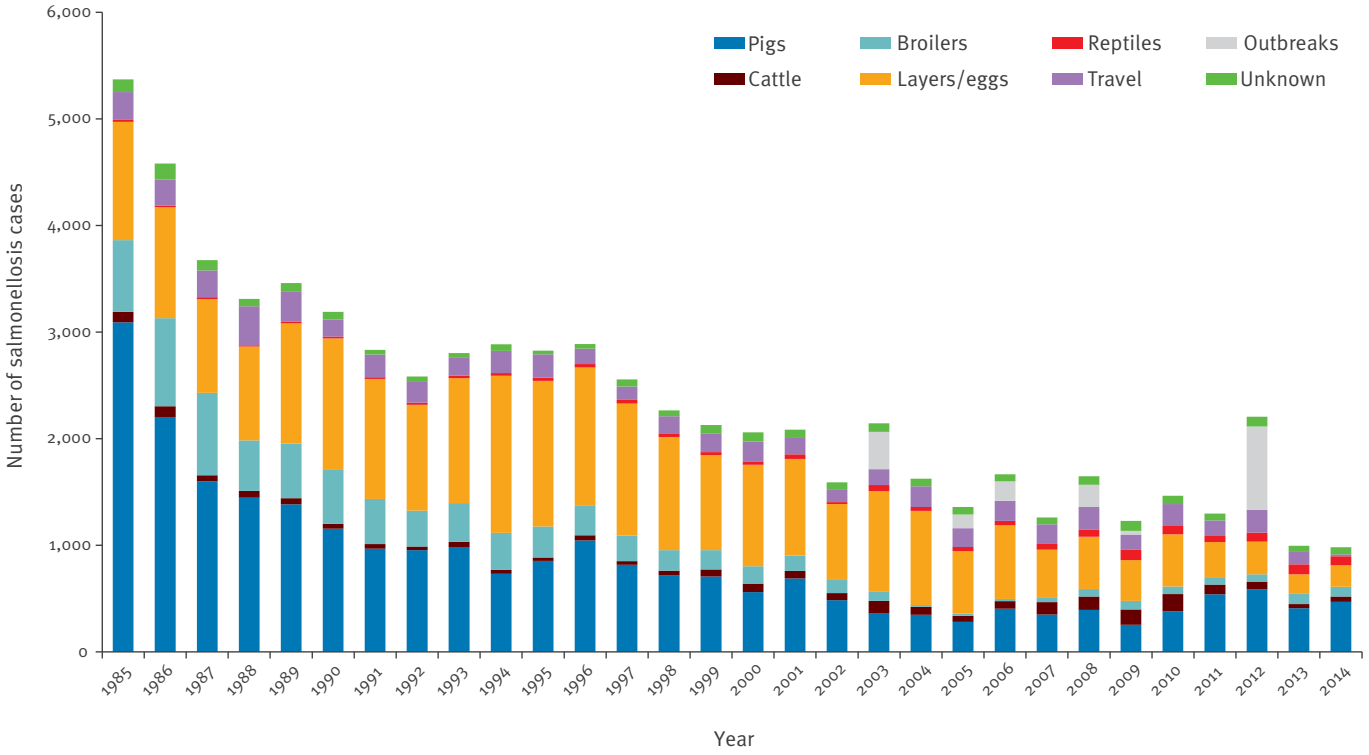
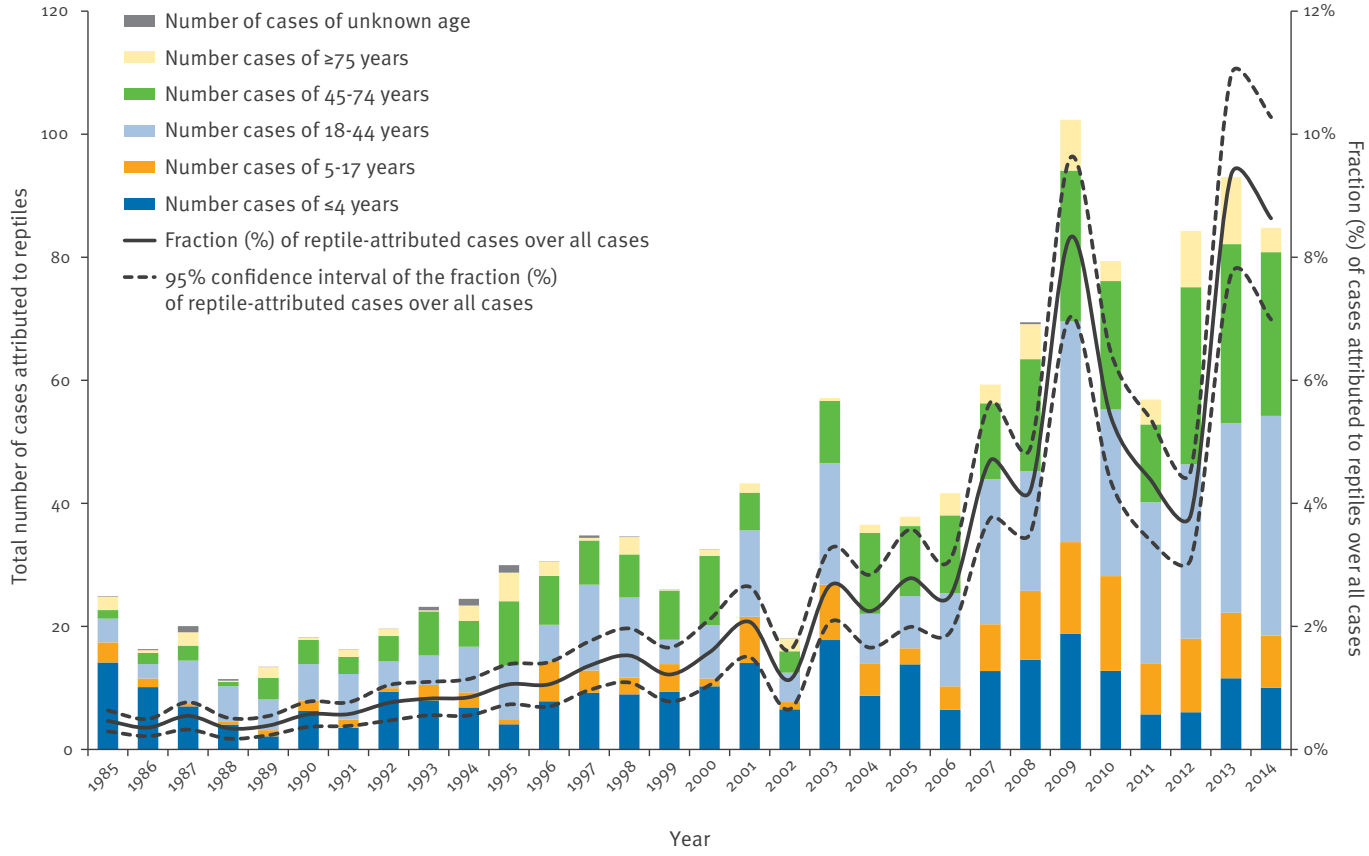


Figure 2

Annual total number of human salmonellosis cases attributed to reptiles, by age group, and estimated fraction of these cases relative to all human salmonellosis cases reported in the Netherlands, 1985–2014 (n = 73,124)



subspecies IIIb (25%). In contrast, the vast majority (>99%) of human isolates and isolates from food-producing animal belonged to subspecies I (Table 2). In total, 2.0% (95% confidence interval (95% CI): 1.3–2.8) of human cases were attributed to reptiles; attributions to the other sources were as follows: layers/eggs 41.3% (95% CI: 36.0–46.5), pigs 40.9% (95% CI: 36.4–45.5), broilers 12.3% (95% CI: 10.3–14.4) and cattle 3.5% (95% CI: 2.5–4.5). The estimated annual fraction of reptile-associated *Salmonella* infections ranged from a minimum of 0.3% (corresponding to 11 cases) in 1988 to a maximum of 9.3% (93 cases) in 2013 (Figure 1). Although human cases decreased over the years (Figure 1), there was a significant increasing trend ($p<0.0001$) in the fraction of reptile-associated *Salmonella* infections (+19% on average each year) (Figure 2). Figure 1 also shows the rise and fall of the *S*. Enteritidis epidemic linked to eggs during the 1990s and the growing importance of pigs since the early 2000s (linked to the emergence of *S*. Typhimurium monophasic variant) after a period of evident decline.

Coloured bars, left vertical axis: number of human salmonellosis cases attributed to reptiles; black line, right vertical axis: estimated proportion of human salmonellosis cases attributed to reptiles.

Looking at the age distribution of reptile-associated *Salmonella* infections over the years (Figure 2), the proportion of cases younger than five years relative to the older age groups decreased significantly by 4% annually ($p<0.0001$), whereas cases in patients aged 45 to 74 years increased by 20% ($p=0.006$) each year.

Discussion

We showed that despite the observed decline in human salmonellosis cases overall, those associated with reptiles are on the rise and increasingly affecting the adult population. This may be explained by the parallel increase in the trade of live (and often wild-caught) reptiles in the European Union (EU). Although the scale of the illegal market is unknown, 5.9–9.8 million reptiles were (legally) imported into the EU in 2009 alone, a substantial rise from the 1.6 million imported in 2005, which coincided with the ban on wild bird imports placed by the EU in 2005 in response to the H5N1 highly pathogenic avian influenza epidemic in poultry [11]. This lends weight to the hypothesis that the shortage of imported wild birds may have played a role in moving the EU exotic pet market towards reptiles so that prospective and established customers may increasingly have embraced reptiles as pets. This is also mirrored in our attributions, as reptile-associated salmonellosis increased steeply after 2005 (Figure 2).

The observed shift in the age groups at highest risk for reptile-associated salmonellosis may be related to the type of reptiles that are currently kept for companionship. In the past, reptile pets consisted mainly of freshwater aquatic baby turtles like the red-eared slider (*Trachemys scripta elegans*), a popular childhood pet and an important source of salmonellosis for children. As an example, in the United States (US) in the early seventies, pet turtles were responsible for ca 18% of salmonellosis cases among children aged one to nine years [12]. This led to a federal ban in 1975 on the sale of turtles with a shell length less than 10 cm, resulting in a 77% decrease in reptile-associated salmonellosis among children of that age [12]. Although baby turtles have become less popular in the Netherlands since the EU ban on imports of red-eared sliders in 1997 for ethical and environmental reasons, a wider variety of reptile species is currently available on the pet market, and most of these species (mainly lizards and snakes) are clearly meant for adult customers rather than children. This is supported by the increased incidence of venomous (pet) snake bites and other injuries in Europe, extremely rare events until the early 2000s. Further, the importation of these animals has been linked to the changed biodiversity of the European household fauna [13,14]. A resurgence of pet reptiles other than baby turtles is also believed to be responsible for the recent trends in reptile-associated salmonellosis in the US [2].

