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Emergence of a new recombinant Sydney 2012 norovirus variant in Denmark, 26 December 2012 to 22 March 2013

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We report here new recombinants between the norovirus II.4 Sydney 2012 and the II.4 New Orleans 2009 variants. This demonstrates that the II.4 Sydney 2012 variant is undergoing further diversification and suggests a potential for rapid evolution. We also provide primers, which allow the amplification and sequencing of both the current New Orleans 2009 and Sydney 2012 variants and the new II.4 New Orleans 2009/II.4 Sydney 2012 recombinants for more accurate surveillance and transmission tracking.

In the period between December 2012 and April 2013, a selection of norovirus (NoV)-positive samples which had been submitted to Statens Serum Institute (SSI) for diagnostic purposes were further characterised by typing sequences of the NoV derived polymerase (pol) and capsid (cap) genes. Based on their characteristics, the pol and cap sequences were respectively assigned to a particular NoV genotype within genogroup I (GI) or genogroup II (GII), including NoV GII, genotype 4 (II.4) variants where applicable. A number of samples tested concomitantly positive for the pol of one NoV variant and the cap of another. For example, the GII, genotype e (II.e) Sydney 2012 NoV pol/II.4 Sydney 2012 NoV cap sequences were co-detected in some samples and in other samples the II.4 New Orleans 2009 NoV pol/II.4 Sydney 2012 NoV cap sequences were co-detected. As this could potentially indicate the presence of recombinant NoV in such samples, primers for a polymerase chain reaction (PCR) assay were designed to investigate if recombinants could be detected in the samples.

Background

The NoV Sydney 2012 variant has since late 2012 attracted global attention and several countries have reported an associated increase in NoV cases and a rapid evolution of this variant [1-4]. The earlier dominant New Orleans 2009 variant [5] has been replaced by the Sydney 2012 variant in the United States [3]. We provide here evidence for the emergence of recombinant Sydney 2012 variants and present primers to enable the amplification and sequencing of these variants.

New recombinants

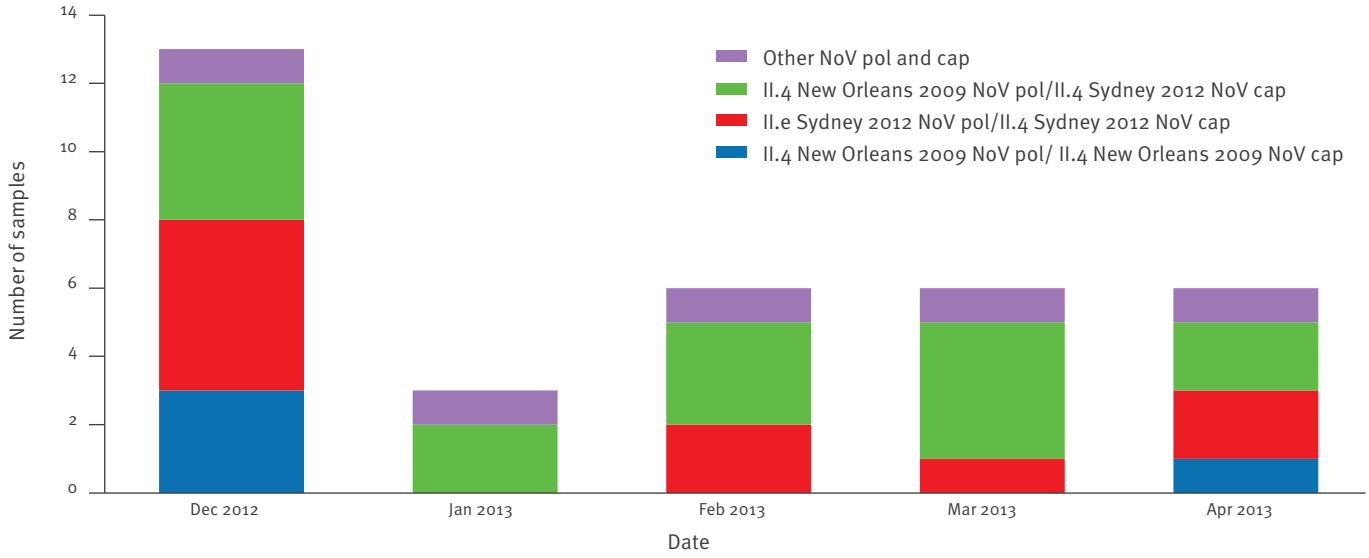
Characterisation of NoV was done by typing of the NoV pol and cap regions. Typing of the pol region was conducted using a first round reverse transcription-PCR (RT-PCR) with the primers NV32,NV32a (forward primers)/NV36 (reverse primer), followed by a second PCR using the primers NV33,NV33a (forward primers)/ NV35,NV35a (reverse primers) [6,7]. The PCR primers in this assay were designed to anneal conserved regions shared by NoV of GI and GII and can amplify both NoV GI and GII viral sequences. Typing of the cap region consisted in a semi-nested PCR for either GI or GII viral sequences. In the first RT-PCR round, the primers G1FF/G1SKR for GI or G2FB/G2SK for GII [8,9] were used, followed by a second round PCR using the primers GIFFN/G1SKR (GI) or GIIFBN/G2SKR (GII) [9,10]. This allowed us to retrieve and analyse the pol and cap genetic sequences derived from individual NoV positive samples submitted to SSI for routine diagnostics, and to respectively assign each sequence to a NoV variant. Co-detection in a sample of a pol sequence characteristic of one NoV variant and a cap sequence characteristic of another could suggest possible NoV recombinants.

The first sample with evidence of possible recombination had been sent to SSI on 21 December 2012. In the period from 8 December 2012 to 12 April 2013, a total of 34 samples were typed in both the pol and cap genes and of these, three (9 %) were II.4 New Orleans 2009 in both pol and cap, 10 (29%) were II.e Sydney 2012 in pol and II.4 Sydney 2012 in cap and 16 (47%) were II.4 New Orleans 2009 in pol and II.4 Sydney 2012 in cap, while the remaining five (15%) were other NoV types (Figure 1).

Interestingly, we did not observe any indications for a recombination of the II.e Sydney 2012 NoV pol and the II.4 New Orleans 2009 NoV cap sequences, which could indicate a strong selection pressure against continued circulation of any NoV variant containing the New Orleans 2009 NoV cap.

Figure 1

Characterisation of norovirus positive samples by analysing respective polymerase and capsid sequences, Denmark, 08 December 2012–12 April 2013 (n=34)



Cap: capsid; NoV: norovirus; pol: polymerase.

The pol and cap type are indicated in the coloured legend.

Table

Primers used for polymerase chain reaction amplification or sequencing of a norovirus contiguous genomic region sequence containing polymerase and capsid regions in order to detect norovirus recombinants

Reaction types	Primer name	Primer orientation	Primer sequence (5´ to 3´)	Annealing position[a]	PCR product size (bp)
First PCR[b]/ sequencing	Pol F1	Forward	CAGAACCACATTTGGCTCAGGTAGTC	4,371	1,325
	Capsid R1	Reverse	CGTGAGAACTCGACAAGAAACTGTGAAGAC	5,696	
Second PCR[b,c]/ sequencing	Pol F2	Forward	GCGACTTCACAATATCAATCAACGAGG	4,434	846
	Capsid R2	Reverse	TGTAAACTCTCCACCAGGGGCTTGTAC	5,280	

PCR: polymerase chain reaction.

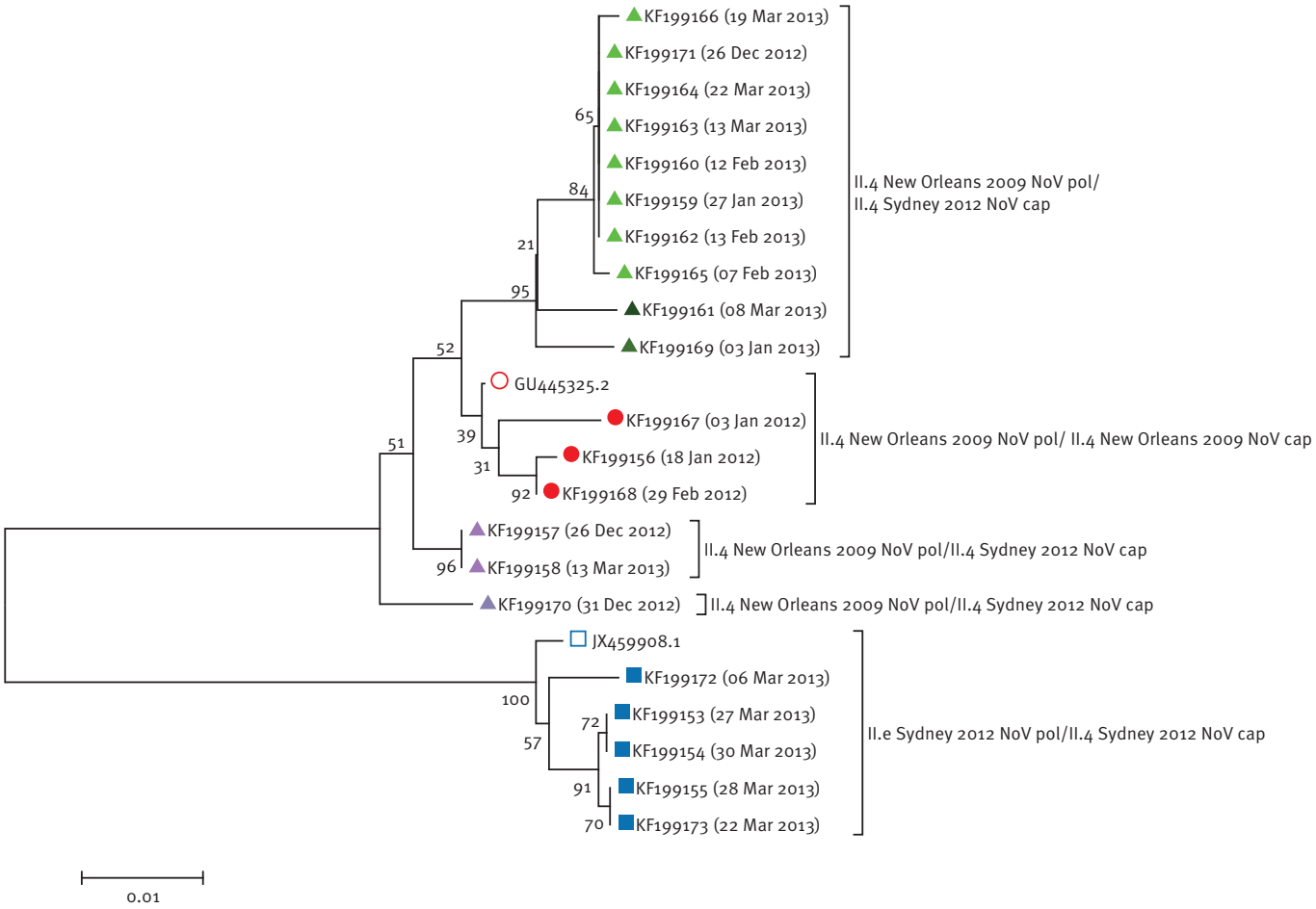
[a] Relative to the II.4 New Orleans 2009 norovirus variant reference sequence (GenBank accession number: GU445325.2).

[b] The primers for this PCR can equally well amplify a genomic region with the New Orleans 2009 polymerase and capsid sequence, a genomic region with the Sydney 2012 polymerase and capsid sequences, or a recombinant genomic region with the New Orleans 2009 polymerase and the Sydney 2012 capsid sequences.

[c] The second PCR is optional and can be used for samples with no product after the first PCR.

Figure 2

Phylogenetic analysis of the contiguous polymerase and capsid sequence regions retrieved from norovirus positive samples, Denmark, 03 January–30 March 2013 (n=21)



Cap: capsid; NoV: norovirus; pol: polymerase.

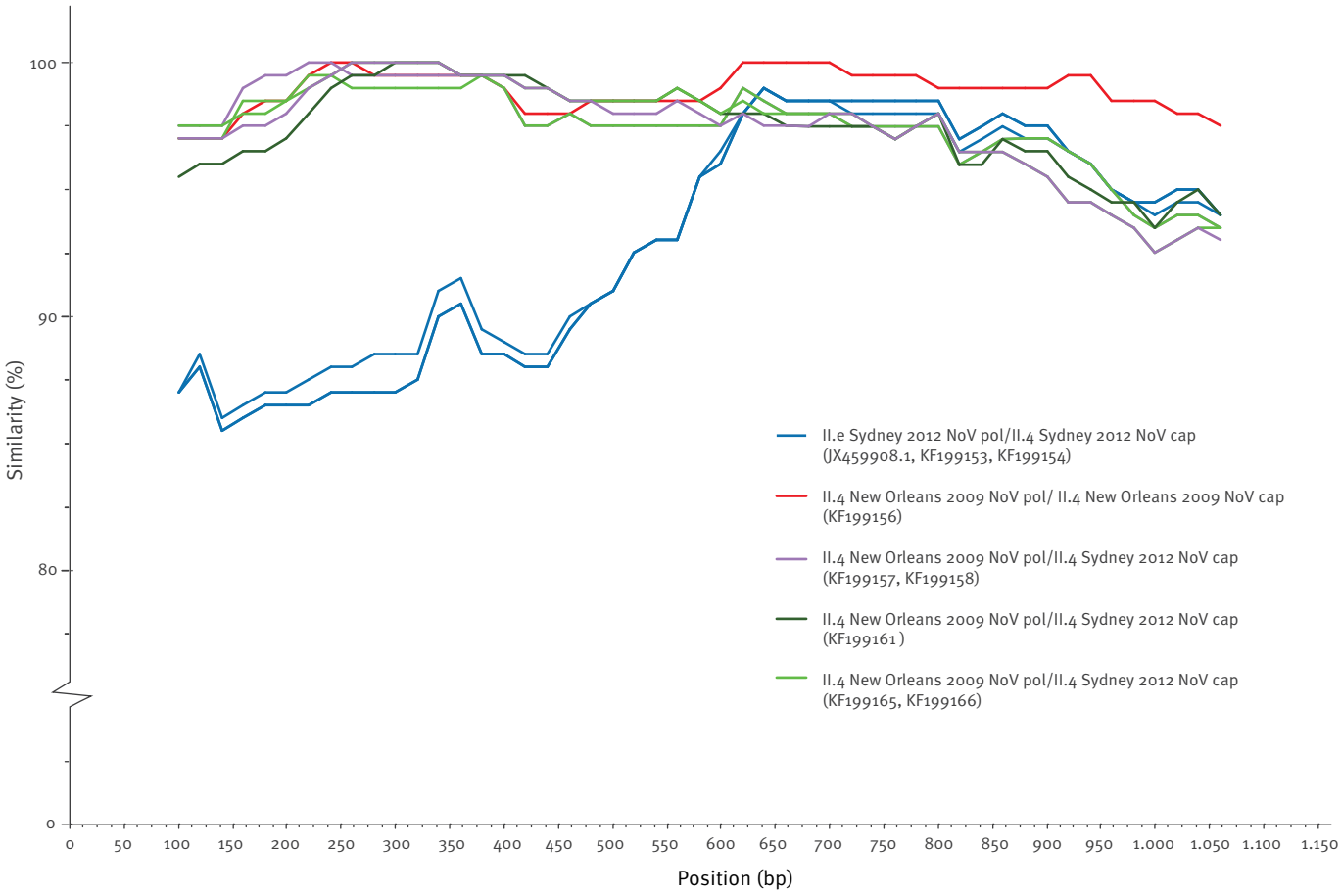
Filled icons represent tested samples in this study; outlined icons represent reference sequences.