Our estimate of 2.0% for reptile-associated salmonellosis is in line with previous estimates based on similar source attribution methods [3], but lower than those based on self-reported exposure to reptiles. For instance, in Sweden, 6% of all salmonellosis cases from 1998 to 2000 reported exposure to reptiles [15]. In the US, the population attributable fraction for reptile/amphibian contact was 6% for all sporadic cases from 1996 to 1997, and 11% among those younger than 21 years [2].

Conclusions

In summary, while human salmonellosis has been decreasing since the 1980s in the Netherlands, we report an increasing trend in reptile-associated salmonellosis and a shift towards adulthood in the age groups at risk, a possible reflection of the increased number and variety of reptiles that are nowadays kept as pets. Although human salmonellosis remains primarily a food-borne disease and the contribution of reptiles is small, our findings call for further attention to the issue of safe reptile ownership in order to target and reinforce current standing recommendations.

Conflict of interest

None declared.

Authors' contributions

LMG and WvP conceived and designed the study. MH produced the laboratory data. LMG performed the statistical analyses and drafted the manuscript. All authors have substantially contributed to critically reviewing the manuscript and approved it as submitted.

References

1. Geue L, Löschner U. Salmonella enterica in reptiles of German and Austrian origin.Vet Microbiol. 2002;84(1-2):79-91. DOI: 10.1016/S0378-1135(01)00437-0 PMID: 11731161
2. Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, et al. Reptiles, amphibians, and human Salmonella infection: a population-based, case-control study. Clin Infect Dis. 2004;38(s3) Suppl 3;S253-61. DOI: 10.1086/381594 PMID: 15095197
3. Bertrand S, Rimhanen-Finne R, Weill FX, Rabsch W, Thornton L, Perevoscikovs J, et al. Salmonella infections associated with reptiles: the current situation in Europe. Euro Surveill. 2008;13(24):18902.PMID: 18761944
4. Mughini-Gras L, Smid J, Enserink R, Franz E, Schouls L, Heck M, et al. Tracing the sources of human salmonellosis: a multi-model comparison of phenotyping and genotyping methods. Infect Genet Evol. 2014;28:251-60. DOI: 10.1016/j.meegid.2014.10.003 PMID: 25315490
5. Mughini-Gras L, Enserink R, Friesema I, Heck M, van Duynhoven Y, van Pelt W. Risk factors for human salmonellosis originating from pigs, cattle, broiler chickens and egg laying hens: a combined case-control and source attribution analysis.PLoS One. 2014;9(2):e87933. DOI: 10.1371/journal.pone.0087933 PMID: 24503703
6. Mughini-Gras L, Barrucci F, Smid JH, Graziani C, Luzzi I, Ricci A, et al. Attribution of human Salmonella infections to animal and food sources in Italy (2002-2010): adaptations of the Dutch and modified Hald source attribution models. Epidemiol Infect. 2014;142(5):1070-82. DOI: 10.1017/S0950268813001829 PMID: 23920400
7. DE Knegt LV, Pires SM, Hald T. Attributing foodborne salmonellosis in humans to animal reservoirs in the European Union using a multi-country stochastic model.Epidemiol Infect. 2015;143(6):1175-86. DOI: 10.1017/S0950268814001903 PMID: 25083551
8. Mughini-Gras L, van Pelt W. Salmonella source attribution based on microbial subtyping: does including data on food consumption matter?Int J Food Microbiol. 2014;191:109-15. DOI: 10.1016/j.ijfoodmicro.2014.09.010 PMID: 25261828
9. van Pelt W, de Wit MA, Wannet WJ, Ligtvoet EJ, Widdowson MA, van Duynhoven YT. Laboratory surveillance of bacterial gastroenteric pathogens in The Netherlands, 1991-2001. Epidemiol Infect. 2003;130(3):431-41.PMID: 12825727
10. van Duijkeren E, Wannet WJ, Houwers DJ, van Pelt W. Serotype and phage type distribution of salmonella strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001.J Clin Microbiol. 2002;40(11):3980-5. DOI: 10.1128/JCM.40.11.3980-3985.2002 PMID: 12409362
11. Wild Pets in the European Union. Horsham: ENDCAP; 2012. Available from: http://endcap.eu/wp-content/uploads/2013/02/Report-Wild-Pets-in-the-European-Union.pdf
12. Cohen ML, Potter M, Pollard R, Feldman RA. Turtle-associated salmonellosis in the United States. Effect of Public Health Action, 1970 to 1976.JAMA. 1980;243(12):1247-9. DOI: 10.1001/jama.1980.03300380027016 PMID: 7359680
13. Schaper A, de Haro L, Desel H, Ebbecke M, Langer C. Rattlesnake bites in Europe--experiences from southeastern France and northern Germany.J Toxicol Clin Toxicol. 2004;42(5):635-41. DOI: 10.1081/CLT-200026962 PMID: 15462156
14. Warwick C, Steedman C. Injuries, envenomations and stings from exotic pets.J R Soc Med. 2012;105(7):296-9. DOI: 10.1258/jrsm.2012.110295 PMID: 22843648
15. de Jong B, Andersson Y, Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis.Emerg Infect Dis. 2005;11(3):398-403. DOI: 10.3201/eid1103.040694 PMID: 15757554
16. European Food Safety Authority (EFSA). Biological hazards reports. National zoonoses country reports. c2004-14. Parma: EFSA. [Acccessed: 29 Feb 2016]. Available from: http://www.efsa.europa.eu/en/zoonosesscdocs/zoonosescomsumrep
17. Bouwknegt M, Dam-Deisz W, Wannet WJB, van Pelt W, Visser G, van de Giessen AW. Surveillance of zoonotic bacteria in farm animals in The Netherlands - Results from January 1998 until December 2002. Bilthoven, The Netherlands: Rijksinstituut voor Volksgezondheid en Milieu (RIVM). 2004. Available from: http://rivm.openrepository.com/rivm/bitstream/10029/8900/1/330050001.pdf
18. Feiten and cijfers - Gezelschapsdierensector 2011. [Facts and figures - Fodder Industry]. Den Haag: Hogeschool HAS Den Bosch, 2012. Dutch. Available from: https://issuu.com/hasdenboschinternational/docs/feiten___cijfers_van_de_gezelschapsdierensector_20
19. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). c1984-14. CITES trade database. Geneva: CITES. [Accessed: 29 Feb 2016]. Available from: http://trade.cites.org/
20. Friesema IH, van Gageldonk-Lafeber AB, van Pelt W. Extension of traditional infectious disease surveillance with a repeated population survey.Eur J Public Health. 2015;25(1):130-4. DOI: 10.1093/eurpub/cku122 PMID: 25085476

License and copyright

National outbreak of *Yersinia enterocolitica* infections in military and civilian populations associated with consumption of mixed salad, Norway, 2014

E MacDonald[1,2], M Einöder-Moreno[1,2], K Borgen[1], L Thorstensen Brandal[3], L Diab[4], Ø Fossli[5], B Guzman Herrador[1], AA Hassan[6], GS Johannessen[7], EJ Johansen[5], R Jørgensen Kimo[4], T Lier[8], BL Paulsen[6], R Popescu[9], C Tokle Schytte[9], K Sæbø Pettersen[7], L Vold[1], Ø Ørmen[4], AL Wester[3], M Wiklund[8], K Nygård[1]

1. Department of Infectious Disease Epidemiology, Norwegian Institute of Public Health, Oslo, Norway
2. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Control and Prevention (ECDC), Stockholm, Sweden
3. Department of Foodborne Infections, Norwegian Institute of Public Health, Oslo, Norway
4. Norwegian Armed Forces, Norway
5. District Office of Midt-Troms, Norwegian Food Safety Authority, Finnsnes, Norway
6. District Office of Tromsø, Norwegian Food Safety Authority, Tromsø, Norway
7. Norwegian Veterinary Institute, Oslo, Norway
8. Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway
9. Regional Office for Troms and Finnmark, Norwegian Food Safety Authority, Kautokeino, Norway

Correspondence: Emily MacDonald (emily.macdonald@fhi.no)

Citation style for this article:
MacDonald E, Einöder-Moreno M, Borgen K, Thorstensen Brandal L, Diab L, Fossli Ø, Guzman Herrador B, Hassan AA, Johannessen GS, Johansen EJ, Jørgensen Kimo R, Lier T, Paulsen BL, Popescu R, Tokle Schytte C, Sæbø Pettersen K, Vold L, Ørmen Ø, Wester AL, Wiklund M, Nygård K. National outbreak of Yersinia enterocolitica infections in military and civilian populations associated with consumption of mixed salad, Norway, 2014. Euro Surveill. 2016;21(34):pii=30321. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.34.30321

Article submitted on 6 July 2015 / accepted on 20 July 2016 / published on 25 August 2016

In May 2014, a cluster of *Yersinia enterocolitica* (YE) O9 infections was reported from a military base in northern Norway. Concurrently, an increase in YE infections in civilians was observed in the Norwegian Surveillance System for Communicable Diseases. We investigated to ascertain the extent of the outbreak and identify the source in order to implement control measures. A case was defined as a person with laboratory-confirmed YE O9 infection with the outbreak multilocus variable-number tandem repeat analysis (MLVA)-profile (5-6-9-8-9-9). We conducted a case–control study in the military setting and calculated odds ratios (OR) using logistic regression. Traceback investigations were conducted to identify common suppliers and products in commercial kitchens frequented by cases. By 28 May, we identified 133 cases, of which 117 were linked to four military bases and 16 were civilians from geographically dispersed counties. Among foods consumed by cases, multivariable analysis pointed to mixed salad as a potential source of illness (OR 10.26; 95% confidence interval (CI): 0.85–123.57). The four military bases and cafeterias visited by 14/16 civilian cases received iceberg lettuce or radicchio rosso from the same supplier. Secondary transmission cannot be eliminated as a source of infection in the military camps. The most likely source of the outbreak was salad mix containing imported radicchio rosso, due to its long shelf life. This outbreak is a reminder that fresh produce should not be discounted as a vehicle in prolonged outbreaks and that improvements are still required in the production and processing of fresh salad products.

Introduction

Yersinia enterocolitica (YE) infection is the fourth most commonly reported cause of bacterial diarrhoeal disease in Norway [1]. Yersiniosis is notifiable to the Norwegian Institute of Public Health (NIPH) via the Norwegian Surveillance System for Communicable Diseases (MSIS). Since 2008, between 40 and 60 cases have been reported annually. More than 80% of yersiniosis cases in Norway are due to serotype O3, which is also the dominant cause of yersiniosis in Canada, Europe, Japan, and parts of the United States [2].The highest isolation rates have been reported during the cold season in temperate climates, including northern Europe and especially Scandinavia. The incubation period is generally under 10 days, but most often between three and seven days. Typical symptoms of yersiniosis include self-limiting acute febrile diarrhoea with abdominal pain, which can mimic appendicitis and has led to appendectomy [3]. YE infections have also been known to lead to sequelae such as reactive arthritis, erythema nodosum and conjunctivitis in up to 12% of cases [4].

Transmission most frequently occurs through eating contaminated food, particularly raw or undercooked pork, as the pig is the only animal consumed by humans which regularly harbours the pathogenic serovars O3 and O9 [2]. Case–control studies in Finland, Germany, New Zealand, Norway and Sweden have found that consumption of pork is associated with sporadic yersiniosis [5-9]. While outbreaks of yersiniosis have also been linked to consumption of pork [10,11], other food

items such as milk, water and fresh vegetables have also been reported as a source of infection, and an outbreak of YE O9 due to imported ready-to-eat salad mix occurred in Norway in 2011 [11]. Most yersiniosis cases are sporadic and outbreaks are rarely reported [12]. Yersiniosis is rarely transmitted through sustained person-to-person transmission, although there have been previous outbreaks in which food handlers have been implicated [13].

The event

On Thursday 8 May 2014, the Food Safety Authorities (FSA) District Office for Midt-Troms reported two cases of YE infections from a military base in northern Norway to the NIPH via the national web-based outbreak reporting system (Vesuv). Three additional cases were suspected at the time of the report. Concurrently, an increase of YE O9 infections was observed in MSIS with nine human isolates of YE O9 from geographically dispersed areas of the country received between 5 and 11 May 2014. The National Reference Laboratory (NRL) identified a common profile for the military and civilian cases through multilocus variable number tandem repeat analysis (MLVA), which had not been observed in Norway before this outbreak. In collaboration with the FSA and the military, an outbreak investigation was initiated to ascertain the extent of the outbreak, determine whether all cases were linked to the military and identify the source of the yersiniosis outbreak in Norway in order to implement control measures and prevent further spread.

Methods

Case finding

Outbreak case definition

For this outbreak a case was defined as any person with laboratory-confirmed YE O9 infection with the outbreak MLVA profile (5-6-9-8-9-9) with onset of symptom between 1 March and 15 June 2014.

Case finding among civilians

In Norway, YE is reportable via MSIS and all isolates of presumptive YE are forwarded from clinical microbiology laboratories to the NRL where they are routinely characterised phenotypically, biotyped, tested for markers of plasmid-associated virulence factors and serogrouped against O3, O5,27, O8 and O9. Isolates can also be tested for a range of other serogroups if needed. The isolates are then MLVA-typed by the method described by Gierczyński et al. [14], locally adjusted to capillary electrophoresis.

Case finding on military bases

The Norwegian Armed Forces is a conscript military with 33 military bases throughout the country. Three military bases in the county of Troms in northern Norway (military bases T1, T2 and T3) and one military base in the county of Hedmark in south-eastern Norway (military base H1) reported cases to the NIPH. Base T1, the largest of the three bases, is located ca 40 km from base T2 and ca 30 km from base T3. The population of the military bases is composed primarily of privates, who are mostly Norwegians completing one year of mandatory military service. The soldiers belonging to each base are organised in companies, typically composed of 100 to 150 people. Bedrooms are typically shared by four to six people; bathrooms can be shared by up to 50 people. Privates and officers eat in the same mess halls, which are organised such that soldiers take food from a buffet table offering several hot and cold meal options, as well as a cold salad bar.

Information about cases on military bases was collected through the Military Health Officer. On 13 May the Military Health Office requested that all soldiers based at the three bases in Troms report to the healthcare centre if they had gastrointestinal symptoms, for isolation and testing. All cases diagnosed with yersiniosis were subsequently sent home from the military base until they provided a stool sample negative for YE. All kitchen staff on base T2 were tested, regardless of presence of symptoms, while kitchen staff from the other bases were only tested if symptomatic.

International enquiry

On 16 May the NIPH sent a message via the European Centre for Disease Prevention and Control (ECDC) Epidemic Intelligence Information System asking whether other European countries were also observing an increase in cases of YE infections.

Investigating the source of infection

Trawling questionnaire and further development of a short questionnaire for civilian cases

The initial cases, both military and civilian, were interviewed using a standardised 22-page trawling questionnaire designed to generate hypotheses for possible sources of infection in a food-borne outbreak. For the identified military cases this questionnaire was administered on base by the local FSA, prior to being sent home from the base. For microbiologically-confirmed outbreak cases identified by the NRL that did not have any connections to a military base, the district FSA would visit the residence of the case to conduct the interview, as well as to collect food samples. The trawling questionnaire included detailed questions about food consumption and purchases, animal contact and environmental exposures in the week before onset of symptoms, as well as clinical and demographic information.

Subsequent to analysing information from the trawling interviews, a shorter questionnaire was developed for civilian cases. This questionnaire focused on foods of most interest, which included pork products and raw vegetables. It also included questions about potential locations of exposure, such as restaurants and cafeterias. The short questionnaire was administered to

seven civilian cases through the FSA either by phone or in person.

Case–control investigation in the military setting

A case–control study was designed in order to identify the vehicle of infection among privates from two of the military bases. Cases identified by 29 May among privates in base T1 and among privates in base T2 were included in the study. Cases from the two most affected companies in base T2 were excluded *a priori* as additional factors affecting the occurrence of disease were suspected, including secondary transmission. Four controls were selected for each case, frequency matched by company. Due to security reasons, access to lists of privates belonging to each company was not provided to the investigators. Therefore, military officials from the relevant bases were given instructions to systematically select controls from an alphabetical list. In total, 21 cases (10 cases from T1 and 11 cases from T2) and 82 controls (44 controls from T1 and 38 controls from T2) were included in the case–control study.

Based on the hypotheses generated from the trawling questionnaire, a short self-administered questionnaire was developed for the case–control study. Menus from the military kitchens were available and used in this process. A total of 36 salad/vegetables items, 17 pork products and seven prepared salads that are served in the mess hall on a regular basis were included in the questionnaire, which was piloted with the head cook of the military kitchens and the brigade veterinarian before dissemination. Given the wide range in onset dates in cases and the anticipated difficulty for military personnel to remember the specific food items consumed from a buffet on specific days, both cases and controls were asked to indicate what food items they consume in a typical two week period in the mess hall.

Data collection

All controls for the case–control study were gathered in groups and interviewed in their respective military bases on 27 and 28 May 2014. Study participants were distributed the paper questionnaire which they were asked to complete. Photographs of different salad types were shown on a projector. Cases were interviewed by telephone by employees of the NIPH between 29 May and 10 June, as they had been asked to return home after being diagnosed and many had left the military base at the time of the study. Cases were sent an email with the same photographs of the salads shown to the controls and were asked to refer to the images while being interviewed.

Data analysis

Data were entered in the web-based questionnaire tool Questback. We calculated the number of people exposed to various food items, number of ill people among the exposed and unexposed and attack rates (AR) for all food items. We first analysed the association of each food item with yersiniosis one by one (univariable analysis). In the next step we selected food items which had odds ratios (OR) with a p-value lower than 0.25 and that had at least 50% of the cases exposed. Of these, we selected the three variables with lower p-value and stratified. Multivariable analysis was performed using logistic regression with OR, adjusted for military camp. We also calculated the dose-response association between the amount of salad consumed (never, once per month, once per week, several times per week and every day) and yersiniosis. This dose-response was also analysed for the amount of pork meat consumed. Descriptive analyses were performed in Excel and Stata 12, and univariable and multivariable analyses were performed in Stata 12.

Microbiological investigation of food samples

During site inspections, food samples were collected from the military base kitchens as well as from several commercial kitchens that had served civilian cases. Food samples were also collected from the homes of civilian cases. Samples were submitted to the Norwegian Veterinary Institute for analysis. The samples were analysed according to the ISO/WD Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presumptive pathogenic YE (version 2012–12–01), which included direct plating and alkali treatment of both peptone-sorbitol-bile (PSB) and irgasan-ticarcillin-potassium chlorate (ITC) enrichment broths [15]. The samples were plated on both cefsulodin-irgasan-novobiocin (CIN) agar and a CHROMagar Yersinia enterocolitica (Paris, France). In addition, the samples were cold-enriched using a modified version of the Nordic Committee on Food Analysis method 117 (NMKL 117) [16]. The PSB enrichment broths and suspicious colonies were examined for the *ail* gene, an indicator for pathogenic YE, by polymerase chain reaction (PCR) [17].

Traceback investigation

The FSA inspected the military base kitchens on 9 May, 13 May as well as 27 and 28 May 2014. A traceback investigation was conducted by the FSA on food items by reviewing documentation for suspected food products delivered to the military kitchens and commercial kitchens/cafeterias where civilian cases had eaten. The FSA contacted the distributers of suspected food items and conducted inspections where necessary.