The tree is a Neighbour Joining tree. Numbers at the nodes indicate supporting bootstrap values obtained from 1,000 replicates. Triangles with the same colour represent sequences differing from each other by no more than five single nucleotide polymorphisms. On the tree leaves the GenBank accession numbers of sequences are shown. When sequences are not reference sequences, the GenBank number is followed by the collection date of the sample from which the sequence was derived.

The pol and cap region types of the sequences (which were determined by typing for non-reference sequences) are shown to the side of the tree.

Figure 3

Simplot showing the percentage similarity between selected sequences retrieved from norovirus positive samples (n=8) in Denmark and the II.4 New Orleans 2009 reference sequence (GenBank accession number: GU445325.2)



Cap: capsid; NoV: norovirus; pol: polymerase.

The New Orleans 2009 norovirus reference sequence (GenBank accession number: GU445352.2) was used as the query sequence. In the legend, the GenBank accession numbers of the sequences are given for all eight sequences obtained from samples in this study and the Sydney 2012 reference sequence (GenBank accession number: JX459908.1) as well as the pol and cap type. For the II.4 New Orleans 2009 NoV pol/II.4 Sydney 2012 NoV cap recombinants, the different line colours indicate that these sequences originate from independent recombination events. The Simplot settings were: Window: 200 bp, Step: 20, GapStrip: On.

Since the pol and cap typing produce two non-overlapping sequences, the possibility of patients having a double infection with both the II.4 New Orleans 2009 NoV pol/II.4 New Orleans 2009 NoV cap and the II.e Sydney 2012 NoV pol/II.4 Sydney 2012 NoV cap variants could not be excluded. Therefore, to confirm recombination, we designed a set of nested primers (Table), allowing for amplification and sequencing of a contiguous genomic region containing both parts of the pol and cap regions used for typing, and the intergenic region.

In total, 21 samples, collected in the period from 03 January 2012 to 30 March 2013 were RT-PCR amplified and directly sequenced using the primers in the Table. Typing was initially performed using the typing tool implemented at the National Institute for Public Health and the Environment (RIVM) in the Netherlands [11]. Of the 21 amplified sequences, 19 were long enough to yield sequencing results for both the pol and cap regions, while two yielded sequencing results for the entire pol sequence but were too short to include the complete cap sequence. These two sequences were respectively derived from two samples which had been included in the 34 samples previously typed by independent amplification of the pol and cap sequences. Therefore the missing cap region of the two incomplete sequences could be obtained from the result of this previous typing approach. Phylogenetic analysis was performed on the sequences (Figure 2) using the Neighbour Joining method with Jukes Cantor implemented in Mega 5 [12].

According to the phylogenetic tree, the sequences which were typed as II.4 New Orleans 2009 pol/II.4 New Orleans 2009 NoV cap or II.e Sydney 2012 NoV pol/II.4 Sydney 2012 NoV cap formed clusters with their respective reference sequences, whereas the sequences typed as II.4 New Orleans 2009 NoV pol/II.4 Sydney NoV 2012 cap did not cluster together with any of these reference sequences. Most (8 of 13) of the recombinant II.4 New Orleans 2009 NoV pol/II.4 Sydney 2012 NoV cap sequences were localised to a single cluster (light green triangles on Figure 2). The samples in this cluster were collected during a period of 86 days (from 26 December 2012 to 22 March 2013) and differ from each other by no more than five single nucleotide polymorphisms (SNPs). These recombinants therefore probably arose from a unique recombinant, circulating in the Danish population during this period, which had been generated from a single recombination event. Another small cluster containing two sequences (pink triangles) and three other unclustered sequences (dark green, green and purple triangles) were distinctively different from the dominant cluster (light green triangles) (Figure 2). These five sequences were all collected during the same time period as the sequences in the majority cluster and probably arose from four individual recombination events. Therefore, this analysis confirms that II.4 New Orleans 2009 NoV pol/II.4 Sydney 2012 NoV cap recombinants have emerged independently and that up to five individual recombination events can be observed among the analysed samples.

Eight selected NoV sequences from this study and the II.e pol/II.4 cap Sydney 2012 reference sequence (GenBank accession number: JX459908.1) were further analysed over a region of 1,161 nucleotides using Simplot 1.3 [13] with a windows size of 200 and a 20 bp step using the New Orleans 2009 reference sequence (GenBank accession number: GU445325.2) as the query sequence (Figure 3). The selected sequences included two sequences from the study defined as having a II.e Sydney 2012 NoV pol and a II.4 Sydney 2012 NoV cap (Figure 3, in blue), one sequence from the study defined as having a II.4 New Orleans 2009 NoV pol and a II.4 New Orleans 2009 NoV cap (Figure 3, in red) and five sequences with a II.4 New Orleans 2009 NoV pol and a II.4 Sydney 2012 cap. These latter five sequences were chosen as representatives of recombinants which arose from three independent pol/cap recombination events (Figure 3, in dark green, light green and purple). The Simplot shows that all the chosen II.4 New Orleans 2009 NoV pol/II.4 Sydney 2012 NoV cap recombinant sequences (dark green, light green and pink lines) are very similar to the II.4 New Orleans 2009 NoV pol/II.4 New Orleans 2009 NoV cap sequences (red line) in the pol region and a have a cap region similar to II.4 Sydney 2012 NoV cap.

The region around position 600 to 625 (position 5,037 to 5,062 on the II.4 New Orleans 2009 reference sequence (GenBank accession number: GU445325.2), where the similarity between the II.e Sydney 2012 NoV pol/II.4 Sydney 2012 NoV cap sequences (Figure 3, blue lines), and the II.4 New Orleans 2009 query sequence, reaches a maximum, corresponds to the beginning of the II.4 Sydney 2012 NoV cap gene sequence. As this sequence is common to the II.4 New Orleans 2009 NoV pol/II.4 Sydney 2012 NoV cap recombinants (dark green, light green and pink lines) this indicates that recombination has occurred in this region.

Conclusion

Recombination is a major diversifying factor in the evolution of several NoV genotypes and II.4 variants [14,15] and might result in the emergence of more virulent variants [16,17]. We report here the emergence of new recombinants of the Sydney 2012 NoV variant, which shows that this variant is undergoing further diversification. It remains to be determined how virulent the new recombinants will be, including their capability to escape the acquired herd immunity. We also here provide primers, which will allow for identification of both the II.4 New Orleans 2009 NoV pol/II.4 New Orleans 2009 NoV cap and II.e Sydney 2012 NoV pol/II.4 Sydney 2012 NoV cap and the recombinant II.4 New Orleans 2009 NoV pol/II.4 Sydney 2012 NoV cap variants for more accurate surveillance and transmission tracking.

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Conflict of interest

None declared.

Authors' contributions

J Fonager: Conceived the idea for the study and performed sequence, phylogenetic and Simplot analysis and drafted the first version of the paper, made revisions, and approved the final version of the paper. TK Fischer: Designed the project together with J Fonager, provided constructive comments and revised the first draft of the paper critically and approved the final version of the paper. S Barzinci: Contributed considerably with the laboratory analyses and approved the final version of the paper.

References

1. van BJ, Ambert-Balay K, Botteldoorn N, Eden JS, Fonager J, Hewitt J, et al. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. Euro Surveill. 2013;18(1):pii=20345. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20345
2. Bennett S, MacLean A, Miller RS, Aitken C, Gunson RN. Increased norovirus activity in Scotland in 2012 is associated with the emergence of a new norovirus GII.4 variant. Euro Surveill. 2013;18(2):pii=20349. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20349 PMid:23324428
3. Centers for Disease Control and Prevention (CDC). Emergence of new norovirus strain GII.4 Sydney--United States, 2012. MMWR Morb Mortal Wkly Rep. 2013;62(3):55. PMid:23344699
4. Fonager J, Hindbaek LS, Fischer TK. Rapid emergence and antigenic diversification of the norovirus 2012 Sydney variant in Denmark, October to December, 2012. Euro Surveill. 2013;18(9):pii=20413. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20413
5. Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention. Updated norovirus outbreak management and disease prevention guidelines. MMWR Recomm Rep. 2011;60(RR-3):1-18.
6. Schreier E, Doring F, Kunkel U. Molecular epidemiology of outbreaks of gastroenteritis associated with small round structured viruses in Germany in 1997/98. Arch Virol. 2000;145(3):443-53. http://dx.doi.org/10.1007/s007050050038 PMid:10795514
7. Oh DY, Gaedicke G, Schreier E. Viral agents of acute gastroenteritis in German children: prevalence and molecular diversity. J Med Virol. 2003;71(1):82-93. http://dx.doi.org/10.1002/jmv.10449 PMid:12858413
8. Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol. 2003;41(4):1548-57. http://dx.doi.org/10.1128/JCM.41.4.1548-1557.2003 PMid:12682144 PMCid:153860
9. Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, et al. Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods. 2002;100(1-2):107-14. http://dx.doi.org/10.1016/S0166-0934(01)00404-9
10. Gallimore CI, Cheesbrough JS, Lamden K, Bingham C, Gray JJ. Multiple norovirus genotypes characterised from an oyster-associated outbreak of gastroenteritis. Int J Food Microbiol. 2005;103(3):323-30. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.02.003 PMid:15967530
11. Kroneman A, Vennema H, Deforche K, v d Avoort H, Penaranda S, Oberste MS, et al. An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol. 2011;51(2):121-5. http://dx.doi.org/10.1016/j.jcv.2011.03.006 PMid:21514213
12. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28(10):2731-9. http://dx.doi.org/10.1093/molbev/msr121 PMid:21546353 PMCid:3203626
13. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol. 1999;73(1):152-60. PMid:9847317 PMCid:103818
14. Eden JS, Tanaka MM, Boni MF, Rawlinson WD, White PA. Recombination within the Pandemic Norovirus GII.4 Lineage. J Virol. 2013;87(11):6270-82. http://dx.doi.org/10.1128/JVI.03464-12 PMid:23536665
15. Bull RA, Tanaka MM, White PA. Norovirus recombination. J Gen Virol. 2007;88(Pt 12):3347-59. http://dx.doi.org/10.1099/vir.0.83321-0 PMid:18024905
16. Mathijs E, Denayer S, Palmeira L, Botteldoorn N, Scipioni A, Vanderplasschen A, et al. Novel norovirus recombinants and of GII.4 sub-lineages associated with outbreaks between 2006 and 2010 in Belgium. Virol J. 2011;8:310. http://dx.doi.org/10.1186/1743-422X-8-310 PMid:21682917 PMCid:3135559
17. Giammanco GM, Rotolo V, Medici MC, Tummolo F, Bonura F, Chezzi C, et al. Recombinant norovirus GII.g/GII.12 gastroenteritis in children. Infect Genet Evol. 2012;12(1):169-74. http://dx.doi.org/10.1016/j.meegid.2011.10.021 PMid:22067516

High prevalence of antibodies against *Leptospira* spp. in male Austrian adults: a cross-sectional survey, April to June 2009

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To assess the distribution of specific antibodies against *Leptospira* spp. in Austrian adults, we conducted an explorative nationwide cross-sectional serological study in 400 healthy individuals. Antibody titres against *Leptospira* spp. were determined in a microscopic agglutination test using a panel of 14 serovar cultures. Sera of 18 participants were excluded because the samples were unsuitable for testing; the remaining 382 participants comprised 166 professional soldiers and 216 civilians. Overall, 88 (23%) individuals tested positive in serological screening. The subjects' sera reacted most frequently with serovars Canicola (16.5%) and Hardjo (11.8%). Epidemiological information was obtained from a questionnaire: no correlation was found for area of residence, travel abroad, regular outdoor activities, occupational animal contact, or ownership of companion animals. The proportion of seropositive samples was significantly lower among professional soldiers (15.7%) than among civilians (28.7%) (p=0.003). Our data demonstrate serological evidence of a high rate of exposure to *Leptospira* spp. among the Austrian population. No increased risk of exposure to *Leptospira* spp. was detected in military personnel.

Introduction

Leptospirosis is a worldwide zoonotic disease caused by bacteria of the genus *Leptospira*. The great majority of infections caused by *Leptospira* spp. are either subclinical or very mild, and patients will probably not seek medical attention [1]. In a smaller proportion of infections, which constitute the majority of cases that are recognised, patients develop a febrile illness with myalgia, followed by jaundice, and severe disease with consecutive multiorgan failure (Weil's disease) may occur. In such patients, the clinical presentation depends upon the predominant organs involved; the case fatality rate could reach 40% or more [2].

There are over 250 known pathogenic serovars, classified into serogroups, for which about 160 mammalian species have been identified as natural hosts; these include feral, semi-domestic, and farm and companion animals as important reservoirs [3]. *Leptospira* organisms shed in the urine of these reservoir animals can survive in the environment for long periods of time [2]. Humans acquire the disease by contact with animal urine in water, soil, or other contaminated material. Direct transmission from animals to humans is common among occupational groups who handle animals and animal tissues, such as butchers, veterinarians, and cattle and pig farmers [2]. Indirect infection through contact with leptospires excreted into the environment is probably the main route of acquiring leptospirosis. High incidence has been recorded among people who are exposed to wet environments in their occupational activities [1]. Similarly, large outbreaks have occurred worldwide after heavy rainfalls or floods, particularly in south-east Asian countries and Central and South America [2]. Furthermore, leptospirosis has been recognised as a potential hazard of recreational exposure to contaminated water, including swimming, canoeing, rafting, fishing, and similar sports [1,4,5].

Although leptospirosis is more common in tropical areas, it is also found in temperate areas, including Europe. The disease has been recognised as an emerging global public health problem because of its epidemic proportions and increasing incidence in both developing and developed countries [2]. In the context of climate change, leptospirosis has also been recognised as a re-emerging infectious disease with

particular interest for Europe and is currently under surveillance in the European Union [6].

In the past decade, with eight to 11 cases annually, the Austrian annual epidemiological reports on communicable disease have shown low incidence rates of leptospirosis compared with other countries [7]. However, because of the high proportion of mild cases and protean manifestations of the disease, surveillance based on clinical cases is likely to underestimate true infection rates. The aim of the present study was therefore to assess the prevalence of specific antibodies against *Leptospira* spp. in healthy adult individuals in Austria.

Methods

Study design and sample population

We conducted an exploratory national cross-sectional serological study in healthy Austrian individuals volunteering for military deployment abroad. In Austria, military service is compulsory for male citizens and voluntary for females. All individuals who have completed military service are eligible to volunteer for military missions abroad. Thus, in addition to professional military personnel, civilians can also volunteer for deployment abroad provided that they have completed initial military service. Before taking part in a mission, all applicants, military personnel as well as civilians undergo a medical check-up including routine laboratory investigations at the Military Hospital Vienna.

As no estimates of the prevalence of anti-*Leptospira* antibodies were available for non-endemic regions, sample size was not formally calculated. We used a convenience sample of 400 individuals. With this sample we could expect a meaningful 95% confidence interval (95% CI) from 3.1% to 7.6%, assuming a seroprevalence of 5%.

Between April and June 2009, 508 applicants from all nine federal states were eligible to volunteer for deployment abroad; 400 of them volunteered to participate in the study. No soldiers undergoing compulsory military service were included in the study. All study participants completed an epidemiological questionnaire on demographic characteristics, domestic animals, occupational animal contact, regular outdoor activities, previous international military operations at any times in the past, and holiday destinations abroad within the previous six months. The time frame of six months was chosen to obtain more reliable data on travel history, as many Austrians travel abroad annually or even more often. The study was approved by the Institutional Review Board of the Austrian Armed Forces.

Serology

A serum sample was obtained from each participant for determination of antibodies indicating previous contact with *Leptospira* spp. Sera were stored at -20°C until testing at the National Reference Laboratory for Leptospirosis at the Austrian Agency for Health and Food Safety (AGES) [5,8-10].

The sera were tested against a panel of 14 live cultures of reference serovars serving as antigens in the microscopic agglutination test (MAT). The cultures were seven days-old and autoagglutination-free, grown in Elinghausen-McCullough-Johnson-Harris (EMJH) medium (Difco, Sparks, United States) enriched with Bacto *Leptospira* Enrichment (Difco, Sparks, United States). The serovar panel comprised Australis, strain Ballico; Autumnalis, strain Akiyani A; Bataviae, strain Swart; Bratislava, strain Jez Bratislava; Canicola, strain Hond Utrecht IV; Copenhageni, strain M20; Grippotyphosa, strain Moskva V; Hardjo, strain Hardjoprajitno; Hebdomadis, strain Hebdomadis; Pomona, strain Pomona; Pyrogenes, strain Selanim, Saxkoebing, strain Mus 24; Tarassovi, strain Mitis Johnson; and Wolffii, strain 3705; The respective serogroups are shown in Table 1.

In the MAT, two doubling dilutions of each serum, 1:25 and 1:50, were used in an initial screening test. In a second step, any sera that tested positive in the first screen were titrated up to dilutions of 1:1,600. A positive and a negative control were included for each serovar in each test. The end point was set as the highest dilution of serum at which 50% agglutination occurred. In accordance with previous serosurveys, a reactive antibody titre of ≥1:100 was considered as evidence of past exposure to *Leptospira* spp. [11]. To reduce the subjective effect of observer variation, all MAT tests were performed by the same person.

Statistics

Data are presented as mean±standard deviation (SD); categorical data are presented as absolute and relative frequencies. The Mann–Whitney U test, chi-squared test or Fisher's exact test were used as appropriate for hypothesis testing to describe differences between negative and positive individuals. Prevalence of anti-*Leptospira* antibodies was described as relative frequency with exact 95% CIs. Odds ratios with exact 95% CIs were calculated using logistic regression models for identification of risk factors for seropositivity to *Leptospira* spp. MS Excel 2011 and Stata 11 for Mac (College Station, United States) were used for data management and analysis. A two-sided p value <0.05 was considered statistically significant. To illustrate co-infections with different serovars, a Venn diagram was created with web tools provided by the Bioinformatics and Systems Biology of Gent (http://bioinformatics.psb.ugent.be/webtools/Venn).

Results

Eighteen of the enrolled 400 participants were excluded from the statistical analysis because of impurities in the serum: such samples were haemolytic or lipaemic or contained protein deposits. The remaining 382 participants comprised 166 (43.5%) professional soldiers and 216 (56.5%) civilians. Subjects were between 18

Table 1

Prevalence of 14 serovars of *Leptospira* in 88 healthy individuals positive for antibodies against *Leptospira* spp., Austria, April–June 2009

Serovars	Serogroup	Number of positive sera [a]	Prevalence [b] % (95% CI)	Relative frequency[c] %
Canicola	Canicola	63	17 (13–20)	72
Hardjo	Sejroe	45	12 (9–15)	51
Copenhageni	Icterohaemorrhagiae	19	5 (3–7)	22
Bratislava	Australis	15	4 (2–6)	13
Tarassovi	Tarassovi	10	3 (1–4)	11
Pyrogenes	Pyrogenes	4	1 (0–2)	5
Saxkoebing	Sejroe	2	0.5 (0–1)	2
Pomona	Pomona	1	0.3 (0–1)	1
Grippotyphosa	Grippotyphosa	1	0.3 (0–1)	1
Bataviae	Bataviae	1	0.3 (0–1)	1
Australis	Australis	0	0	0
Autumnalis	Rachmati	0	0	0
Hebdomadis	Hebdomadis	0	0	0
Wolffii	Borgpetersenii	0	0	0

CI: confidence interval.

[a] Antibodies against multiple (at least two) serovars were found in 41 individuals.

[b] Prevalences represent unadjusted univariate estimates and do not total in the overall seroprevalence found (23%) because some sera reacted with more than one serovar.

[c] Percentages do not total 100 because some sera reacted with more than one serovar.

and 57 years-old (mean age: 29.5 years), 371 (97.1%) were male and 11 (2.9%) female. Because the small number of female participants, no sex-stratified risk analysis for infection with *Leptospira* spp. was performed. A total of 146 (38.2%) persons declared previous military assignments abroad.

Of the 382 serum samples included, 88 (23.0%, 95% CI: 18.8–27.3%) were positive for antibodies against one or more serovars of *Leptospira* in the MAT. Among the 14 serovars tested, 10 were detected in the samples. The numbers of positive sera for each serovar, together with the prevalence among the tested individuals and the relative proportion of the different serovars among seropositive samples are shown in Table 1. The prevalences were, in descending order: Canicola, Hardjo, Copenhageni, Bratislava, Tarassovi, Pyrogenes, Saxkoebing, Pomona, Grippotyphosa, and Bataviae (Table 1).

Of the 382 serum samples tested, 41 (10.7%, 95% CI: 0.08–13.85%) contained antibodies against two or more serovars: 21 samples were positive for two serovars, 13 samples for three, six samples for four, and one sample was positive for six serovars. Serovar Canicola was distinctly dominant, accounting for 71% of all positive sera, followed by Hardjo with 51%. Some serovars, such as Australis, Autumnalis, Hebdomadis and Wolffii were not represented at all.

The serovar combinations in cross-reacting sera are shown as a cross-table (Table 2). The most frequently combined serovars were Copenhageni with Canicola or Hardjo: of the 19 samples positive for Copenhageni, 17 were also positive for Canicola and 15 for Hardjo. The combinations of the five most frequent serovars (Canicola, Hardjo, Copenhageni, Bratislava and Tarassovi) are shown as a Venn diagram in the Figure. Titres above 1:100 (1:200 to ≥1:800) were found only for serovar Bratislava, in four samples.

The mean age of individuals who tested positive was 30.1 years (SD: 9.4 years) versus 29.3 years (SD: 9.7 years) for those with negative screening results, thus no association was found between age and seropositivity for *Leptospira* spp. (p=0.53).

Univariate associations between potential risk factors for positive *Leptospira* serology are shown in Table 3. There was no statistically significant difference in seroprevalence between the different regions of Austria, travel activity abroad within the six months before the screening, occupational animal contact, or ownership of companion animals. However, the seropositivity rate in people keeping aquarium fish was significantly

Table 2

Distribution of 14 serovars of *Leptospira* in 88 healthy Austrians testing positive for antibodies against *Leptospira* spp., Austria, April–June 2009

Table 2

Distribution of 14 serovars of *Leptospira* in 88 healthy Austrians testing positive for antibodies against *Leptospira* spp., Austria, April–June 2009

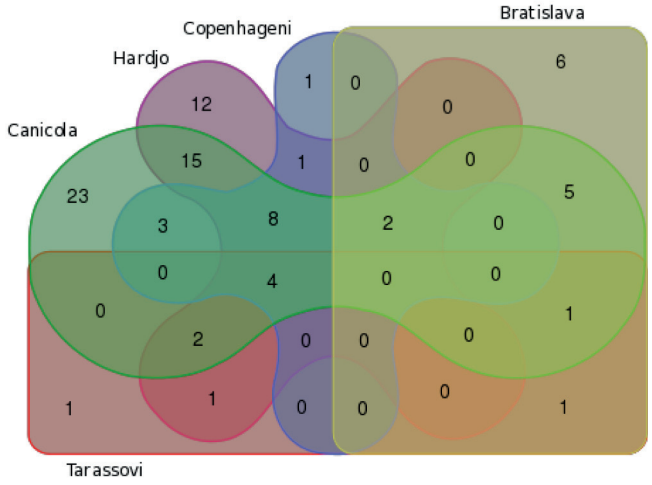
	Canicola	Hardjo	Copenhageni	Bratislava	Tarassovi	Pyrogenes	Saxkoebing	Grippotyphosa	Pomona	Bataviae	Wolffii	Australis	Autumnalis	Hebdomadis
Canicola	63	45	19	15	10	4	2	1	1	1	0	0	0	0
Hardjo	45	45	15	8	7	3	2	1	1	0	0	0	0	0
Copenhageni	19	15	19	3	4	1	1	0	1	0	0	0	0	0
Bratislava	15	8	3	15	2	0	0	1	0	0	0	0	0	0
Tarassovi	10	7	4	2	10	1	0	0	1	0	0	0	0	0
Pyrogenes	4	3	1	0	1	4	1	0	1	0	0	0	0	0
Saxkoeing	2	2	1	0	0	1	2	0	0	0	0	0	0	0
Grippotyphosa	1	1	0	1	0	0	0	1	0	0	0	0	0	0
Pomona	1	1	1	0	1	1	0	0	1	0	0	0	0	0
Bataviae	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Wolffii	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Australis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Autumnalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hebdomadis	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The numbers may not sum up in the totals (shown in grey) because some serum samples were positive for multiple serovars.

The numbers may not sum up in the totals (shown in grey) because some serum samples were positive for multiple serovars.

Figure

Venn diagram showing the distribution of the five most frequent *Leptospira* serovars reacting with serum samples from 88 healthy individuals, Austria, April–June 2009



Numbers indicate the number of sera positive for the respective serovar.

higher (3/3 versus 85/377 (22%), p=0.01). The seropositivity rate was significantly lower among professional soldiers than in civilians (26/166 (16%) versus 62/216 (29%), odds ratio (OR): 0.46, 95% CI: 0.28–0.77, p=0.003). Concerning previous military missions abroad, a significant risk factor was found only for previous deployment in the Middle East (18/50 (36%) versus 70/332 (21%), OR: 2.11, 95% CI: 1.12–3.97, p=0.03).

For seropositivity against two or more serovars, professional soldiers had again a significantly lower risk than civilians (10/166 (6.02%) versus 31/216 (14.35%), OR: 0.38, 95% CI: 0.18–0.80, p=0.01). However, travel to Asia/Australia within the six months before the screening was a significant risk factor (3/5 (60.0%) versus 38/377 (10.1%), OR: 13.38, 95% CI: 2.17–82.62, p=0.01) for multiple seropositivity.

Discussion

Human leptospirosis is considered a rare disease in Austria. Nine cases were reported in 2011, representing an annual incidence of 0.13/100,000. Considering these low numbers, the 23% seropositivity found in the present study were unexpectedly high and are comparable with findings in tropical and subtropical countries of high endemicity [2].

One factor contributing to the high rate of seropositivity could be the study population: nearly all individuals tested were men aged between 18 and 57 years. The predominance of men among clinical cases is well recognised [12,13] and has been explained by their greater tendency to participate in outdoor activities at high risk for exposure [5,13]. Similarly, case rates among adults between 20 and 50 years of age are also consistently the highest reported [12,13]. Nonetheless, in the present serosurvey, no association between regular outdoor activities and antibodies against *Leptospira* spp. could be detected.

In a previous study performed in the year 2000 in 149 hunters considered a high-risk group (146 men, mean age: 50 years) in south-eastern Austria [8], the infection rate (MAT titre ≥100) was found to be 10% among the hunters and 0% in a control group of 50 individuals (seropositivity was determined in the MAT in the same reference laboratory as in the present study). Thus, the findings of the present study may indicate an increase in seropositivity to *Leptospira* spp. in Austria in the past decade.

Notably, the national rate for clinical cases of leptospirosis reported in Austria has increased in the past two decades, from between zero and three cases in the 1990s to between eight and 11 cases in the last decade. Unfortunately, no information on routes of transmission, causative serovars, or clinical manifestations is available for these patients, except for one report on a waterborne outbreak in 2010 involving four athletes who had competed in a triathlon [5] and three cases requiring intensive care in 2004 [10]. Moreover, the number of reported cases is small and the observed increase in incidence does not necessarily reflect a concomitant increase in seroprevalence.

Since 1998, increasing numbers of clinical cases have been reported in neighbouring Germany [14]. Increasing travel abroad may have contributed to more frequent exposure to *Leptospira* spp., as growing numbers of imported cases have been reported in Austria and Germany [9]. Nevertheless, travel in general was not associated with a higher risk for seropositivity in our study, with the limitation that travel histories were only collected for the period six months before serum collection. However, previous travel to Asia/Australia was a significant parameter for being positive to multiple serovars, indicating high exposure. In particular, leptospirosis is highly endemic in south-east Asia, where numerous outbreaks and seroprevalences up to 50% are reported [2].