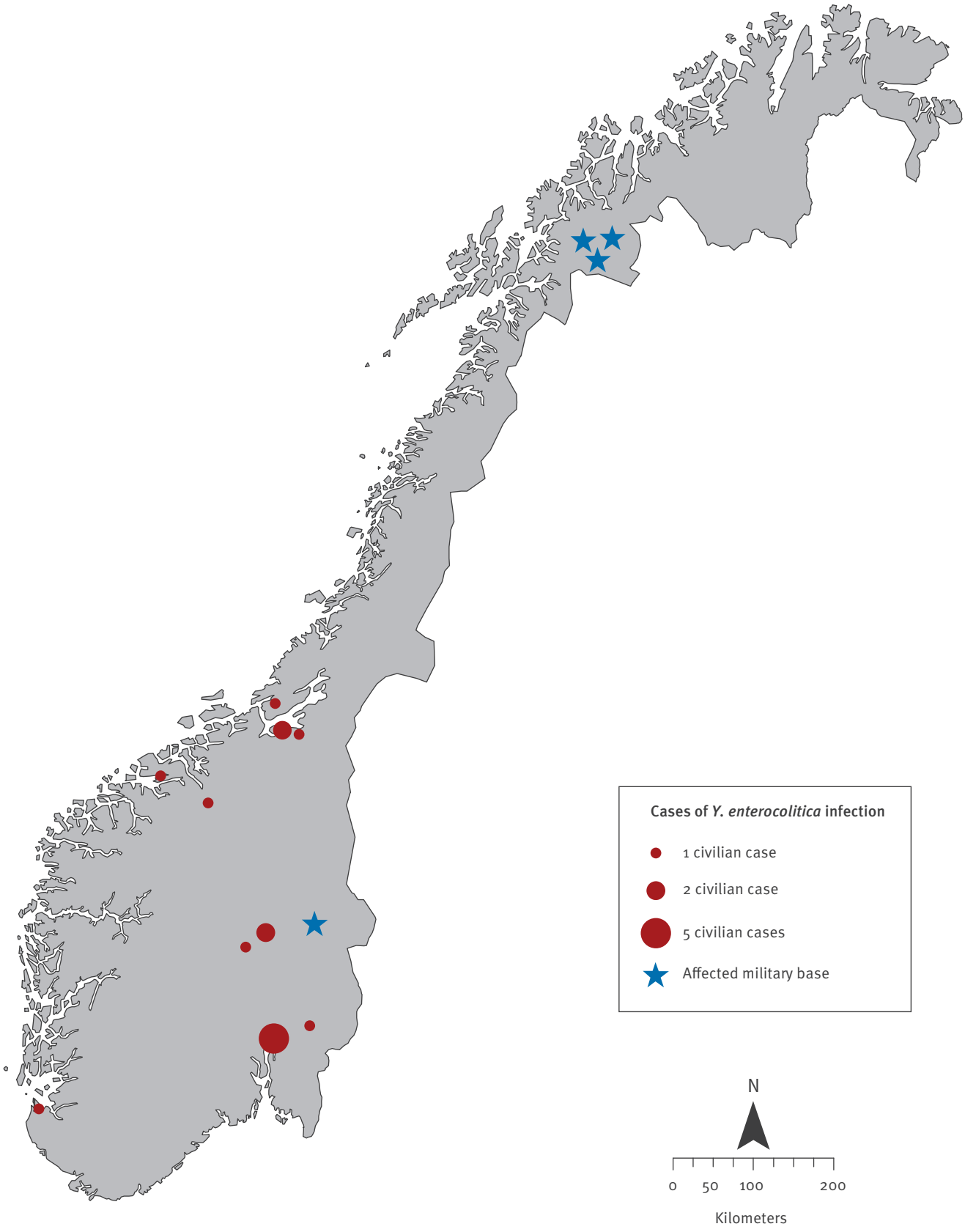
Results

Description of the outbreak

As of 29 July 2014, 133 confirmed cases of YE O9 infections were reported to the NIPH. Almost 90% of the confirmed cases (n = 117) had a confirmed link to one of four different military bases (Figure 1). Sixteen cases had no reported links to a military case. These cases resided in six different counties in Norway – Oslo (n = 5), Sør-Trøndelag (n = 4), Oppland (n = 3), Møre og Romsdal (n = 2), Akershus (n = 1), and Rogaland (n = 1). The 16 civilian cases ranged in age from 24 to 95 years (median: 39 years) and just over half were female (n = 9).

Figure 1

Geographical distribution of cases of *Yersinia enterocolitica* infection by military base and municipality of residence, Norway 2014



Of the 117 cases from the military bases with identical MLVA profiles, almost all were reported from three bases in Troms county: T1 (n=14), T2 (n=88) and T3 (n=3). Four cases were reported from military base H1 in Hedmark county and three cases had links to more than one military base (including either T2 or H1). For five cases the base was unknown. Cases linked to the military bases ranged in age from 19 to 57 years (median age: 21) and 21% were female (n=24). Military cases belonged to at least seven battalions and fourteen companies. At least 32% (n=37) of all military cases belonged to Company X of military base T2. Although the exact number of privates in the company is unknown, assuming a total company membership of between 100 and 150, the attack rate for Company X would have been between 25% and 37%. The company with the second highest attack rate, also from base T2, had 10 cases reported, corresponding to an attack rate of 7% to 10%. Seven kitchen personnel were diagnosed with yersiniosis, of which two were asymptomatic. All of these employees worked in base T2. Symptomatic kitchen staff were not identified at any of the other bases.

Symptom onset among cases with this information available (n=102) ranged from 9 April to 28 May 2014 (Week 15 to Week 21) (Figure 2). For civilian cases, most (n=12) had symptom onset from Week 15 to Week 17, while over 90% of military cases (n=81) had symptom onset between Week 17 and Week 20.

International requests for information produced no reports of similar yersiniosis outbreaks in other European countries.

Investigating the source of the outbreak

Trawling interviews

Eighteen military cases, as well as nine of the total 16 civilian cases were interviewed using the hypothesis-generating questionnaire. The results of the trawling questionnaires from the military bases indicated that almost all soldiers ate all their meals in the same mess halls. The military mess halls offered a buffet, which meant that soldiers could choose what to take, but most cases were unaware of how food was prepared and which ingredients were used. Many cases reported consuming salad from the salad bar. The results of the trawling questionnaires for civilian cases suggested that all but one of the cases had eaten from restaurants or commercial kitchens. In particular, 12 of the 16 civilian cases interviewed reported eating from salad bars at workplace cafeterias.

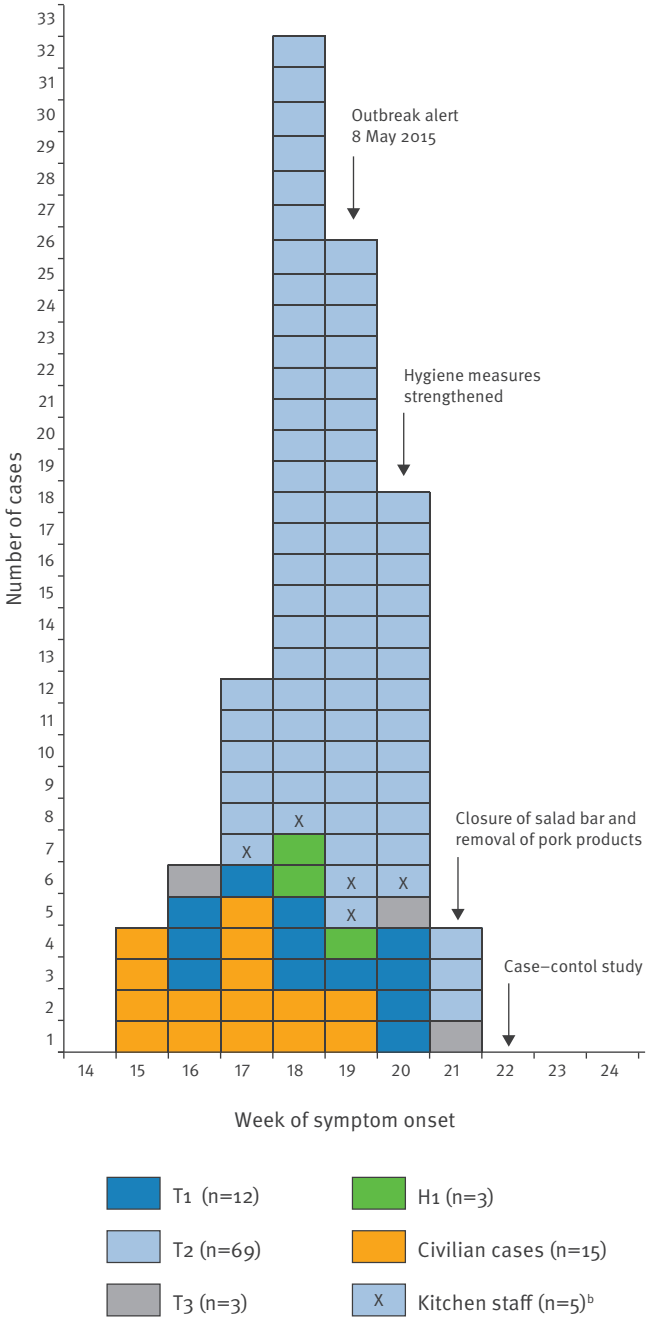
Case–control study

In the case–control study, 10 food items had at least 50% of cases exposed and had a p-value<0.25 in the univariable analysis (Table). These were included in the multivariable analysis.

Of the 10 significant food items in univariable analysis, salad mix and arugula were the most likely to be associated with illness in multivariable analysis. Cases were 10 times more likely to have eaten salad mix than controls (OR: 10.26; 95% confidence interval

Figure 2

Distribution of cases of *Yersinia enterocolitica* infection by week of symptom onset, Norway 2014 (n=102)[a]



Week 14 starts on 31 March.

In the legend military bases where cases occurred are indicated by T1, T2, T4 (for bases in Troms county) and H1 (for a base in Hedmark county).

[a] Of a total 133 outbreak cases, date of symptom onset was available for 102.

[b] The five kitchen staff are included among the 69 cases in military base T2.

(CI): 0.85–123.57, p-value: 0.067) and 95% of the cases (20/21) had eaten the salad mix compared with 70% of the controls (57/82). Cases were almost six times more likely to have eaten arugula than controls (OR: 5.48; 95%CI: 1.19–25.19, p-value: 0.029) and 52% of the cases (11/21) had eaten arugula compared with 24% of the controls (20/82). A dose-response relationship was observed between consumption of salad and illness. We observed that for every day salad was eaten, the risk increased by 9%.

Testing food samples

Fifteen food samples were taken from mess halls, cafeterias and private homes at the time of inspection on 27 May 2014. These included three samples of fresh-cut mixed salads, one shredded iceberg lettuce, six samples of other types of leafy greens (whole heads), one sample of carrots (snacks carrots), three samples of ham and one of bacon. The relevant batches of the consumed food products were already eaten or destroyed at the time of inspection. All food samples tested negative for pathogenic YE.

Traceback investigation

Initial traceback investigations indicated that at least 14 of 16 civilian cases had eaten at kitchens or cafeterias that were supplied by the same distributor of fresh fruits and vegetables as the military kitchens in all four bases. This distributor holds ca 1% of the Norwegian market and uses only Norwegian produce during the summer, but imports 70–80% of produce during the winter months. At the time of the outbreak, the distributor had not yet begun using Norwegian products exclusively. Further investigation found that almost all kitchens could document receiving salad mix (which contains 80% iceberg lettuce and 20% radicchio rosso), whole iceberg lettuce or whole radicchio rosso with produce originating from one of two countries. Information about the imports of radicchio rosso showed that the import on 6 April 2014 came from the previous harvesting season, while the import on 16 April 2014 was from a new harvesting season. The ingredients were washed and salad mixes were assembled at a processing factory in Norway that belongs to a subsidiary company to the distributor. An inspection of the processing factory where the salad mixes were produced for the distributor found significant lapses in hygiene, including not changing water in rinsing tanks on a regular basis.

As control measures, the distributor improved hygiene measures, and the military bases thoroughly cleaned the bathrooms and kitchens, and increased awareness on hand hygiene among the soldiers. From Week 21, the kitchens in all three bases in Troms voluntarily elected to close the salad bar and refrain from serving pork products until the outbreak was resolved.

Discussion

This outbreak of yersiniosis infection among civilians and members of the military was likely associated with consumption of fresh salad products. The geographically widespread occurrence of the yersiniosis cases and the prolonged duration indicated that the source of the product was widely distributed and available for a sustained period of time, which does not immediately suggest fresh produce as a source. However, the traceback investigations' results for both the civilian and military cases strongly indicate that almost all cases were exposed to salad products supplied by the same distributer. Although the specific food item responsible for the outbreak could not be identified, the traceback investigation points towards one of two types of salad vegetables as the source: radicchio rosso and iceberg lettuce. Of these, radicchio rosso was considered to the be the most biologically plausible ingredient as it was the only salad component that keeps long enough to fit with the duration of this outbreak. Radicchio

Table

Univariable results of the case–control study of military bases T1 and T2, outbreak of *Yersinia enterocolitica* O9 infections, April–June 2014, Norway

Exposure	Cases (n = 21)		Controls (n = 82)		OR (95% CI)	P-value
	Total	Exposed N (%)	Total	Exposed N (%)		
Salad mix	21	20 (95)	82	57 (70)	8.77 (1.24–377.81)	0.015
Iceberg salad	21	20 (95)	82	62 (76)	6.45 (0.90–280.52)	0.046
Cooked ham	21	20 (95)	81	63 (78)	5.71 (0.79–249.76)	0.067
Onion	21	19 (91)	81	54 (67)	4.75 (1.01–44.52)	0.031
Arugula	21	11 (52)	82	20 (24)	3.41 (1.12–10.35)	0.013
Red salad leaves	21	11 (52)	81	20 (25)	3.36 (1.10–10.19)	0.014
Chopped ham	21	18 (86)	82	54 (66)	3.11 (0.80–17.71)	0.077
Salami	21	19 (91)	81	63 (78)	2.71 (0.56–26.03)	0.192
Roast beef	21	17 (81)	81	55 (68)	2.01 (0.57–8.97)	0.242
Cauliflower	21	11 (52)	81	54 (67)	0.55 (0.19–1.65)	0.225

CI: confidence interval; OR: odds ratio.

rosso has a shelf life of up to 150 days [18], while iceberg lettuce has a shelf life of less than two weeks. In addition, radicchio rosso is stored at +1 °C before it is supplied to the market. These storage conditions allow the growth of YE as this bacterium is able to grow down to –2 °C. If the implicated radicchio rosso was from the previous harvesting season and was imported before the changeover to Norwegian produce, it may have been stored for a long period of time, facilitating microbiological growth. Given the uncommon serotype and novel MLVA profile, it is suspected that the contamination of an imported salad product occurred outside of Norway, but potential lapses in processing after importation may have contributed to the spread.

All samples of salad products were negative for pathogenic *Yersinia*, but it is often challenging to isolate pathogenic YE from food samples. In a 2011 outbreak of yersiniosis associated with pre-mixed salad in Norway, non-pathogenic *Yersinia* was found in packaged salads [11], indicating that the long-term storage of this food product is conducive to the persistence of the bacterium. In this previous outbreak, non-pathogenic and environmental strains of *Yersinia*, including YE biotype 1A and *Y. kristensenii* were identified in samples of mixed salad and radicchio rosso. Radicchio rosso was considered to be the most likely source of contamination, given the microbiological results and the traceback investigation, although this could not be conclusively determined. In addition, several outbreaks of *Y. pseudoturberculosis* associated with consumption of vegetables such as carrots have also suggested the capacity for *Yersinia* bacteria to multiply in contaminated produce stored at cold temperatures [19-21]. Arugula is probably the most recognisable of the salads that were shown in the pictures, which may explain why it was significant in multivariable analysis. Salad mixes of the type we suspect to be implicated often contain arugula, although the traceback investigation indicates that arugula was not distributed to all the implicated commercial kitchens.

The case–control study in the military camps demonstrated that cases were 10 times more likely to have eaten salad mix than controls. Despite not reaching statistical significance, this result, along with other epidemiological evidence, excludes pork and supports salad as the likely source of infection. However, the findings do not allow for incrimination of a specific type of salad leaf. In the questionnaires we asked the respondents to indicate what they consume in a typical two week period rather than the two weeks before the onset of symptoms. While this supports that the cases eat all types of salads more frequently than controls, framing the questions in this way may have obscured an exposure that was specific to the period before the outbreak. In addition, as the salads were prepared in commercial kitchens, the study participants were not responsible for preparing the salads and may have been unable to discern the different types of salad. Although we tried to minimise this problem by showing photographs of different salad types to the study participants, many of the leaves have a similar appearance and cannot easily be distinguished.

Outbreaks of gastroenteritis at military bases of differing aetiologies, particularly norovirus, are not uncommon [22-27], but to our knowledge only one previous military outbreak of yersiniosis has been reported, from naval troops and infantry in Finland in 1973 [28]. Military bases present a unique opportunity for epidemiological investigation and for implementation of control measures as the population is well defined, attends the same healthcare facility and is responsive to requests to participate in investigations. The military may also have additional incentives to prevent and quickly control outbreaks that occur, as a matter of national security. Due to the communal living space, washrooms and kitchens, outbreaks in military camps can spread quickly through person-to-person transmission and the implementation of control measures can be difficult. In a recent study among military personnel deployed as part of the Ebola response in Sierra Leone, incidence of gastroenteritis was found to be lower than in military personnel deployed in Afghanistan [29]. Hygiene policies were similar in both contexts with the exception of hand washing, which occurred much more frequently in Sierra Leone. Although the deployment context is different than being on base, these results reinforce the importance of basic hygiene practices in reducing the spread of gastroenteritis in a military context. For these reasons, emphasis on personal hygiene, isolation of symptomatic cases and extensive disinfection of common areas, kitchens and washrooms are important measures to implement quickly.

However, few accounts of person-to-person transmission of YE infection exist and are limited to exposures in nosocomial or family settings [30,31]. A study in Denmark on the occurrence of household outbreaks associated with different pathogens found that the tendency for YE to cause household outbreaks was low compared with other bacteria, like *Salmonella* Enteritidis and *Shigella sonnei* [32]. Although it was not possible to document the proportion of cases who may have been infected through person-to-person transmission, this transmission route may have propagated the outbreak. It is possible that different approaches to hygiene may have been taken by individuals and groups within specific companies, which may have explained the differences in attack rates in different companies. Varying approaches to testing among the leadership of different companies within the military or different levels of worry among some companies may have also led to increased interaction with the healthcare services. In any case, the importance of hand hygiene, safe food preparation measures and appropriate cleaning routines for washrooms during gastroenteritis outbreaks in military camps, regardless of the aetiology, cannot be ignored.

The role of food handlers in this outbreak is unknown. As all seven positive food handlers worked in camp T2, this might explain the concentration of cases in that camp. Food handlers have been implicated in very few outbreaks of yersinosis. In 1981, investigators of an outbreak of YE O8 at a summer diet camp concluded that a food handler may have introduced the bacteria during food preparation [13]. In the 1973 yersiniosis outbreak in Finland, two civilian food handlers had positive faecal samples, but their role in the transmission was also unclear [28]. Concerning the military cases in Norway, infected food handlers may have propagated the outbreak by contaminating food that was served in the camp's kitchen but it is unlikely that a food handler introduced the infection as the outbreak commenced simultaneously in several locations. In addition, the role of infected food handlers cannot explain the high attack rate among specific companies within camp T2.

Other factors, including differences between companies in exposure to contaminated products, were also considered. As members of the same company tend to eat at the same time, it is possible that specific groups could have been more exposed to a contaminated batch depending on when they came to the mess hall or which batches were served to different companies. However, if this type of disproportionate exposure occurred, it cannot fully explain the prolonged duration of the outbreak.

Conclusions and recommendations

This outbreak was the largest outbreak of YE infection in Norway as of 2014 and the first to be reported from a military context. The identification of the most likely source of infection, mixed salad, required combining information from the epidemiological, environmental, and traceback investigations from both civilian and military contexts. Person-to-person transmission may also have played a role in propagating the outbreak. The results of the investigation highlight that fresh produce should not be dismissed as possible sources in prolonged outbreaks. The implication of salad mix reinforces the need for improved control measures in the production chain for fresh produce.

Conflict of interest

None declared.

Authors' contributions

EM drafted the manuscript. EM, M E-M, BGH, KB, LV and KN were responsible for the study design and contributed to the epidemiological investigation. M E-M conducted the statistical analysis. ØF, LD, RJK and ØØ managed the outbreak response in the military camps. AAH, E-J J, B-L P, RP and CTS conducted interviews, environmental investigations and traceback investigations. TL, MW, ALW and LTB conducted laboratory investigations on human specimens. GSJ and KSP conducted laboratory investigations on food samples. All authors contributed to the writing of this manuscript and approved the final version.