Based on clinical cases and outbreak reports [15-21], military personnel are considered at risk for exposure to *Leptospira* spp. because of field activities during exercises and deployment in endemic countries. However, nearly all reports of outbreaks among military personnel have focused on symptomatic cases and little is known about exposure among asymptomatic individuals. In the present study, seropositivity among professional soldiers was unexpectedly lower than among civilians. Although there have not been any studies comparing exposure to *Leptospira* in military personnel

Table 3

Risk factors for seropositivity to *Leptospira* spp., Austria, April–June 2009 (n=382)

Risk factor	Level	Positives/total (%)	Odds ratio (95% CI)	p value[a]
Male	Yes No	85/371 (23) 3/11 (27)	0.79 (0.21–3.05)	0.72
Outdoor activities	Yes No	73/309 (24) 15/73 (21)	1.20 (0.64–2.24)	0.65
Professional soldier	Yes No	26/166 (16) 62/216 (29)	0.46 (0.28–0.77)	0.003[b]
Previous mission abroad	Yes No	35/146 (24) 53/236 (22)	1.09 (0.67–1.77) 1 (reference)	0.80
Africa	Yes No	0/4 (0) 88/378 (23)	NA	0.58
Middle East	Yes No	18/50 (36) 70/332 (21)	2.11 (1.12–3.97)	0.03[b]
Southern Europe	Yes No	20/118 (17) 68/264 (26)	0.59 (0.34–1.02)	0.07
Asia	Yes No	2/6 (33) 86/376 (23)	1.69 (0.30–9.36)	0.63
Travel	Yes No	17/59 (29) 71/323 (22)	1.44 (0.77–2.68)	0.25
Africa	Yes No	1/9 (11) 87/373 (23)	0.41 (0.05–3.33)	0.69
America	Yes No	4/14 (29) 84/368 (23)	1.35 (0.41–4.42)	0.75
Asia/Australia	Yes No	3/5 (60) 85/377 (23)	5.15 (0.85–31.34)	0.08
Europe	Yes No	11/34 (32) 77/348 (22)	1.68 (0.08–3.61)	0.20
Occupational animal contact	Yes No	5/16 (31) 83/366 (23)	1.55 (0.52–4.59)	0.38
Meat processing	Yes No	0/1 (0) 88/381 (23)	NA	0.99
Canine unit	Yes No	0/1 (0) 88/381 (23)	NA	0.99
Kitchen	Yes No	3/5 (60) 85/377 (23)	5.15 (0.85–31.34)	0.08
Agriculture	Yes No	2/6 (33) 86/376 (23)	1.69 (0.30–9.36)	0.63
Companion animals	Yes No	40/173 (23) 48/209 (23)	1.01 (0.63–1.63)	0.99
Fish keeping	Yes No	3/3 (100) 85/379 (22)	NA	0.01[b]
Arthropod	Yes No	1/3 (33) 87/379 (23)	1.68 (0.15–18.73)	0.55
Rabbit	Yes No	3/12 (25) 85/370 (23)	1.12 (0.30–4.22)	0.99
Dog	Yes No	19/82 (23) 69/300 (23)	1.01 (0.57–1.80)	0.99
Cat	Yes No	24/116 (21) 64/266 (24)	0.82 (0.48–1.40)	0.51
Rodent	Yes No	3/10 (30) 85/372 (23)	1.45 (0.37–5.72)	0.70
Horse	Yes No	0/4 (0) 88/378 (23)	NA	0.58
Reptile	Yes No	0/8 (0) 88/374 (24)	NA	0.21
Bird	Yes No	2/3 (67) 86/379 (23)	6.81 (0.61–76.05)	0.13

CI: confidence interval; NA: not applicable.

[a] Fisher's exact test.

[b] A p value <0.05 was considered statistically significant.

and civilians, the few available epidemiological studies in military personnel support the present finding of a low overall exposure among soldiers. Three studies comparing new recruits and soldiers who had served longer found significantly lower seroprevalences among newly arrived recruits [15,22,23]

Although seropositivity in the present study was lower among professional soldiers, we found a significant association for *Leptospira* seropositivity among individuals, military and civilians, previously deployed in the Middle East. Thus, although particular military activities and specific deployments may be associated with exposure to *Leptospira*, this does not apply to all soldiers and care should be taken when defining military personnel as an occupational high-risk group.

Concerning other possible risk factors for exposure to *Leptospira* surveyed in the present study, occupational animal contact, ownership of companion animals, and regular outdoor activities did not reveal any significant association with seropositivity. The significant association observed for the keeping of fish merits further investigation, although the number (three of three fish keepers were seropositive) is too small to permit firm conclusions. There are no reports of leptospirosis contracted by fish keepers, but fish handlers have been identified as having an occupational exposure risk because of indirect contact with contaminated water [24]. Moreover, fish may be carriers of *Leptospira* spp. [21].

In addition to the unexpectedly high overall seroprevalence, another key finding of the present study was the identification of the predominant serovars. In 2000, the predominant serovar in Austria was Bratislava (73%), followed by Hardjo (20%) [8]; the present study shows that the common serovars in Austria are now Canicola (71%), Hardjo (51%), Copenhageni (22%), and Bratislava (12.5%). Canicola and Copenhageni, but also Bratislava, are common serovars in dogs [2,25].

Domestic animals in general, and particularly dogs, represent important reservoir hosts for *Leptospira* spp. In neighbouring Germany, seroprevalence rates up to 30% have been reported for canine leptospirosis [14]. It has been postulated that the resurgence of canine leptospirosis together with increasing rat populations may be spreading the disease in temperate countries, particularly in urban areas [14]. In the present study, however, no association was found between dog ownership and *Leptospira* seropositivity, and we were not able to identify whether seropositivity was associated with rural or urban residency in Austria. Further epidemiological studies are required to address this important issue.

Of note, the serovars Canicola and Bratislava are also an important cause of porcine reproductive failure [26]. Horses, too, have recently been shown to act as relevant maintenance hosts for serovar Bratislava [27]. Moreover, in Germany, serovar Bratislava has also been identified in wild boars, which were found to be a source of human infection [28]. Seropositivity to serovar Hardjo may suggest exposure to soil or water contaminated by cattle [2]. Rodents, often incriminated as the source of infection in humans, are associated with serovar Copenhageni, although other serovars have also been isolated [29].

It should be noted that the MAT is a serogroup-specific assay and interpretation of results may be complicated by a high degree of cross-reactivity between different *Leptospira* serogroups in acute-phase sera [1]. However, serogroup specificity in convalescent-phase samples is higher. Thus, in a single serum sample, positive titres to different serogroups suggest that individuals have probably been exposed to more than one *Leptospira* species [26].

Although antibodies against serovar Canicola were the most frequently detected, titres above 1:100 (1:200 to ≥1:800) were found only for serovar Bratislava, in four serum samples. According to the current case definition from the US Centers for Disease Control and Prevention, a titre of ≥1:200 (in combination with compatible clinical symptoms) is used to define a probable case [30]. However, none of these four individuals had clinical or laboratory signs of acute infection. Stronger reactivity with the reference serovar or cross-reactivity with other reference serovars used in the MAT could be possible explanations for the higher titres of Bratislava antibodies that were found.

The limitations of the study should be noted. Firstly, the sample population comprised almost exclusively healthy male adults. Thus, the results cannot be generalised to the overall Austrian population. Secondly, because only individuals who have completed the mandatory military service in their adolescence are eligible to volunteer for military assignments abroad, the civilian group had also experienced previous military training at least once in their lifetime. Thirdly, the survey on previous travel abroad covered only the previous six months, thus limiting its value in discerning travel as a risk for acquisition of infection.

Given the large number of potential risk factors and the small number of predictors, we did not use multivariable methods to adjust for potential confounders. Nevertheless, although we could not identify candidate confounder variables from the univariable analyses, residual confounding cannot be excluded. Lastly, although for epidemiological serosurveys the MAT is the most appropriate test for gaining a general impression of *Leptospira* serogroups present in a population [31], it is difficult to compare the results from different studies because the specificity of the MAT is directly linked to the type and quality of the antigens used in the test. It is also crucial that appropriate positive and negative control sera are used to ensure an acceptable level of sensitivity and specificity. There is a critical

need for an international bank of validated reference sera to provide a common standard for *Leptospira* serological assays.

In conclusion, the present study indicates increasing human exposure to *Leptospira* spp. in Austria in the past decade and underlines the fact that surveillance of clinical cases and hospitalisations may greatly underestimate the true rates of infection. Subclinical or mild infections could be common and, because of the non-specific clinical features, cases of leptospirosis may often be misdiagnosed. Physicians should therefore have a high index of suspicion in patients with acute febrile illness. Increased efforts and epidemiological studies are necessary for better understanding of the ecology and mechanisms of disease transmission. The development of control tools, including more effective animal vaccines, is also required for the interruption of transmission cycles.

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Conflict of interest

None declared.

References

1. Levett P.N. Leptospirosis. Clin Microbiol Rev. 2001;14(2):296-326. http://dx.doi.org/10.1128/CMR.14.2.296-326.2001
2. Vijayachari P, Sugunan AP, Shriram AN. Leptospirosis: an emerging global public health problem. J Biosci. 2008;33(4):557-69. http://dx.doi.org/10.1007/s12038-008-0074-z PMid:19208981
3. European Centre for Disease Prevention and Control (ECDC). Leptospirosis: Factsheet for health professionals. Stockholm: ECDC. Available from: http://ecdc.europa.eu/en/healthtopics/leptospirosis/basic_facts/pages/factsheet_health_professionals.aspx
4. Brockmann S, Piechotowski I, Bock-Hensley O, Winter C, Oehme R, Zimmermann S, et al. Outbreak of leptospirosis among triathlon participants in Germany, 2006. BMC Infect Dis. 2010;10:91. http://dx.doi.org/10.1186/1471-2334-10-91 PMid:20380736 PMCid:2858141
5. Radl C, Muller M, Revilla-Fernandez S, Karner-Zuser S, de Martin A, Schauer U, et al. Outbreak of leptospirosis among triathlon participants in Langau, Austria, 2010. Wien Klin Wochenschr. 2011;123(23-24):751-5. http://dx.doi.org/10.1007/s00508-011-0100-2 PMid:22105111
6. Dujardin JC, Campino L, Canavate C, Dedet JP, Gradoni L, Soteriadou K, et al. Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. Emerg Infect Dis. 2008;14(7):1013-8. http://dx.doi.org/10.3201/eid1407.071589 PMid:18598618 PMCid:2600355
7. European Centre for Disease Prevention and Control (ECDC). Annual epidemiological report 2011. Reporting on 2009 surveillance data and 2010 epidemic intelligence data. Stockholm: ECDC; 2011. Available from: http://www.ecdc.europa.eu/en/publications/Publications/1111_SUR_Annual_Epidemiological_Report_on_Communicable_Diseases_in_Europe.pdf
8. Deutz A, Fuchs K, Schuller W, Nowotny N, Auer H, Aspock H, et al. Seroepidemiological studies of zoonotic infections in hunters in southeastern Austria--prevalences, risk factors, and preventive methods. Berl Munch Tierarztl Wochenschr. 2003;116(7-8):306-11. PMid:12894685
9. Hoffmeister B, Peyerl-Hoffmann G, Pischke S, Zollner-Schwetz I, Krause R, Muller MC, et al. Differences in clinical manifestations of imported versus autochthonous leptospirosis in Austria and Germany. Am J Trop Med Hyg. 2010;83(2):326-35. http://dx.doi.org/10.4269/ajtmh.2010.10-0040 PMid:20682876 PMCid:2911179
10. Scharfetter A, Mühlhans M, Payer S, Wenisch C. Three cases of leptospirosis requiring intensive care. Eur J Clin Microbiol Infect Dis. 2004;23(12):905-8. PMid:15599652
11. Faine S (editor). Guidelines for the control of leptospirosis. Geneva: World Health Organization; 1982. Available from: http://whqlibdoc.who.int/offset/WHO_OFFSET_67_%28p1-p98%29.pdf
12. Ciceroni L, Stepan E, Pinto A, Pizzocaro P, Dettori G, Franzin L, et al. Epidemiological trend of human leptospirosis in Italy between 1994 and 1996. Eur J Epidemiol. 2000;16(1):79-86. http://dx.doi.org/10.1023/A:1007658607963 PMid:10780347
13. Katz AR, Buchholz AE, Hinson K, Park SY, Effler PV. Leptospirosis in Hawaii, USA, 1999-2008. Emerg Infect Dis. 2011;17(2):221-6. http://dx.doi.org/10.3201/eid1702.101109 PMid:21291592 PMCid:3204774
14. Jansen A, Schoneberg I, Frank C, Alpers K, Schneider T, Stark K. Leptospirosis in Germany, 1962-2003. Emerg Infect Dis. 2005;11(7):1048-54. http://dx.doi.org/10.3201/eid1107.041172 PMid:16022779 PMCid:3371786
15. Tan DS, Lopes DA. A preliminary study of the status of leptospirosis in the Malaysian Armed forces. Southeast Asian J Trop Med Public Health. 1972;3(2):208-11. PMid:5082848
16. Allen GL, Weber DR, Russell PK. The clinical picture of leptospirosis in American soldiers in Vietnam. Mil Med. 1968;133(4):275-80. PMid:4967463
17. Mackenzie RB, Reiley CG, Alexander AD, Bruckner EA, Diercks FH, Beye HK. An outbreak of leptospirosis among U. S. army troops in the Canal Zone. Am J Trop Med Hyg. 1966;15(1):57-63. PMid:5901631
18. Corwin A, Ryan A, Bloys W, Thomas R, Deniega B, Watts D. A waterborne outbreak of leptospirosis among United States military personnel in Okinawa, Japan. Int J Epidemiol. 1990;19(3):743-8. http://dx.doi.org/10.1093/ije/19.3.743 PMid:2262273
19. Johnston JH, Lloyd J, McDonald J, Waitkins S. Leptospirosis--an occupational disease of soldiers. J R Army Med Corps. 1983;129(2):111-4. PMid:6620235