References

1. Norwegian Institute of Public Health (NIPH). Outbreaks of infectious diseases in Norway. Annual report 2013. [Utbrudd av smittsomme sykdommer i Norge. Årsrapport 2013]. Oslo: NPIH; 2014.
2. Norwegian Scientific Committee for Food Safety. Panel on Biological Hazards. A preliminary risk assessment of Yersinia enterocolitica in the food chain: some aspects related to human health in Norway. 04/103. [Accessed 5 May 2011]. Available from: http://www.vkm.no/dav/d165b9d426.pdf
3. Naktin J, Beavis KG. Yersinia enterocolitica and Yersinia pseudotuberculosis.Clin Lab Med. 1999;19(3):523-36, vi.PMID: 10549424
4. Rosner BM, Werber D, Höhle M, Stark K. Clinical aspects and self-reported symptoms of sequelae of Yersinia enterocolitica infections in a population-based study, Germany 2009-2010. BMC Infect Dis. 2013;13(1):236. DOI: 10.1186/1471-2334-13-236 PMID: 23701958
5. Huovinen E, Sihvonen LM, Virtanen MJ, Haukka K, Siitonen A, Kuusi M. Symptoms and sources of Yersinia enterocolitica-infection: a case-control study.BMC Infect Dis. 2010;10(1):122. DOI: 10.1186/1471-2334-10-122 PMID: 20487529
6. Rosner BM, Stark K, Höhle M, Werber D. Risk factors for sporadic Yersinia enterocolitica infections, Germany 2009-2010.Epidemiol Infect. 2012;140(10):1738-47. DOI: 10.1017/S0950268811002664 PMID: 22313798
7. Satterthwaite P, Pritchard K, Floyd D, Law B. A case-control study of Yersinia enterocolitica infections in Auckland.Aust N Z J Public Health. 1999;23(5):482-5. DOI: 10.1111/j.1467-842X.1999.tb01303.x PMID: 10575769
8. Ostroff SM, Kapperud G, Hutwagner LC, Nesbakken T, Bean NH, Lassen J, et al. Sources of sporadic Yersinia enterocolitica infections in Norway: a prospective case-control study. Epidemiol Infect. 1994;112(1):133-41. DOI: 10.1017/S0950268800057496 PMID: 8119353
9. Boqvist S, Pettersson H, Svensson A, Andersson Y. Sources of sporadic Yersinia enterocolitica infection in children in Sweden, 2004: a case-control study.Epidemiol Infect. 2009;137(6):897-905. DOI: 10.1017/S0950268808001209 PMID: 18789174
10. Grahek-Ogden D, Schimmer B, Cudjoe KS, Nygård K, Kapperud G. Outbreak of Yersinia enterocolitica serogroup O:9 infection and processed pork, Norway.Emerg Infect Dis. 2007;13(5):754-6. DOI: 10.3201/eid1305.061062 PMID: 17553258
11. MacDonald E, Heier BT, Nygård K, Stalheim T, Cudjoe KS, Skjerdal T, et al. Yersinia enterocolitica outbreak associated with ready-to-eat salad mix, Norway, 2011. Emerg Infect Dis. 2012;18(9):1496-9. DOI: 10.3201/eid1809.120087 PMID: 22932318
12. European Centre for Disease Prevention and Control (ECDC). Annual epidemiological report 2014 - Food- and waterborne diseases and zoonoses. Stockholm: ECDC; 2014.
13. Morse DL, Shayegani M, Gallo RJ. Epidemiologic investigation of a Yersinia camp outbreak linked to a food handler.Am J Public Health. 1984;74(6):589-92. DOI: 10.2105/AJPH.74.6.589 PMID: 6721015
14. Gierczyński R, Golubov A, Neubauer H, Pham JN, Rakin A. Development of multiple-locus variable-number tandem-repeat analysis for Yersinia enterocolitica subsp. palearctica and its application to bioserogroup 4/O3 subtyping.J Clin Microbiol. 2007;45(8):2508-15. DOI: 10.1128/JCM.02252-06 PMID: 17553973
15. International Organization for Standardization (ISO). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica. ISO 10273. 2nd Edition. Geneva: ISO; 2003.
16. Nordic Committee on Food Analysis (NMKL). Yersinia enterocolitica. Detection in foods. NMKL no. 117, 3rd ed. NMKL; 1996.
17. Nordic Committee on Food Analysis (NMKL). Pathogenic Yersinia enterocolitica and Yersinia pseudotuberculosis – real time PCR methods for detection in food, feed and environmental samples. NMKL no. 117, 2nd ed. NMKL; 2013.
18. Thompson K. Controlled Atmosphere Storage of Fruits and Vegetables. 2nd Edition. CAB International, Oxfordshire, UK; 2010, pp. 179.
19. Kangas S, Takkinen J, Hakkinen M, Nakari UM, Johansson T, Henttonen H, et al. Yersinia pseudotuberculosis O:1 traced to raw carrots, Finland. Emerg Infect Dis. 2008;14(12):1959-61. DOI: 10.3201/eid1412.080284 PMID: 19046537
20. Jalava K, Hakkinen M, Valkonen M, Nakari UM, Palo T, Hallanvuo S, et al. An outbreak of gastrointestinal illness and erythema nodosum from grated carrots contaminated with Yersinia pseudotuberculosis. J Infect Dis. 2006;194(9):1209-16. DOI: 10.1086/508191 PMID: 17041846

21. Rimhanen-Finne R, Niskanen T, Hallanvuo S, Makary P, Haukka K, Pajunen S, et al. Yersinia pseudotuberculosis causing a large outbreak associated with carrots in Finland, 2006. Epidemiol Infect. 2009;137(3):342-7. DOI: 10.1017/S0950268807000155 PMID: 18177523
22. Grotto I, Huerta M, Balicer RD, Halperin T, Cohen D, Orr N, et al. An outbreak of norovirus gastroenteritis on an Israeli military base. Infection. 2004;32(6):339-43. DOI: 10.1007/s15010-004-4002-3 PMID: 15597223
23. Jelastopulu E, Venieri D, Komninou G, Kolokotronis T, Constantinidis TC, Bantias C. Outbreak of acute gastroenteritis in an air force base in Western Greece.BMC Public Health. 2006;6(1):254. DOI: 10.1186/1471-2458-6-254 PMID: 17044937
24. Lee VJ, Ong AE, Auw M. An outbreak of Salmonella gastrointestinal illness in a military camp.Ann Acad Med Singapore. 2009;38(3):207-11.PMID: 19347073
25. Wadl M, Scherer K, Nielsen S, Diedrich S, Ellerbroek L, Frank C, et al. Food-borne norovirus-outbreak at a military base, Germany, 2009. BMC Infect Dis. 2010;10(1):30. DOI: 10.1186/1471-2334-10-30 PMID: 20163705
26. Mayet A, Andreo V, Bedubourg G, Victorion S, Plantec J, Soullie B, et al. Food-borne outbreak of norovirus infection in a French military parachuting unit, April 2011. Euro Surveill. 2011;16(30):19930.PMID: 21813082
27. Yap J, Qadir A, Liu I, Loh J, Tan BH, Lee VJ. Outbreak of acute norovirus gastroenteritis in a military facility in Singapore: a public health perspective.Singapore Med J. 2012;53(4):249-54. PMID: 22511047
28. Lindholm H, Visakorpi R. Late complications after a Yersinia enterocolitica epidemic: a follow up study.Ann Rheum Dis. 1991;50(10):694-6. DOI: 10.1136/ard.50.10.694 PMID: 1958092
29. Tuck JJ, Williams JR, Doyle AL. Gastro Enteritis in a military population deployed in West Africa in the UK Ebola response; was the observed lower disease burden due to handwashing?Travel Med Infect Dis. 2016;14(2):131-6. DOI: 10.1016/j.tmaid.2015.12.009 PMID: 26827135
30. Ratnam S, Mercer E, Picco B, Parsons S, Butler R. A nosocomial outbreak of diarrheal disease due to Yersinia enterocolitica serotype o:5, biotype 1.J Infect Dis. 1982;145(2):242-7. DOI: 10.1093/infdis/145.2.242 PMID: 7054326
31. Schmitz AT, Tauxe RV. Yersinia enterocolitica Infections. In: Brachman PS, Abrutyn E, editors. Bacterial Infections of Humans. 4th ed. New York: Springer; 2009.
32. Ethelberg S, Olsen KE, Gerner-Smidt P, Mølbak K. Household outbreaks among culture-confirmed cases of bacterial gastrointestinal disease.Am J Epidemiol. 2004;159(4):406-12. DOI: 10.1093/aje/kwh049 PMID: 14769645

License and copyright

Identifying components for programmatic latent tuberculosis infection control in the European Union

A Sandgren[1], JM Vonk Noordegraaf-Schouten[2], AM Oordt-Speets[2], GB van Kessel[2], SJ de Vlas[3], MJ van der Werf[1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. Pallas health research and consultancy BV, Rotterdam, the Netherlands
3. Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands

Correspondence: Marieke J van der Werf (marieke.vanderwerf@ecdc.europa.eu)

Citation style for this article:
Sandgren A, Vonk Noordegraaf-Schouten JM, Oordt-Speets AM, van Kessel GB, de Vlas SJ, van der Werf MJ. Identifying components for programmatic latent tuberculosis infection control in the European Union. Euro Surveill. 2016;21(34):pii=30325. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.34.30325

Article submitted on 6 July 2015 / accepted on 29 April 2016 / published on 25 August 2016

Individuals with latent tuberculosis infection (LTBI) are the reservoir of *Mycobacterium tuberculosis* in a population and as long as this reservoir exists, elimination of tuberculosis (TB) will not be feasible. In 2013, the European Centre for Disease Prevention and Control (ECDC) started an assessment of benefits and risks of introducing programmatic LTBI control, with the aim of providing guidance on how to incorporate LTBI control into national TB strategies in European Union/European Economic Area (EU/EEA) Member States and candidate countries. In a first step, experts from the Member States, candidate countries, and international and national organisations were consulted on the components of programmatic LTBI control that should be considered and evaluated in literature reviews, mathematical models and cost-effectiveness studies. This was done through a questionnaire and two interactive discussion rounds. The main components identified were identification and targeting of risk groups, determinants of LTBI and progression to active TB, optimal diagnostic tests for LTBI, effective preventive treatment regimens, and to explore the potential for combining LTBI control with other health programmes. Political commitment, a solid healthcare infrastructure, and favourable economic situation in specific countries were identified as essential to facilitate the implementation of programmatic LTBI control.

Introduction

Control of latent tuberculosis infection

The epidemiological situation of tuberculosis (TB) in the European Union/European Economic Area (EU/EEA) is heterogeneous. Substantial differences are seen in the TB notification rates and different countries face different challenges such as TB among migrants [1]. In 2013, 18 EU/EEA countries had less than 10 TB cases per 100,000 population [1] and are considered to have entered the TB elimination phase [2]. To reach TB elimination, a comprehensive package of interventions is required that includes addressing latent tuberculosis infection (LTBI) [3]. Individuals with LTBI represent a source from which active TB disease arises [4] and LTBI control is therefore an important condition for TB elimination. Detection of individuals with LTBI and provision of preventive treatment to these cases are key principles of LTBI control. Some countries have implemented LTBI interventions, for example in the United Kingdom (UK), individuals infected with human immunodeficiency virus (HIV) are tested for LTBI, and in the Netherlands, specific high-risk groups are targeted for LTBI screening [5,6]. As more countries are reaching the elimination phase, it is relevant to consider implementing a programmatic approach to LTBI control in the EU/EEA and candidate countries, which implies a national level comprehensive and systematic strategy.

Aim and scope of the ECDC project

The European Centre for Disease Prevention and Control (ECDC) has embarked on a project to provide EU/EEA Member States and candidate countries with scientific advice and guidance on programmatic LTBI control. In 2013, ECDC therefore initiated a comprehensive assessment of the potential benefits and risks of introducing programmatic LTBI control in national TB prevention and control strategies. The assessment is being carried out by a consortium consisting of Pallas health research and consultancy and the Department of Public Health at Erasmus Medical Center, both located in Rotterdam, the Netherlands. The goal of this assessment is to develop guidance that provides options for programmatic LTBI control. The assessment includes the following activities:

1. Inventory of expert opinions on components of LTBI control to consider in the assessment, collected through a questionnaire and two interactive rounds during a workshop;
2. Systematic literature reviews on scientific evidence for relevant components of LTBI control;
3. Mathematical modelling and cost-effectiveness studies on LTBI control;
4. Expert panel meeting to discuss the results of activities 2 and 3;

5. Strategy synthesis and guidance development with the options for introducing programmatic LTBI control in the EU/EEA.

Here we provide an overview of the inventory of expert opinions (activity 1).

Methods for inventory of expert opinions

The experts' opinions were collected in a modified Delphi approach with three rounds (round 1: questionnaire, round 2: Treasure Hunt and round 3: Idea Factory). The objectives of this inventory were: (i) to define the components of programmatic LTBI control in the EU/EEA to be considered and evaluated during the assessment and (ii) to develop research questions on each component of programmatic LTBI control for the systematic reviews.

The questionnaire round was held in the months preceding the workshop meeting to collect the opinions and visions on LTBI control of 27 experts from EU/EEA Member States and candidate countries as well as six additional stakeholders in the field of TB (see acknowledgements for the list of participants). Country experts were nominated by the ECDC advisory forum (one expert per country) and stakeholder experts were selected by ECDC. The questionnaire collected background information about the expert, the TB situation in their country, an appraisal of the relevance, importance, efficacy, cost-effectiveness, acceptability, and feasibility of possible components of LTBI control, and the expected developments in TB epidemiology, control, and interventions. It also included questions about the best approaches for LTBI control in the expert's country and in the EU/EEA as a whole. The outcomes of this questionnaire gave insight into the aspects the experts agreed and disagreed upon. These were used to define the components of programmatic LTBI control to be further evaluated in the second and third round of the Delphi process.

The questionnaire round was followed by a workshop meeting on 19–20 September 2013. During the workshop, the participants received a summary of the questionnaire results, which they further discussed using the methods 'Treasure Hunt' (round 2) and 'Idea Factory' [7] (round 3). The methods and themes for discussion were adapted using the results of the questionnaire round. Both interactive methods allowed the participants to further identify and refine relevant components of LTBI control and to assess what aspects were key for the successful implementation of the components.

Treasure Hunt is a method that allows for an efficient and intense exchange of ideas. It comprises of a number of interactive rounds on central themes or questions which are discussed in small groups. In our project, six questions were discussed, aiming at: (i) the difference between TB and LTBI control, (ii) interventions with impact on LTBI incidence, (iii) risk groups, (iv) country-specific factors for LTBI control, (v) new diagnostics for LTBI and (vi) current developments in the EU/EEA regarding TB/LTBI. The Idea Factory is an interactive process in which the experts, divided into small teams, are asked to develop proposals regarding several themes defined beforehand. The themes for round 3 were adapted to the outcomes of round 2. The process is shaped as a competition where proposals are evaluated by a review team. In developing the proposals, the participants were asked to elaborate on the following aspects: specific conditions needed for successful implementation of interventions in LTBI control programmes, circumstances that should be taken into account during the implementation of the intervention, and who should have the lead. In a concluding session, the experts were asked to suggest research questions relevant for the next steps in the assessment, in particular for the systematic reviews to be performed.

Inventory of expert opinions

Questionnaire

In total 23 of the 27 experts filled out the questionnaire. The appraisal considered contact tracing a very important intervention to control LTBI. Chemoprophylaxis (for individuals at risk of TB infection) and preventive therapy (for individuals with LTBI) were seen as relevant interventions by most experts, but not as feasible interventions. Screening programmes to detect LTBI among high-risk groups were also frequently seen as a relevant and important intervention, but were considered not always feasible. Vaccines against TB infection were valued as an acceptable intervention, but not as a feasible intervention. The results of the questionnaire showed that LTBI control should mainly focus on high risk groups such as (but not limited to) HIV-infected individuals, healthcare workers and immunocompromised patients, but not on travellers to countries with a high TB incidence or people who abuse alcohol. During the meeting, TB contacts and migrants or refugees were also suggested as a target group for LTBI control. Overall, more than 90% of the experts thought that programmatic LTBI control in their country and in the EU/EEA would be relevant, important and effective. LTBI screening (in high-risk groups) was considered the best and most complete intervention to control LTBI in their country and in the EU/EEA.

Treasure Hunt

During the Treasure Hunt, six questions were discussed by the experts:

1. What is the difference between TB and LTBI control?

The experts indicated that TB and LTBI control are closely related in terms of case finding, treatment, treatment-related side-effects, risk groups, stigma and the goal of the control programme (i.e. decreasing TB incidence). However, there are also important differences between the two: active TB disease is infectious, the methods of diagnosis and treatment of TB and LTBI are distinct, the ethics regarding treatment (treating

ill persons with TB vs 'not ill' persons with LTBI) and the acceptability of possible side effects of TB or LTBI treatment differ. As a result, the perception and understanding of infection vs disease among policymakers and healthcare workers are also different. The participants emphasised that they expected that evidence-based data for LTBI control strategies are currently not widely available.

2. What do you consider the most important interventions with impact on LTBI incidence?

The interventions that the participants considered to have the largest impact on LTBI in Europe within the next 10 years were contact tracing, LTBI screening and preventive therapy for LTBI, especially in risk groups. Furthermore, a need for developing better tests for LTBI diagnosis was identified, as was a need for prognostic tools that predict the chance of active TB developing in individuals infected with *Mycobacterium tuberculosis*. Also, better insight into the prevalence and determinants of progression to TB disease was considered desirable.

3. What are the risk groups that should be prioritised for LTBI control interventions?

According to the experts, besides TB contacts and immunocompromised patients, HIV patients as well as migrants and refugees should have the highest priority in programmatic LTBI control. The risk group consisting of travellers to countries with high TB incidence should have the least priority. LTBI interventions should be country-specific, however.

4. What are country-specific factors for LTBI control that should be taken into consideration?

Although the meeting participants considered it possible to identify LTBI control interventions that could be implemented in the EU/EEA in general, there are country-specific factors that should be taken into account to successfully implement these interventions, such as the epidemiological situation (e.g. overall TB incidence, incidence of multidrug-resistant TB (MDR-TB)), healthcare structure and infrastructure (e.g. health priorities, medical partnerships, local feasibility), cultural aspects (e.g. acceptance by clinicians of LTBI treatment as a useful intervention) and available resources.

5. What are the arguments for investment in new diagnostics for LTBI?

The expert groups were asked to provide arguments both for and against the proposition that extra attention and financial resources should be invested in new diagnostics for LTBI. All expert groups provided arguments in favour of the proposition that there is a need to develop better diagnostic tests and prognostic tools. The most important arguments mentioned were: to save costs, to have a better tool for LTBI diagnosis and to be able to gain better insight in LTBI such as the prevalence and determinants of progression to TB disease. Half of the expert groups also provided arguments against the proposition. These were that money and focus could better be used for other investigations such as studies on MDR-TB and the development of a vaccine.

6. What are the key future developments in the EU/EEA regarding TB/LTBI?

When considering LTBI prevention strategies, it is important to take future developments in Europe into account, such as changes in migration patterns, incidence of MDR-TB and of HIV/TB co-infection, and waning TB expertise among healthcare professionals as TB becomes less common.

Idea Factory

During the Idea Factory, proposals for the implementation of the following seven themes and an open category regarding programmatic LTBI control in the EU/EEA were developed by the participants: contact tracing, chemoprophylaxis, preventive therapy, screening, education and information, programmatic LTBI control in the EU/EEA and integration of latent TB control in other healthcare interventions. The main proposals are summarised in Box 1. An important condition for successful implementation mentioned in most of the proposals was to ensure political will and commitment.

Box 1

Main proposals developed during the Idea Factory for the implementation of programmatic control of latent tuberculosis infection[a]

- To develop a systematic approach to implement contract tracing in programmatic LTBI control;
- To develop monitoring and evaluation systems for contact tracing, for LTBI cases and for outcome of preventive treatment;
- To use social networks to improve contact tracing;
- To identify the target groups for chemoprophylaxis and preventive treatment;
- To collect more evidence on the compliance with and outcome of LTBI treatment in the different target groups;
- To ensure education and training for all levels of society, including specific groups such as policy makers, healthcare workers and community workers;
- To develop methods and content for the information and education strategy and take into consideration the specifities of the target groups;
- To integrate LTBI control in healthcare programmes for other diseases;
- To invest in research and development of better drugs;
- To provide support and technical assistance for development and implementation of guidelines;
- To develop a decision-making support tool for LTBI control.

[a] Experts elaborated on conditions needed for successful implementation of interventions in LTBI control programmes, circumstances that should be taken into account during the implementation and who should have the lead (not reported in this box).

Research questions for systematic reviews

Based on the outcomes of the questionnaire and the two rounds of interactive discussion, the participants developed topics for research questions to be addressed in the systematic literature reviews. The main themes of the research questions on LTBI control were: identification of the most important LTBI risk groups, prevalence of LTBI in different risk groups and the general population, risk of active TB over time after infection, risk of TB after exposure to an infectious index case with or without preventive therapy, risk of developing TB related to the country of origin when migrating to a low incidence area, current most optimal diagnostic test or combination of diagnostic tests for diagnosing LTBI, efficacy of and current most optimal LTBI preventive treatment regimens in different risk groups, major and minor adverse events related to LTBI preventive treatment, adherence to LTBI preventive treatment in different risk groups, effective interventions to improve LTBI treatment adherence, access to risk groups for screening and treatment, impact of combining LTBI screening with other health programmes and increasing awareness and knowledge of LTBI.