20. Russell KL, Montiel Gonzalez MA, Watts DM, Lagos-Figueroa RC, Chauca G, Ore M, et al. An outbreak of leptospirosis among Peruvian military recruits. Am J Trop Med Hyg. 2003;69(1):53-7. PMid:12932097
21. Hadad E, Pirogovsky A, Bartal C, Gilad J, Barnea A, Yitzhaki S, et al. An outbreak of leptospirosis among Israeli troops near the Jordan River. Am J Trop Med Hyg. 2006;74(1):127-31. PMid:16407357
22. Lettieri C, Moon J, Hickey P, Gray M, Berg B, Hospenthal D. Prevalence of leptospira antibodies in U.S. Army blood bank donors in Hawaii. Mil Med. 2004;169(9):687-90. PMid:15495719
23. Olson JG, Irving GS, Bourgeois AL, Hodge FA, Van Peenen PF. Seroepidemiological evidence of infectious diseases in United States Marine Corps personnel, Okinawa, Japan, 1975--1976. Mil Med. 1979;144(3):175-6. PMid:107484
24. Waitkins SA. Leptospirosis as an occupational disease. Br J Ind Med. 1986;43(11):721-5. PMid:3790454 PMCid:1007745
25. Scanziani E, Origgi F, Giusti AM, Iacchia G, Vasino A, Pirovano G, et al. Serological survey of leptospiral infection in kennelled dogs in Italy. J Small Anim Pract. 2002;43(4):154-7. http://dx.doi.org/10.1111/j.1748-5827.2002.tb00048.x PMid:11996391
26. Jori F, Galvez H, Mendoza P, Cespedes M, Mayor P. Monitoring of leptospirosis seroprevalence in a colony of captive collared peccaries (Tayassu tajacu) from the Peruvian Amazon. Res Vet Sci. 2009;86(3):383-7. http://dx.doi.org/10.1016/j.rvsc.2008.09.009 PMid:19000627
27. Baverud V, Gunnarsson A, Engvall EO, Franzen P, Egenvall A. Leptospira seroprevalence and associations between seropositivity, clinical disease and host factors in horses. Acta Vet Scand. 2009;51:15. http://dx.doi.org/10.1186/1751-0147-51-15 PMid:19331656 PMCid:2679755
28. Jansen A, Luge E, Guerra B, Wittschen P, Gruber AD, Loddenkemper C, et al. Leptospirosis in urban wild boars, Berlin, Germany. Emerg Infect Dis. 2007;13(5):739-42. http://dx.doi.org/10.3201/eid1305.061302 PMid:17553254 PMCid:2738438
29. Matthias MA, Levett PN. Leptospiral carriage by mice and mongooses on the island of Barbados. West Indian Med J. 2002;51(1):10-3. PMid:12089866
30. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. MMWR Recomm Rep. 1997;46(RR-10):1-55.
31. Levett PN. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. Clin Infect Dis. 2003;36(4):447-52. http://dx.doi.org/10.1086/346208 PMid:12567302

Laboratory preparedness for detection and monitoring of Shiga toxin 2-producing *Escherichia coli* O104:H4 in Europe and response to the 2011 outbreak

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A hybrid strain of enteroaggregative and Shiga toxin 2-producing *Escherichia coli* (EAEC-STEC) serotype O104:H4 strain caused a large outbreak of haemolytic uraemic syndrome and bloody diarrhoea in 2011 in Europe. Two surveys were performed in the European Union (EU) and European Economic Area (EEA) countries to assess their laboratory capabilities to detect and characterise this previously uncommon STEC strain. Prior to the outbreak, 11 of the 32 countries in this survey had capacity at national reference laboratory (NRL) level for epidemic case confirmation according to the EU definition. During the outbreak, at primary diagnostic level, nine countries reported that clinical microbiology laboratories routinely used Shiga toxin detection assays suitable for diagnosis of infections with EAEC-STEC O104:H4, while 14 countries had NRL capacity to confirm epidemic cases. Six months after the outbreak, 22 countries reported NRL capacity to confirm such cases following initiatives taken by NRLs and the European Centre for Disease Prevention and Control (ECDC) Food- and Waterborne Disease and Zoonoses laboratory network. These data highlight the challenge of detection and confirmation of epidemic infections caused by atypical STEC strains and the benefits of coordinated EU laboratory networks to strengthen capabilities in response to a major outbreak.

Introduction

Between May and August 2011, an outbreak of Shiga toxin 2-producing *Escherichia coli* (STEC) affected over 4,000 individuals in Europe. It was associated with the highest number of cases of haemolytic uraemic syndrome (HUS) reported to date (782 confirmed and 119 suspected cases) in the European Union (EU)/European Economic Area (EEA) [1]. The first cases were reported from Germany, where the laboratory characterisation of the causative bacterial strain was conducted [2,3]. The outbreak strain was identified as STEC with unusual characteristics. These included the rare serotype O104:H4, lack of attaching/effacing pathogenicity island of virulent STEC strains, as indicated by the lack of the *eae* gene, but harbouring virulence markers of enteroaggregative *E. coli*, e.g. presence of *aggR* gene, and exhibiting a multidrug resistance phenotype, including production of CTX-M-15 extended spectrum beta-lactamase (ESBL) [4-6]. At the beginning of June 2011, the European Centre for Disease Prevention and Control (ECDC) published an EU epidemic case definition for this outbreak strain [7] to allow standardised reporting by the EU/EEA countries and comparison of data at EU level for outbreak monitoring.

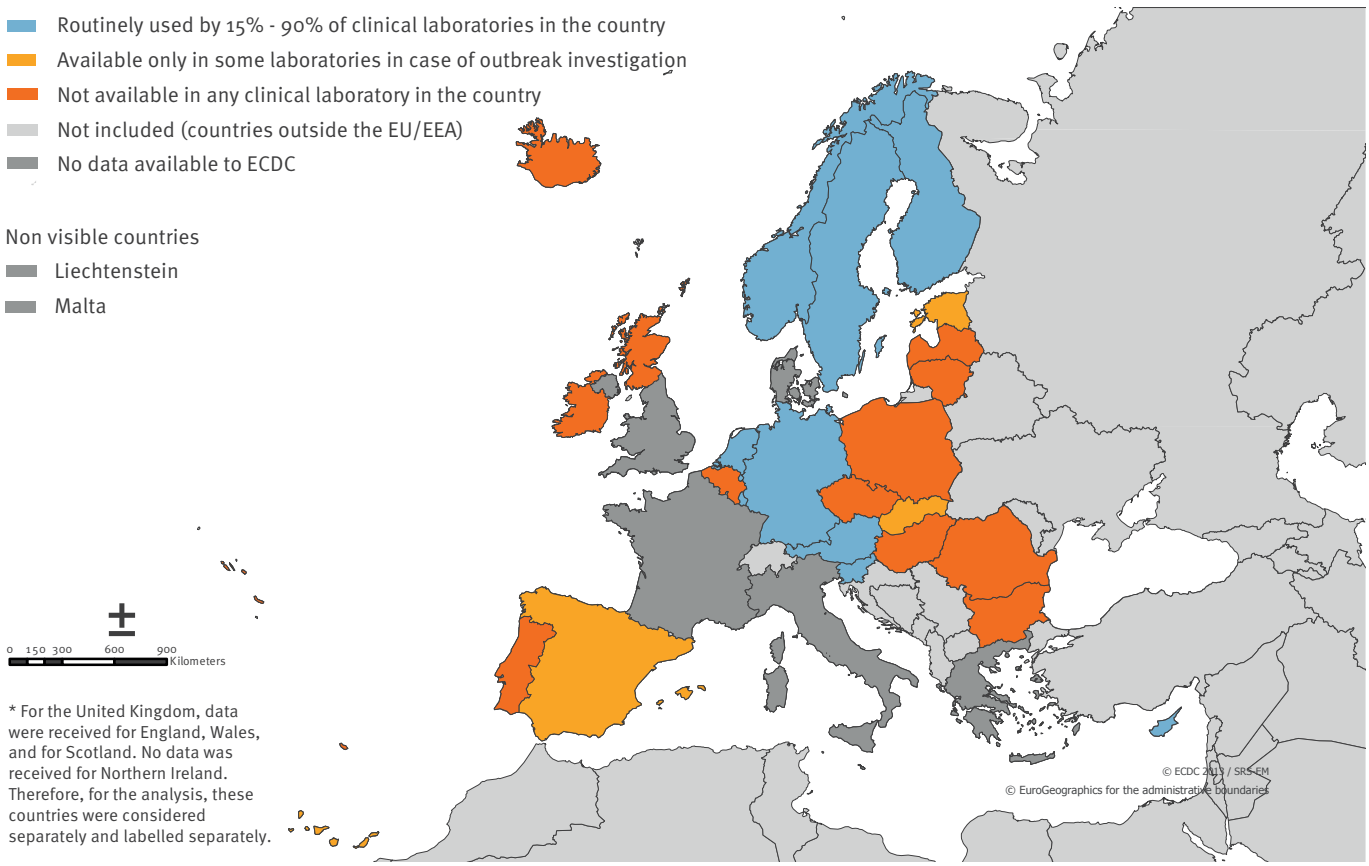
Epidemiological investigations conducted in Germany, France, Denmark and other countries indicated contaminated fenugreek sprouts as likely vehicle of the infections [8,9]. A trace-back global exercise, led by the European Food Safety Authority (EFSA), showed that an implicated lot of fenugreek seeds had been imported to 24 EU Member States [10].

In addition to the risk of spread of STEC O104:H4 , illustrated by STEC O104:H4 cases in Bordeaux [11], in the early phases of this outbreak, several points raised public health concern: (i) the complexity of the detection and identification of STEC that made it difficult to diagnose cases and hampered the assessment of the effect of disease control measures, (ii) the limited sensitivity of routine diagnostic methods for detecting this serotype and pathotype that suggested potential surveillance gaps [12-15], and (iii) the unusually high rate of renal and neurological complications and death among adult cases [16-18].

The mission of ECDC is to identify, assess and communicate current and emerging threats to human health

Figure 1

Availability of Shiga toxin detection tests at primary diagnostic level in European Union and European Economic Area, June 2011



ECDC: European Centre for Disease Prevention and Control; EU/EEA: European Union/European Economic Area.

posed by infectious diseases in the EU/EEA. To adress the concerns raised in terms of laboratory diagnostics, ECDC investigated how well the EU/EEA countries were able to diagnose and confirm STEC O104:H4 cases according to the EU epidemic case definition and whether rapid laboratory capacity building initiatives were needed. In this article, we present the results of two laboratory capacity surveys in the EU/EEA before, during, and after the 2011 STEC O104:H4 outbreak respectively, and describe capacity building activities taken at national and European levels in response to the outbreak.

Methods

A short questionnaire to survey laboratory practices for enabling application of the epidemic case definition and identification of the epidemic STEC O104:H4 strain was sent by email to the STEC/VTEC contact points of the European Food- and Waterborne Diseases and Zoonoses Network (FWD-Net) on 2 June 2011 (first survey). The survey focused on availability of the following strain characterisation tests at national reference laboratory (NRL) level: O serogrouping, H serotyping, Shiga toxin 1 (*stx1*) and 2 gene (*stx2*) detection and subtyping, *eae*, *aggR* and *EAggEC* virulence gene detection, multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) analysis with *Xba*I macrorestriction. The survey also inquired about information available at national level on diagnostic capabilities in clinical microbiology practice (i.e. at primary level) for Shiga toxin and gene detection and asked to indicate the source of this informarion on primary level diagnostic capabilities. Data received by 2 July 2011 were included for the purpose of this analysis.

A follow-up survey was sent in January 2012 (second survey), assessing the NRL capabilities for STEC detection and characterisation available in April 2011 (before the outbreak) and December 2011 (after the outbreak). The survey also addressed actions taken at national level from June to December 2011 to stengthen STEC O104:H4 diagnostic capabilities and/or reporting of cases. Furthermore, the survey participants were asked to answer whether they need services for STEC strain characterisation and to indicate the type of support expected from ECDC in STEC outbreaks and

in general for investigation of outbreaks of emerging, highly virulent pathogenic organisms of international concern. All responses received by the end of March 2012 were included in the analysis presented here.

For the United Kingdom, data were received for England and Wales, and for Scotland. No data were received for Northern Ireland. Therefore, England and Wales, and Scotland were considered as separate countries, hence the survey comprised 32 instead of 30 EU/EEA countries. For the analysis of the data, the denominator used was 32 to allow for comparison of data between the two surveys.

To compare the proportion of countries reporting confirmed epidemic cases to the laboratory capability at clinical diagnostic level, Fisher's exact test was used with two-tailed probability.

Results

In the first survey in mid 2011, 24 of 32 countries responded to the questionnaire and in the second survey in early 2012, responses came from 29 countries, leading to response rates of 75% and 91% respectively.

First survey (mid 2011)

Availability of Shiga toxin detection tests at primary diagnostic level

Data showed that in seven countries, information available was based on national external quality assessment results and/or national surveys of testing practices of clinical laboratories, conducted in 2010 and/or 2011.

Table

Routine testing for Shiga toxin genes at clinical microbiology level in European Union and European Economic Area countries reporting or not reporting confirmed epidemiological cases of Shiga toxin 2-producing *Escherichia coli* O104:H4, July 2011

Shiga toxin diagnostic testing at clinical laboratories	Number of countries (n=32)[a]		Total
	Reporting	Not reporting	
Available in more than 15%	6	3	9
Available only in some, in case of outbreaks	1	2	3
Not available	2	10	12

[a] For the United Kingdom, data were received for England and Wales, and for Scotland. No data were received for Northern Ireland. Therefore, England and Wales, and Scotland were considered as separate countries, hence the survey comprised 32 instead of 30 European Union and European Economic Area countries.

For 17 countries, the information was based on personal communication to NRL.

Based on the information available to the NRLs, *stx1* and *stx2* and/or Stx 1/2 assays were routinely used in clinical laboratories in nine of the 32 countries. For these nine countries, the percentage of clinical laboratories using these assays routinely varied between 15 to 90% laboratories per country with a median of 65%. According to NRLs, in three countries, such tests were used by clinical laboratories occasionally, e.g. in the case of outbreaks. Shiga toxin 1 and 2 toxin and/or gene tests were not used in clinical laboratories in 12 countries. Figure 1 shows the availability of these tests at clinical laboratory level by country in the European EU/EEA.

Of note, six of nine countries with clinical microbiology Stx/*stx* detection capacity had reported STEC O104:H4 epidemic cases as compared with two of 12 countries with no capacity ($p<0.05$, Table).

STEC O104:H4 case confirmation capabilities at national reference laboratory level at the time of the 2011 outbreak

The responses demonstrated that in 18 of 32 countries, O104 serogrouping test was available, while H4 serotyping test was available in 14 countries. Tests for detection of *stx1/stx2*, *eae* and *aggR* were available in 20, 19, and 14 countries, respectively.

Analyses with PFGE using *Xba*I macrorestriction according to the PulseNet protocol were available in 18 NRLs and MLST was performed only in five laboratories .

The data on the availability of the above tests showed that 14 NRLs were capable of confirming an outbreak-related case according to the EU epidemic case definition. In four additional countries, the NRLs were able to confirm cases only if epidemiological criteria were met. In six countries the NRLs reported lack of specific tests for confirmation of epidemic STEC O104:H4 cases.

Second survey (early 2012)

Capabilities of NRLs for case detection and identification of STEC O104:H4 before and after the 2011 outbreak

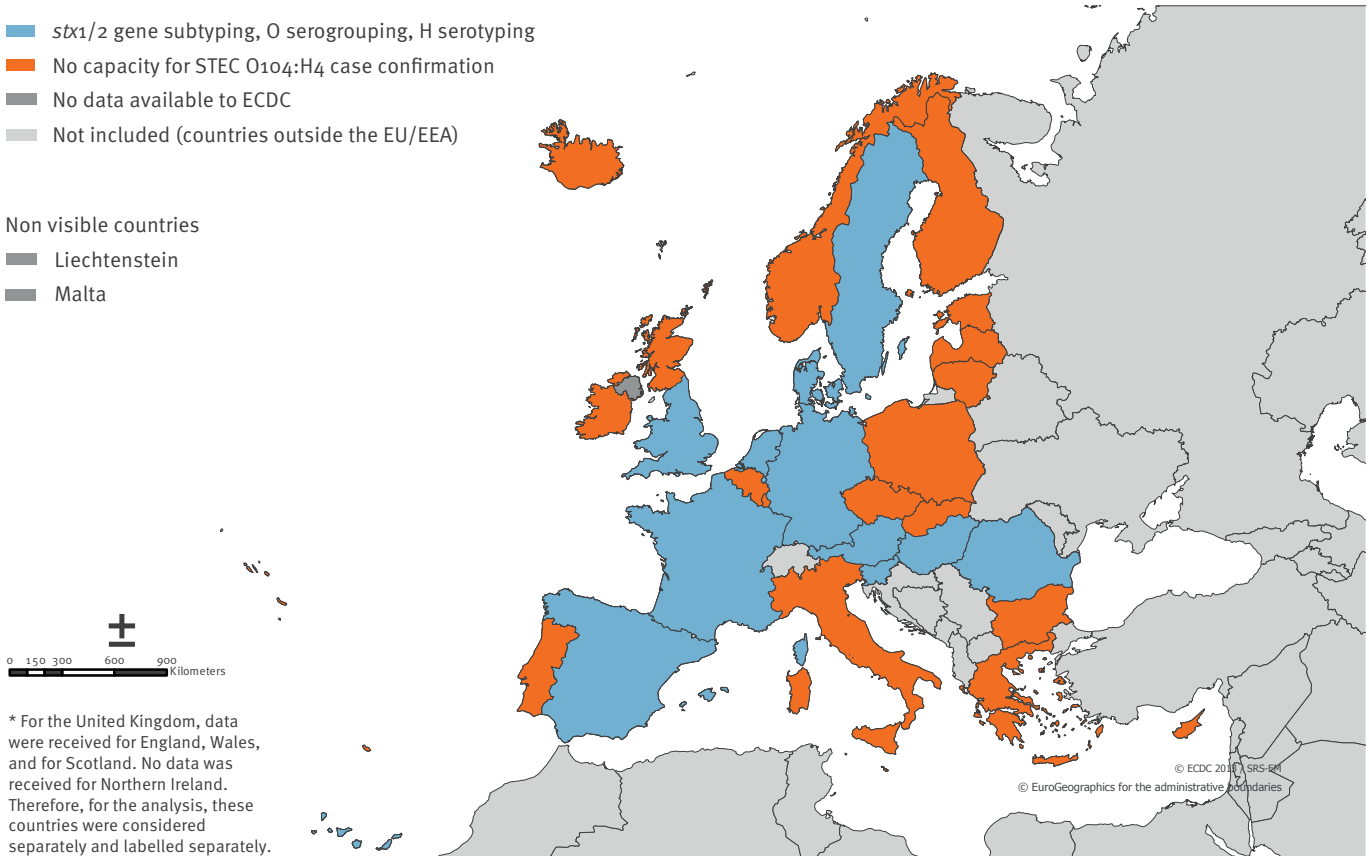
Data obtained revealed that prior to the STEC O104:H4 outbreak in April 2011, 11 of 32 countries had NRL capacity to confirm STEC O104:H4 cases. In December 2011, five months after the outbreak, 22 countries reported such NRL capacity ($p<0.05$, Figure 2).

There was an increase in the number of STEC detection and identification methods at NRL level in December 2011 compared with April 2011 (Figure 3). The change was most pronounced for methods required for STEC O104:H4 case confirmation. From April to December 2011, 15 countries had developed O104 serogrouping capability, eight H4 serotyping capability, six *aggR* detection test and four *stx1/2* and *eae* detection tests.

Figure 2

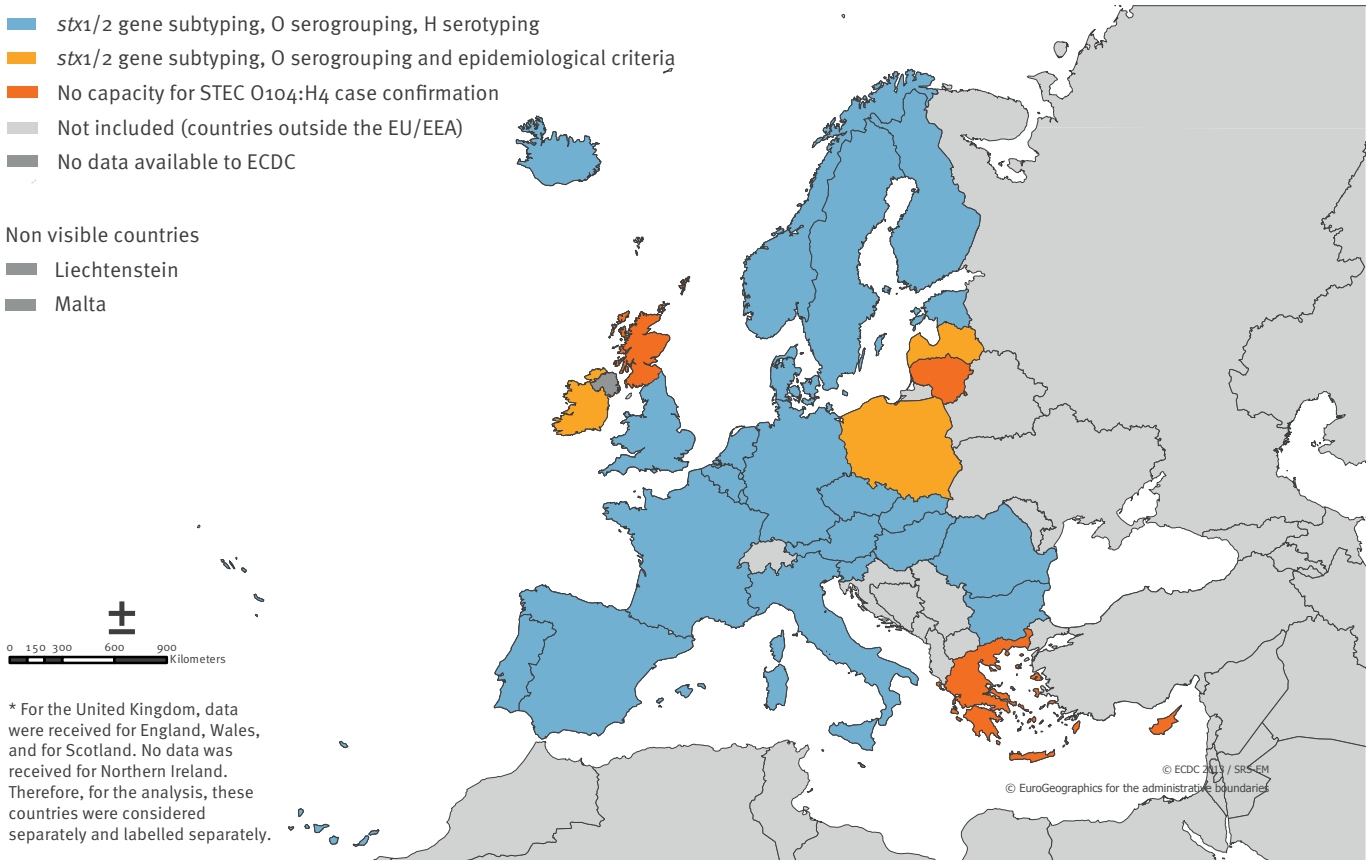
Capabilities of national reference laboratories in the European Union and European Economic Area for case detection and identification of Shiga toxin 2-producing *Escherichia coli* O104:H4 before and after the 2011 outbreak, March 2012

A: April 2011



* For the United Kingdom, data were received for England, Wales, and for Scotland. No data was received for Northern Ireland. Therefore, for the analysis, these countries were considered separately and labelled separately.

B: December 2011

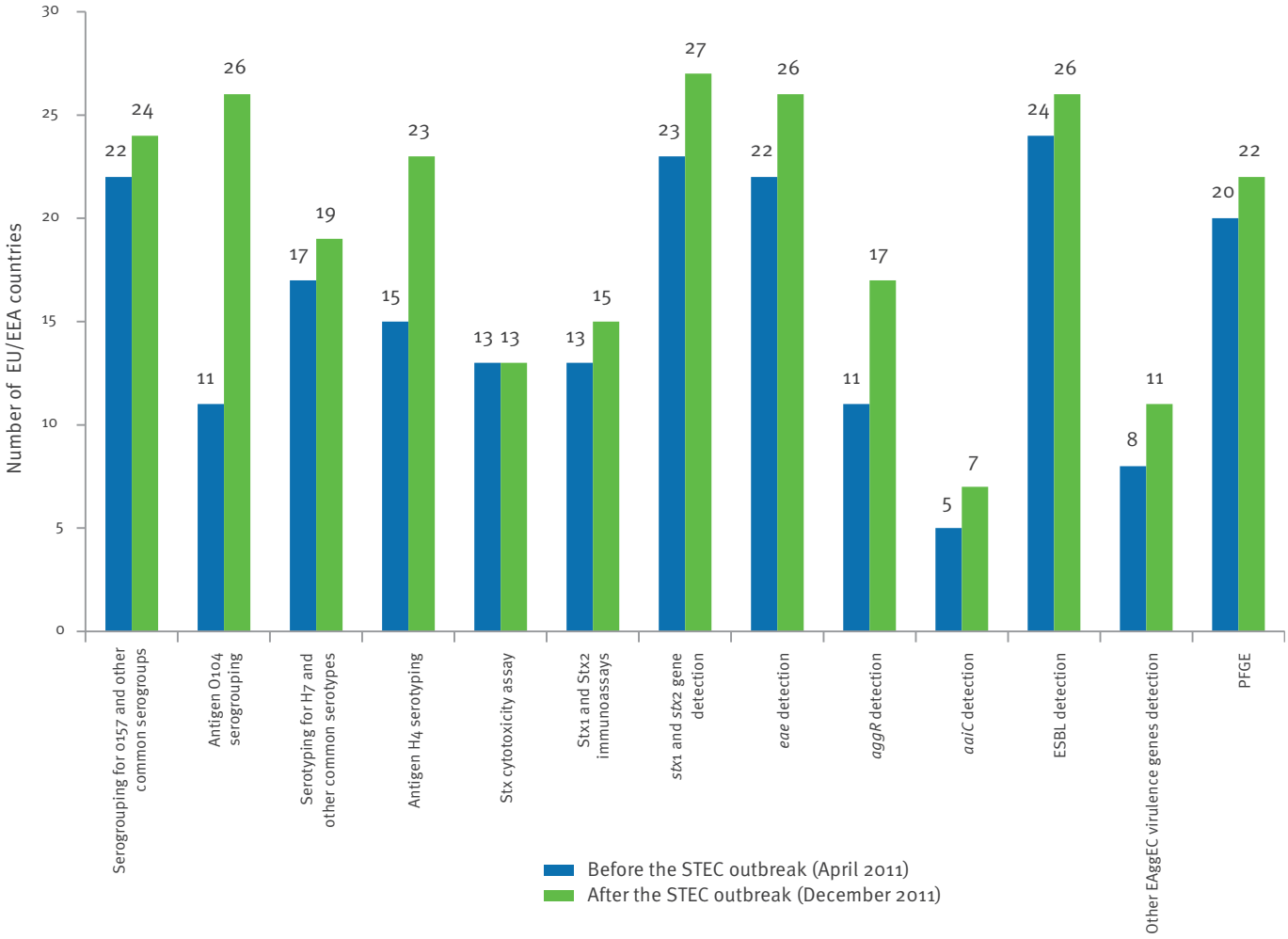


* For the United Kingdom, data were received for England, Wales, and for Scotland. No data was received for Northern Ireland. Therefore, for the analysis, these countries were considered separately and labelled separately.

ECDC: European Centre for Disease Prevention and Control; EU/EEA: European Union/European Economic Area; STEC: Shiga toxin 2-producing *Escherichia coli*.

Figure 3

Number of Shiga toxin 2-producing *Escherichia coli* detection and identification methods at national reference laboratories in the European Union and European Economic Area before and after the 2011 outbreak in Europe



ESBL: extended spectrum beta-lactamase; EU/EEA: European Union/European Economic Area; PFGE: pulsed-field gel electrophoresis; STEC: Shiga toxin 2-producing *Escherichia coli*.

In April 2011, 24 countries reported capabilities for assessing ESBL production: 12 countries reported testing for both gene detection and phenotypic characterisation, and another 12 used phenotypic characterisation alone. In December 2011, 26 countries tested for ESBL at NRL level: 14 used phenotypic detection alone, 11 countries used both phenotypic characterisation and genotypic detection and one tested for ESBL production based on gene detection alone.

Twenty-six countries indicated that specific actions had been taken at national level during and/or following the outbreak to increase STEC O104:H4 detection capabilities. Such actions were directed at primary microbiology diagnostic services in the form of (i) guidance on diagnostic methods for 23 countries, (ii) pathogen isolation and referral of isolates in 21, and (iii) case reporting in 20 countries. Recommendations regarding *E. coli* strain characterisation methods as well as participation in ad hoc external quality assessment (EQA) schemes for diagnostic services were given to clinical laboratories in 10 EU/EEA countries.

Needs for inter-laboratory services for Shiga toxin-producing *E. coli* strain characterisation

Despite these efforts to increase capability for STEC detection and characterisation, nine countries reported needs for access to strain characterisation services from a reference laboratory in another country, including for serotyping of rare STEC serogroups and MLST. Two countries reported NRL service arrangements with reference laboratories in other countries for testing rare STEC serotypes.

Initiatives expected from ECDC to support national response to outbreaks of emerging, highly virulent pathogenic organisms of international concern

The needs most frequently mentioned included (i) early warning and information exchange (17 countries), (ii) protocols for pathogen isolation and detection (13 countries), and (iii) provision of diagnostic materials and reagents (12 countries). One country did not expect any support.