Summary of main components for the assessment

According to the consulted experts, there are a number of issues that ECDC and the EU/EEA Member States need to assess and get a more comprehensive perspective about before deciding to include programmatic LTBI control in the EU/EEA.

Firstly, the prevalence of LTBI in specific risk groups and the respective risk of progression to active TB disease should be assessed. This includes assessing factors and determinants that influence the prevalence of LTBI (in particular changing migration patterns), and the risk of developing active TB over time in infected persons, with or without chemoprophylaxis or preventive treatment.

Regarding the diagnosis of LTBI, experts considered it important to identify the most reliable tests with the highest yield in different epidemiological settings and populations (e.g. immunocompromised patients, HIV patients, children, migrants and close contacts of TB patients). Also, the best strategy for LTBI and TB screening and case finding should be assessed, as well as the potential for combining this with other health programmes. An assessment of the legislation and potential changes needed to implement screening programmes was also suggested.

Furthermore, assessing the best preventive treatment regimens for LTBI in different situations and in different target groups, considering efficacy and adverse effects will be important. The effectiveness of different interventions to improve LTBI treatment uptake and adherence should be assessed, such as directly observed treatment (DOT) and incentives, including making LTBI diagnosis and treatment free of charge. It is likely that programmatic LTBI control will focus on risk groups for TB. The experts therefore suggested that questions on how to best target risk groups and improve their access to LTBI screening and treatment should be addressed.

The experts further suggested to look at interventions based on information and education to increase awareness and knowledge of LTBI and TB, targeting different groups such as policymakers, healthcare workers, medical students, community workers, risk groups and the population as a whole. The assessment should consider what the content of the education and information strategy should be, what the most effective methods for distributing information are and whether social networks can be used. Furthermore, the existence of guidelines and standardised methods for a programmatic LTBI control approach was highlighted as important, as well as the processes for evaluating the implementation of LTBI treatment programmes.

Finally, political will and commitment, the healthcare infrastructure, the economic situation, and other country-specific conditions and circumstances within EU/EEA Member States will have an impact on the implementation of programmatic LTBI control.

Concurrent developments

In the concluding discussions of the workshop, experts emphasised the importance of harmonising and coordinating the assessment undertaken by ECDC with other activities in the area of TB elimination and LTBI control e.g. by the World Health Organization (WHO) and the European Respiratory Society. In keeping with this conclusion, when WHO embarked on developing a guideline on the management of LTBI in 2013, it did so in bilateral collaboration with ECDC and similarly, the ECDC project on programmatic LTBI control has been undertaken in collaboration with WHO. Since 2014, WHO and ECDC have collaborated and shared the evidence base on LTBI management and control which was collected through a series of systematic reviews. This information was used in WHOs 2015 guidelines on management on LTBI [8] and will be used by ECDC for further assessment (including mathematical modelling and cost-effectiveness analyses) and the development of guidance for programmatic LTBI control tailored to the EU/EEA.

Concluding remarks

The workshop helped facilitate an exchange of insights between experts on different areas of LTBI control in Europe. It also created a platform for raising support for programmatic LTBI control that should increase the likelihood of cooperation and implementation during later phases of the process. Key areas that need further attention in the assessment of the potential benefits and risks of introducing programmatic LTBI control in the TB prevention and control strategy of the EU/EEA were identified and agreed upon. The input of the experts during the putting together of the inventory was not exhaustive, however, and the assessment

that followed this process took into consideration additional relevant components and aspects, in collaboration with WHO.

Since the development of the inventory, the assessment has continued and a series of systematic literature reviews has been performed [9-14]. The next step will be to conduct mathematical modelling and cost-effectiveness studies. This work will contribute towards a guidance document that elaborates on the available options when considering programmatic LTBI control in the EU/EEA.

Acknowledgements

The two-day workshop meeting was organised and facilitated by the consortium consisting of Pallas health research and consultancy and the Department of Public Health, Erasmus MC in close collaboration with ECDC. We acknowledge the contributions of Delphine Antoine (France), Ágnes Bakos (Hungary), Olivera Bojovic (Montenegro), Graham Bothamley (Tuberculosis Network European Study Group Clinical Trials), Nicoleta Cioran (Romania), Pierpaolo de Colombani (World Health Organisation Regional Office for Europe), Ioannis Demetriades (Cyprus), Joan O'Donnel (Ireland), Raquel Duarte (Portugal), Connie Erkens (The Netherlands), Brian Farrugia (Malta), Haileyesus Getahun (World Health Organisation Headquarters), Barbara Hauer (Germany), Einar Heldal (The International Union against TB and Lung Diseases), Rein Houben (Tuberculosis Modelling and Analysis Consortium), Jerker Jonsson (Sweden), Rania Kalkouni (Greece), Mitja Košnik (Slovenia), Troels Lillebæk (Denmark), Phil LoBue (US Centers for Disease Control and Prevention), Giovanni Battista Migliori (European Respiratory Society), Vladimir Milanov (Bulgaria), Howard Njoo (Public health agency Canada), Vija Riekstina (Latvia), Lidija Ristic (Serbia), Laura Sánchez-Cambronero (Spain), Aleksandar Šimunović (Croatia), Hanna Soini (Finland), Ivan Solovic (Slovakia), Elena Suciliene (Lithuania), Martina Vasakova (Czech Republic), Maryse Wanlin (Belgium), Brita Askeland Winje (Norway), Maja Zakoska (Former Yugoslav Republic of Macedonia), and Dominik Zenner (United Kingdom). We also thank Gerard Muller who facilitated the workshop.

Support Statement: This work was performed under the ECDC Framework Contracts FWC/ECDC/2013/005 and FWC/ECDC/2014/032 awarded to the consortium consisting of Pallas health research and consultancy and the Department of Public Health, Erasmus MC, Rotterdam.

Conflict of interest

None declared.

Authors' contributions

Andreas Sandgren wrote the first draft of the manuscript. All other co-authors have contributed to the writing and have approved the final version.

References

1. European Centre for Disease Prevention and Control (ECDC)/ World Health Organization Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe 2015. Stockholm: ECDC; 2015. Available from: http://ecdc.europa.eu/en/publications/Publications/tuberculosis-surveillance-monitoring-Europe-2015.pdf
2. Young DB, Gideon HP, Wilkinson RJ. Eliminating latent tuberculosis.Trends Microbiol. 2009;17(5):183-8. DOI: 10.1016/j.tim.2009.02.005 PMID: 19375916
3. Lönnroth K, Migliori GB, Abubakar I, D'Ambrosio L, de Vries G, Diel R, et al. Towards tuberculosis elimination: an action framework for low-incidence countries. Eur Respir J. 2015;45(4):928-52.PMID: 25792630
4. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC, WHO Global Surveillance and Monitoring Project. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country.JAMA. 1999;282(7):677-86. DOI: 10.1001/jama.282.7.677 PMID: 10517722
5. Capocci S, Smith C, Morris S, Bhagani S, Cropley I, Abubakar I, et al. Decreasing cost effectiveness of testing for latent TB in HIV in a low TB incidence area. Eur Respir J. 2015;46(1):165-74. DOI: 10.1183/09031936.00067114 PMID: 25882810
6. Erkens CG, Slump E, Verhagen M, Schimmel H, de Vries G, Cobelens F, et al. Monitoring latent tuberculosis infection diagnosis and management in the Netherlands. Eur Respir J. 2016;47(5):1492-501. DOI: 10.1183/13993003.01397-2015 PMID: 26917614
7. Muller G. Idea-Factory method. Utrecht: Hepta Aps. [Accessed: 6 December 2013]. Available from: http://www.idea-factory.org/
8. Getahun H, Matteelli A, Abubakar I, Aziz MA, Baddeley A, Barreira D, et al. Management of latent Mycobacterium tuberculosis infection: WHO guidelines for low tuberculosis burden countries. Eur Respir J. 2015;46(6):1563-76. DOI: 10.1183/13993003.01245-2015 PMID: 26405286
9. Stuurman AL, Vonk Noordegraaf-Schouten M, van Kessel F, Oordt-Speets AM, Sandgren A, van der Werf MJ. Interventions for improving adherence to treatment for latent tuberculosis infection: a systematic review.BMC Infect Dis. 2016;16(1):257.
10. Sandgren A, Vonk Noordegraaf-Schouten M, van Kessel F, Stuurman A, Oordt-Speets A, van der Werf MJ. Initiation and completion rates for latent tuberculosis infection treatment: a systematic review.BMC Infect Dis. 2016;16(1):204.
11. Stagg HR, Zenner D, Harris RJ, Muñoz L, Lipman MC, Abubakar I. Treatment of latent tuberculosis infection: a network meta-analysis.Ann Intern Med. 2014;161(6):419-28.
12. den Boon S, Matteelli A, Getahun H. Rifampicin resistance after treatment for latent tuberculous infection: a systematic review and meta-analysis.Int J Tuberc Lung Dis. 2016;20(8):1065-71.
13. Sotgiu G, Matteelli A, Getahun H, Girardi E, Sañé Schepisi M, Centis R, et al. Monitoring toxicity in individuals receiving treatment for latent tuberculosis infection: a systematic review versus expert opinion. Eur Respir J. 2015;45(4):1170-3.
14. Den Boon S, Matteelli A, Ford N, Getahun H. Continuous isoniazid for the treatment of latent tuberculosis infection in people living with HIV.AIDS. 2016;30(5):797-801.

ESCAIDE 'late-breaker' abstract call opens 1 September 2016

Eurosurveillance editorial team [1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Correspondence: Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)

Citation style for this article:
Eurosurveillance editorial team. ESCAIDE 'late-breaker' abstract call opens 1 September 2016. Euro Surveill. 2016;21(34):pii=. DOI: http://dx.doi.org/30322

Article published on 25 August 2016

The 2016 European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) will take place from 28 to 30 November 2016.

The later breaker call for abstracts will open on 1 September and may only be submitted online, through the ESCAIDE booking site.

Late breaker abstracts may be eligible for both oral and poster presentation at the Conference. To be eligible, the abstract must fulfil all the following criteria:

- a) report on acute urgent public health problems OR b) contain novel, surprising findings; AND
- report data or information that was unavailable before 11 May 2016 (the deadline for submission in the general call for abstracts for ESCAIDE), AND
- have not been published before.

More information about the late breaker call is available here.

The programme and conference registration instructions are available on the ESCAIDE conference website. For further information, contact: escaide.conference@ecdc.europa.eu