Discussion

Human infections caused by STEC O157:H7 account for nearly half of reported cases of STEC disease in the EU/EEA countries [19]. Non-O157 serotypes are much less commonly reported as causes of human disease, mainly due to weight of diagnostics towards identification of O157 serogroup. The large STEC outbreak in 2011 in the EU/EEA was caused by the rare serotype and atypical enteroaggregative pathotype STEC O104:H4. Considering the impact of this outbreak and its spread across borders, it was essential to assess the capacity of EU/EEA countries to confirm epidemic cases and to target any necessary capacity building activities. The results from such assessement presented here show that during this STEC O104:H4 outbreak, several countries lacked the capacity at national level to detect and characterise STEC O104:H4 cases according to the EU epidemic case definition [7]. Importantly, even greater case detection gaps existed at primary diagnostic level, based on the information reported by NRLs. Diagnostic capability at clinical laboratory level by use of Shiga toxin detection for non-O157 STEC was associated with more frequent reporting of epidemic cases of this rare STEC serotype. Diagnostic and characterisation capacity at NRL level may compensate in part for lack of routine Shiga toxin testing at primary level. However, at the onset of the outbreak, two-thirds of the EU/EEA countries reported no NRL capacity for confirmation of STEC O104:H4 cases, which along with the lack of routine Shiga toxin screening in clinical laboratories, raises the possibility that previous outbreaks of STEC strains of rare serotypes/pathotypes, might have not been detected. This indicates an inadequate level of preparedness of Europe's public health microbiology system to detect early unusual events, such as the emergence of new pathotypes of STEC. It underlines the need to strengthen the microbiology laboratory capacity for timely communicable disease alert and response.

Our study has some limitations: information on clinical laboratories testing practices was based to a large extend on personal communication to NRLs (i); availability of Shiga toxin gene detection tests at NRL level and not that of Stx1 and Stx2 immunoassays was assessed in the first survey (ii); the results of the first survey should be interpreted with caution since it was conducted during the outbreak when testing practice was subject to changes and because it received a lower response rate than the second survey (iii). Nevertheless, our findings of low capacity for detection of non-O157 STEC infections are in line with observations made by others. A survey of laboratory practices for identification of STEC as part of the United States (US) Foodborne Diseases Active Surveillance Network showed that only 11% of laboratories used a method that would detect non-O157 STEC [20]. It is noteworthy that in this survey, conducted in 2007 only half of the laboratories routinely tested all specimens for non-O157 STEC. Another study conducted within the same network, showed that besides the available laboratory capacities, the correct identification of STEC infections also depended on physicians knowledge of STEC and ability to correctly interpret a positive Shiga toxin test result [21]. Thus, laboratory diagnostic and reference testing capacity would not be sufficient for timely and reliable surveillance unless clinical samples and isolates are collected for testing.

Amplification of DNA can be useful for rapid STEC detection in stool specimens [22,23] and has been shown to be cost-effective for diagnosing infections with STEC O157 and other intestinal pathogens [24]. However, when used without culture confirmation, such tests could generate high rates of false positive results, leading to over-reporting and unnecessary treatment and public health measures [25]. In addition to that, referral of STEC isolates to NRLs is needed to perform epidemiological typing in support to cluster detection and source tracing. Due to the ongoing consolidation of clinical laboratory services and increased use of culture-independent diagnostic techniques, the importance of culture confirmation could be overlooked and thus impede microbiological outbreak investigations. Although we could not find any published data illustrating how epidemiological investigations were hampered due to lack of timely culture confirmation in Europe, such information is available from the US [20,25]. In this context, the post-outbreak increase in NRL capabilities to characterise STEC O104:H4 isolates does not guarantee preparedness of EU/EEA public health microbiology system to detect this rare serotype and pathotype. It critically depends on primary case ascertainment by clinical diagnostic testing practice for non-O157 STEC and referral of isolates for reference testing.

In Europe, external quality assessment of NRL capabilities to characterise non-O157 STEC in the FWD-Net laboratories, showed that typing proficiency varied depending on serotype with better performance for STEC O157:H7 than other, less commonly reported serotypes [26]. Another survey of services of NRLs in EU/EEA countries for detection and characterisation of STEC, Campylobacter, *Listeria monocytogenes*, Salmonella, Shigella and Yersinia showed gaps in the capacity and reproducibility of methods used for their early detection and characterisation [27]. Capacity strengthening actions and method harmonisation are undertaken at EU/EEA level, via the ECDC Food- and Waterborne Diseases and Zoonoses programme and

FWD-Net, to ensure that Europe has a reliable surveillance system for enteric pathogens.

This study shows increased EU/EEA NRL capabilities for STEC detection and characterisations following the 2011 STEC O104:H4 outbreak. This is due to efforts taken at national and EU/EEA levels. At national level, measures were implemented to increase referral of specimens from suspected cases of STEC O104:H4 to the NRL and increase the NRL's capabilities to detect epidemic cases. At EU level, rapid development of novel PCR-based diagnostic methods and sharing of these protocols through the laboratories in the FWD-Net contributed to strengthening the capacity to detect and confirm STEC O104:H4 cases in NRLs [6,28,29]. In early June, ECDC published the EU epidemic case definition and issued technical guidance on microbiological testing methods on its website and provided links to expert sources of information in the EU/EEA countries [30]. In addition, ECDC supplied the NRLs in the EU/EEA countries with specific diagnostic reagents and reference materials as requested. Eighteen NRLs received O104 anti-serum and 19 received K9 anti-serum and enteroaggregative STEC control strains, with different O:H combinations and *stx* subtypes, including a STEC O104:H4 strain.

These rapid and coordinated capacity building efforts for STEC detection and characterisation illustrate the importance of the EU network and participating NRLs to work in close collaboration with the EU food safety experts and respective national laboratories. The rapid exchange of information at EU level during this outbreak was key in the coordination of such efforts. The existing Epidemic Intelligence Information System for FWD (EPIS FWD) facilitated the cooperation between FWD-Net laboratories. The EU Reference Laboratory for STEC/VTEC, operating in the area of food and feed safety, rapidly developed a validated laboratory protocol to detect this particular pathotype in food and environmental samples and shared this protocol through EPIS FWD [29]. ECDC also produced rapid risk assessments and EU epidemiological updates during the outbreak. A toolkit for investigation of and response to food- and waterborne disease outbreaks with an EU dimension was published in February 2012 and is available on the ECDC website. For enhanced surveillance of STEC/VTEC, the European Surveillance System (TESSy) metadataset was revised by including new variables on specific genes, i.e. *aggR* and *aaiC*, for reporting of STEC.

In conclusion, Europe's public health microbiology laboratory capacity such as that reported for monitoring emerging STEC outbreaks has been improved via dedicated resources at national level and via cross-sector and cross-border collaborations conducted by public health institutions to support timely and reliable surveillance for disease control.

The STEC/VTEC experts of the European Food- and Waterborne Diseases and Zoonoses Network

The members of the network, who provided the survey data are:
Austria - Sabine Schlager at the National Reference Center (NRC) for Escherichia coli including Verotoxin producing E. coli;
Belgium - Denis Pierard at the NRC VTEC/STEC, Universitair Ziekenhuis Brussel;
Bulgaria - Petar Petrov of the NRL for Enteric Pathogens, Sofia
Cyprus - Panayiota Maikanti-Charalampous at the Reference laboratory for Salmonella and other enteric pathogens;
Czech Republic - Monika Marejkova at the NRL for E. coli and Shigella;
Germany - Angelika Fruth and Rita Prager at the NRC for Salmonella and other enteric bacteria at the Robert Koch Institute;
Denmark - Flemming Scheutz of the WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella;
Estonia - Rita Peetso at the Central Laboratory of Communicable diseases, Health Board,
Finland - Anja Siitonen and Ulla-Maija Nakari at the Bacteriology Unit, National Institute for Health and Welfare;
France - Malika Gouali at the Centre National de Référence des Salmonella, E. coli et Shigella, Institut Pasteur
Greece – Kassiani Mellou from the Greek Centre for Disease Control in collaboration with Reference Laboratories as the Head of Foodborne Diseases Section,
Hungary - Mária Herpay at the National reference laboratory for enteric pathogens;
Iceland - Hjordis Hardardottir at the Department Of Clinical Microbiology, Landspitali University Hospital;
Ireland - Anne Carroll and Eleanor McNamara of the Public Health Laboratory;
Italy - Alfredo Caprioli at Istituto Superiore di Sanità,
Latvia - Solvita Selderina at the East Clinical University Hospital „Infectology Center of Latvia"
Lithuania - Ruta Jankauskiene of the Lithuanian National Public Health Surveillance Laboratory;
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England and Wales - Claire Jenkins at the Gastrointestinal Infections Reference Unit;
Scotland - Mary Hanson at the Scottish E. coli O157/ VTEC Reference Laboratory.

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References

1. European Centre for Disease Prevention and Control [ECDC]. Epidemiological Updates. Shiga toxin-producing E. coli (STEC): Update on outbreak in the EU (27 July 2011, 11:00). Stockholm: ECDC. Available from: http://ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f%2D77d4%2D4ad8%2Db6d6%2Dbf0f23083f30&ID=1166&RootFolder=%2Fen%2Factivities%2Fsciadvice%2FLists%2FECDC%20Reviews
2. Frank C, Faber MS, Askar M, Bernard H, Fruth A, Gilsdorf A, et al. Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011. Euro Surveill. 2011;16(21):pii=19878. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19878
3. Frank C, Werber D, Cramer JP, Askar M, Faber M, an der Heiden M, et al. Epidemic profile of Shiga-toxin-producing Escherichia coli O104:H4 outbreak in Germany. N Engl J Med. 2011;365(19):1771-80. http://dx.doi.org/10.1056/NEJMoa1106483. PMid:21696328.
4. Robert Koch Institute (RKI). Final presentation and evaluation of epidemiological findings in the EHEC O104:H4 Outbreak, Germany 2011. Berlin: RKI. Sept 2011. Available from: http://www.rki.de/EN/Home/EHEC_final_report.pdf;jsessionid=504CAC52EE1C9F1B9CAD341A52E23699.2_cid390?__blob=publicationFile
5. Bielaszewska M, Mellmann A, Zhang W, Kock R, Fruth A, Bauwens A, et al. Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infect Dis. 2011;11(9):671-6. PMid:21703928.
6. Scheutz F, Møller Nielsen E, Frimodt-Møller J, Boisen N, Morabito S, Tozzoli R, et al. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing Escherichia coli O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill. 2011;16(24):pii=19889. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19889. PMid:21699770.
7. European Centre for Disease Prevention and Control (ECDC). EU case definition for diarrhoea and haemolytic uremic syndrome (HUS) caused by the epidemic strain Shiga toxin 2-producing Escherichia Coli (STEC) O104:H4. Stockholm: ECDC. 2011. Available from: http://ecdc.europa.eu/en/healthtopics/escherichia_coli/epidemiological_data/Pages/EU_case_definition.aspx
8. Buchholz U, Bernard H, Werber D, Bohmer MM, Remschmidt C, Wilking H, et al. German outbreak of Escherichia coli O104:H4 associated with sprouts. N Engl J Med. 2011;365(19):1763-70. http://dx.doi.org/10.1056/NEJMoa1106482. PMid:22029753.
9. King LA, Nogareda F, Weill FX, Mariani-Kurkdjian P, Loukiadis E, Gault G, et al. Outbreak of Shiga toxin-producing Escherichia coli O104:H4 associated with organic fenugreek sprouts, France, June 2011. Clin Infect Dis. 2012;54(11):1588-94. http://dx.doi.org/10.1093/cid/cis255. PMid:22460976.
10. European Food Safety Authority (EFSA). Technical Report. Tracing seeds, in particular fenugreek (Trigonella foenum-graecum) seeds, in relation to the Shiga toxin-producting E. coli (STEC) O104:H4 2011 Outbreaks in Germany and France. Parma: EFSA; 2011. Available from: http://www.efsa.europa.eu/en/supporting/pub/176e.htm
11. Gault G, Weill FX, Mariani-Kurkdjian P, Jourdan-da Silva N, King L, Aldabe B, et al. Outbreak of haemolytic uraemic syndrome and bloody diarrhoea due to Escherichia coli O104:H4, south-west France, June 2011. Euro Surveill. 2011;16(26):pii=19905. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19905
12. Blanco JE, Blanco M, Alonso MP, Mora A, Dahbi G, Coira MA, et al. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing Escherichia coli isolates from human patients: prevalence in Lugo, Spain, from 1992 through 1999. J Clin Microbiol. 2004;42(1):311-9. http://dx.doi.org/10.1128/JCM.42.1.311-319.2004. PMid:14715771. PMCid:321739.
13. Buvens G, De Gheldre Y, Dediste A, de Moreau AI, Mascart G, Simon A, et al. Incidence and virulence determinants of verocytotoxin-producing Escherichia coli infections in the Brussels-Capital Region, Belgium, in 2008-2010. J Clin Microbiol. 2012;50(4):1336-45. http://dx.doi.org/10.1128/JCM.05317-11. PMid:22238434. PMCid:3318570.
14. Caprioli A, Tozzi AE, Rizzoni G, Karch H. Non-O157 Shiga toxin-producing Escherichia coli infections in Europe. Emerg Infect Dis. 1997;3(4):578-9. http://dx.doi.org/10.3201/eid0304.970425. PMid:9366613. PMCid:2640089.
15. van Duynhoven YT, Friesema IH, Schuurman T, Roovers A, van Zwet AA, Sabbe LJ, et al. Prevalence, characterisation and clinical profiles of Shiga toxin-producing Escherichia coli in The Netherlands. Clin Microbiol Infect. 2008;14(5):437-45. http://dx.doi.org/10.1111/j.1469-0691.2008.01963.x. PMid:18325039.
16. Askar M, Faber MS, Frank C, Bernard H, Gilsdorf A, Fruth A, et al. Update on the ongoing outbreak of haemolytic uraemic syndrome due to Shiga toxin-producing Escherichia coli (STEC) serotype O104, Germany, May 2011. Euro Surveill. 2011;16(22):pii=19883. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19883
17. Cordesmeyer S, Peitz U, Gödde N, Kasper HU, Hoffmann MW, Allemeyer E. Colonic ischaemia as a severe Shiga toxin/verotoxin producing Escherichia coli O104:H4 complication in a patient without haemolytic uraemic syndrome, Germany, June 2011. Euro Surveill. 2011;16(25):pii=19895. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19895. PMid:21722614.

18. Magnus T, Rother J, Simova O, Meier-Cillien M, Repenthin J, Moller F, et al. The neurological syndrome in adults during the 2011 northern German E. coli serotype O104:H4 outbreak. Brain. 2012;135(Pt 6):1850-9. http://dx.doi.org/10.1093/brain/aws090. PMid:22539260.
19. European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA). Shiga toxin/verotoxin-producing Escherichia coli in humans, food and animals in the EU/EEA, with special reference to the German outbreak strain STEC O104. Stockholm: ECDC; 2011. Available from: http://ecdc.europa.eu/en/publications/Publications/1106_TER_EColi_joint_EFSA.pdf
20. Hoefer D, Hurd S, Medus C, Cronquist A, Hanna S, Hatch J, et al. Laboratory practices for the identification of Shiga toxin-producing Escherichia coli in the United States, FoodNet sites, 2007. Foodborne Pathog Dis. 2011;8(4):555-60. http://dx.doi.org/10.1089/fpd.2010.0764. PMid:21186994.
21. Clogher P, Hurd S, Hoefer D, Hadler JL, Pasutti L, Cosgrove S, et al. Assessment of physician knowledge and practices concerning Shiga toxin-producing Escherichia coli infection and enteric illness, 2009, Foodborne Diseases Active Surveillance Network (FoodNet). Clin Infect Dis. 2012;54 Suppl 5:S446-52. http://dx.doi.org/10.1093/cid/cis246. PMid:22572668.
22. El Sayed Zaki M, El-Adrosy H. Diagnosis of Shiga toxin producing Escherichia coli infection, contribution of genetic amplification technique. Microbes Infect. 2007;9(2):200-3. http://dx.doi.org/10.1016/j.micinf.2006.11.005. PMid:17223372.
23. Gerritzen A, Wittke JW, Wolff D. Rapid and sensitive detection of Shiga toxin-producing Escherichia coli directly from stool samples by real-time PCR in comparison to culture, enzyme immunoassay and Vero cell cytotoxicity assay. Clin Lab. 2011;57(11-12):993-8. PMid:22239032.
24. Abubakar I, Irvine L, Aldus CF, Wyatt GM, Fordham R, Schelenz S, et al. A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. Health Technol Assess. 2007;11(36):1-216. PMid:17803865.
25. Importance of culture confirmation of shiga toxin-producing Escherichia coli infection as illustrated by outbreaks of gastroenteritis--New York and North Carolina, 2005. MMWR Morb Mortal Wkly Rep. 2006;55(38):1042-5. PMid:17008867.
26. European Centre for Disease Prevention and Control (ECDC). External quality assurance scheme for typing of verocytotoxin-producing E. coli (VTEC). Stockholm: ECDC; 2012. Available from: http://ecdc.europa.eu/en/publications/Publications/1204-TER-EQA-VTEC.pdf
27. European Centre for Disease Prevention and Control (ECDC) . Survey of National Reference Laboratory capacity for six FWD in EU/EEA countries. Stockholm: ECDC; 2012. Available from: http://ecdc.europa.eu/en/publications/Publications/Survey-NRL-capacity-for-food-waterborne-agents.pdf
28. Struelens MJ, Palm D, Takkinen J. Enteroaggregative, Shiga toxin-producing Escherichia coli O104:H4 outbreak: new microbiological findings boost coordinated investigations by European public health laboratories . Euro Surveill. 2011;16(24):pii=19890. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19890. PMid:21699771.
29. Istituto Superiore di Sanità (ISS). EU Reference Laboratory for E. coli. Department of Veterinary Public Health and Food Safety Unit of Foodborne Zoonoses. Detection and identification of Verocytotoxin-producing Escherichia coli (VTEC) O104:H4 in food by Real Time PCR. 13 Jun 2011. Rome: ISS. Available from: http://www.iss.it/binary/vtec/cont/Lab_proc_O104_rev2.pdf
30. European Centre for Disease Prevention and Control (ECDC). Escherichia coli. Stockholm: ECDC. [Accessed 20 Jun 2013]. Available from: http://ecdc.europa.eu/en/healthtopics/escherichia_coli/Pages/index.aspx

Evidence-Based Medicine applied to the control of communicable disease incidents when evidence is scarce and the time is limited

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Control of acute communicable disease incidents demands rapid risk assessment, often with minimal peer-reviewed literature available but conducted in the public's view. This paper explores how methods of evidence-based medicine (EBM) can be applied in this scenario to improve decision making and risk communication. A working group with members from EBM organisations, public health institutions and the European Centre for Disease Prevention and Control used a six-stage framework for rapid risk assessments: preparation, risk detection/verification, risk assessment, development of advice, implementation, and evaluation. It concluded that data from observational studies, surveillance and modelling play a vital role in the evidence base. However, there is a need to further develop protocols and standards, to perform, report and register outbreak investigations more systematically and rigorously, and to allow rapid retrieval of the evidence in emergencies. Lack of evidence for risk assessment and advice (usual for new and emerging diseases) should be made explicit to policy makers and the public. Priorities are to improve templates for reporting and assessing the quality of case and outbreak reports, apply grading systems to evidence generated from field investigations, improve retrieval systems for incident reports internationally, and assess how to communicate uncertainties of scientific evidence more explicitly.

Introduction

Public health agencies responsible for the control of public health emergencies are expected to work according to the best standards of scientific evidence. They need to be explicit about the source, type, quality, scope and completeness of the evidence, so that policy makers, politicians and the public can understand the evolving nature of evidence, its strengths and limitations [1]. Even in the acute situation of infectious disease emergencies such as an influenza pandemic, agreed protocols for developing policy and advice should be followed. However, there are two important challenges: reliance upon limited field investigations and population surveillance data, and the speed with which evidence has to be identified and synthesised.

In 2010 the European Centre for Disease Prevention and Control (ECDC) set up a working group to review the potential utility of currently used evidence-based medicine (EBM) tools and risk assessment tools in realistic communicable disease outbreak scenarios, and to propose new tools [2]. A group of experts from 12 countries working in EBM and public health institutions or at ECDC, with a broad range of experience in public health methodology and infectious diseases, were appointed to give guidance on how to strengthen the scientific work at ECDC by adapting and applying EBM methods that were practical and applicable in the environment of infectious diseases and public health.

In this paper we report the conclusions on how to apply the principles of EBM in situations where rapid risk assessment is needed.

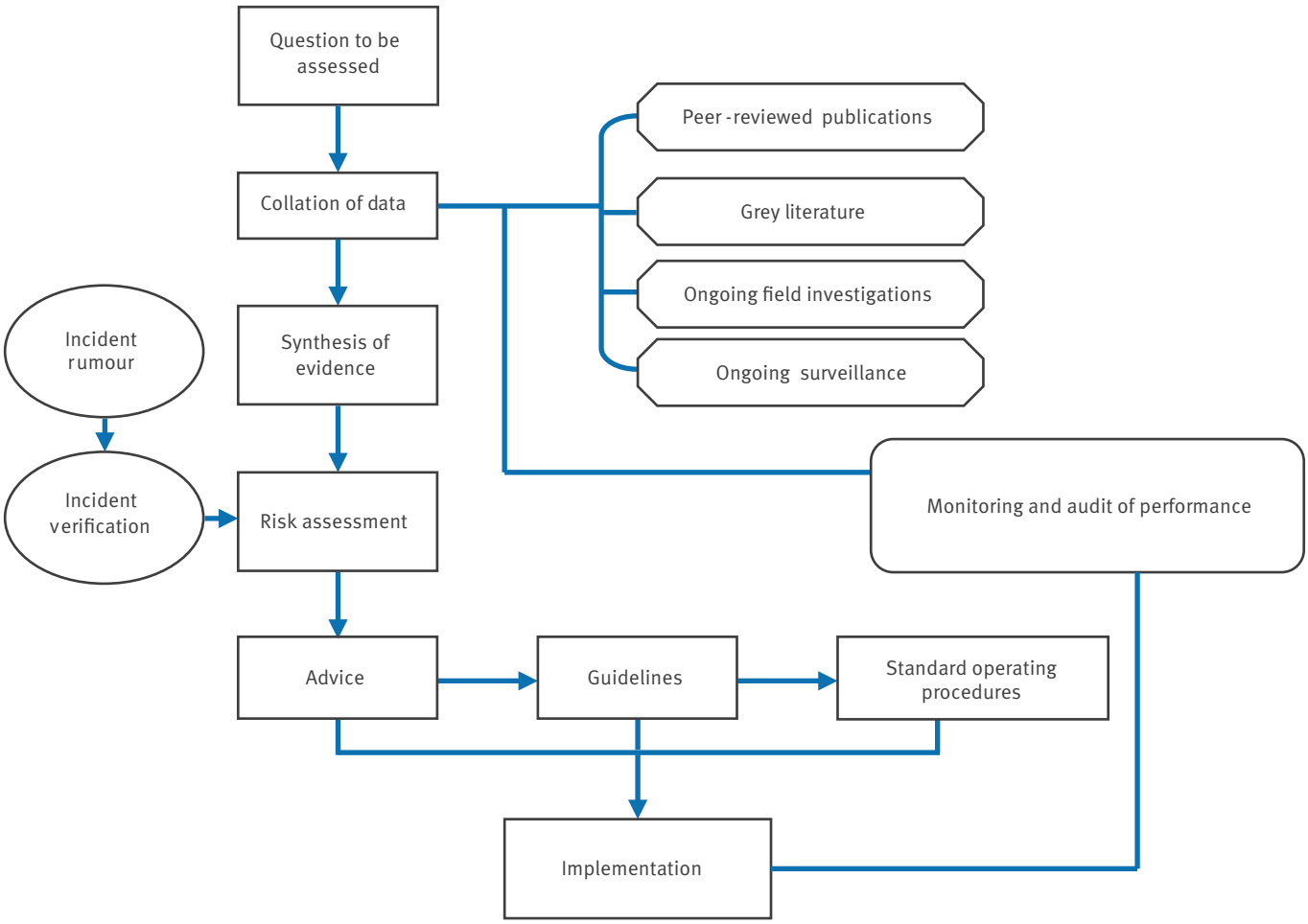
Methods and results

The working group presented the experiences of Member States in providing evidence-based guidance in circumstances when time was short, including the influenza pandemic in 2009 [3] and the Q fever epidemic in the Netherlands [4]. Consensus within the group was reached through informal group processes, through plenary and smaller group discussions, and by review of draft texts by the members and work colleagues in their institutions. The group members are listed at the end of the article.

The development of evidence for control of any incident, outbreak or pandemic was conceptualised as a knowledge cycle in which data are collated from surveillance and field investigation reports and peer-reviewed literature, rapidly appraised and used to

Figure 1

Evidence cycle in outbreak recognition, investigation, control and review



assess risks, develop advice and implement control measures. Continued surveillance, monitoring and auditing further consolidate the evidence base and allow refinement of risk assessment and evaluation of the effectiveness of interventions (Figure 1). Usually in the acute incident the knowledge cycle is entered at the risk assessment stage, when a report of an incident has to be verified, evidence collated and synthesised, and the risk assessed.

We identified six stages that need to be considered when preparing a rapid risk assessment under time constraints, and the need for improvement in each. They are summarised in the Table and described in detail below.

Stage 1: Preparatory phase

Alerting and surveillance systems should be set up that are regularly reviewed for fitness for purpose [5]. For newly emerging infections, the published data available to carry out systematic reviews will necessarily be very limited. It is therefore vital that critical summaries of evidence about epidemiology and control of these diseases are kept up to date and accessible internationally, including specifying key gaps in knowledge and suggesting appropriate models for risk assessment. Outbreak investigations are vital for defining epidemiological characteristics of specific pathogens (e.g. reproduction number) and can be used to evaluate the success of interventions [6,7]. However, to the best of our knowledge there are no agreed international standards for outbreak investigation and reporting. The value of field investigations would be greatly improved if a standardised framework for conducting, reporting, and synthesising data from outbreak investigations was used. Such standards exist for strengthening the reporting of observational studies in epidemiology (STROBE) [8], for the transparent reporting of evaluations of non-randomised designs (TREND) [9], and for meta-analysis of observational studies (MOOSE) [10]. Fine-tuning and evaluation for their application to outbreak situations has been undertaken for hospital

outbreaks (the outbreak reports and intervention studies of nosocomial infection (ORION) statement) [11]. For outbreak reports to be useful to others in a timely way, there needs to be an international repository of such reports and international agreement to make data rapidly available to investigators.

We identified tools and decision aids that we think would greatly improve public health decision making in acute outbreak situations.

- Up-to-date critical summaries of evidence from epidemiology and control of infectious diseases;
- Quality standards for performance and reporting of surveillance and field investigations;
- An international database of outbreak reports, accessible for all and with a user-friendly search function.

Stage 2: Incident verification

The critical step at this stage is to recognise the alert signal among the background noise of information. The agreed terminology outlining the epidemic intelligence process is the following:

- A signal needs to be filtered;
- An event needs to be validated;
- A validated event needs to be analysed.

In order to reduce the risk of bias, reproducible, transparent and explicit incident verification protocols should be followed. The process of verification requires rapid international communication networks of communicable diseases units. Algorithms should include trigger levels for upscaling, and stopping rules, to allow control agencies to agree that further investigation or more detailed risk assessment are not considered appropriate so that resources can be prioritised efficiently [12].

Tools required for this stage:

- International alerting and verification systems (e.g. the European Union's Early Warning and Response System [13]),
- Effective communication platforms (e.g. The European Union's Epidemic Intelligence Information System [14]).

Stage 3: Assessment of risk

This stage follows the verification of a threat and should address specific population groups at risk of more severe disease/outcome (e.g. pregnant women, the elderly, young children and immune-compromised individuals), and those at increased risk of exposure (e.g. healthcare workers). For rare, new and emerging infections there may be little or no peer-reviewed literature, and assessments will depend on field investigations, data from ongoing surveillance, and communication with experts in other countries. A comprehensive international database of outbreaks does currently not exist. Systematic methods for rapid searching and appraisal need to be developed that are appropriate to the time scales involved.

In order to reduce bias and to provide transparent quality assurance, risk assessment protocols and algorithms should be followed, and these should explicitly include frameworks for the synthesis of different types of evidence in relation to public health questions (e.g. risk of influenza A(H1N1) infection to pregnant women at different stages of pregnancy), admit to gaps and uncertainties in the evidence and possible alternative explanations of findings. Evidence should be classified by type (e.g. case report, population surveillance, field investigation) and study quality assessed through evidence-based checklists or tools such as the graphic approach to epidemiology (GATE) instrument for critical appraisal [15] and rapid risk assessment algorithms [16].

Table

Conceptual stages in rapid risk assessment and proposed evidence-based medicine tools

Stage	Task	Tools
Stage 1	Preparatory phase	Summaries of evidence from epidemiology and infectious disease control Quality standards for performance and reporting of surveillance and field investigations An international database of outbreak reports
Stage 2	Incident verification	Alerting and verification systems Effective communication platforms
Stage 3	Assessment of risk	A protocol for rapid searching for relevant peer-reviewed and grey literature Checklists and templates for rapid appraisal of the evidence An international database on incidents and reports A rapid risk assessment procedure and tool
Stage 4	Developing advice	Guidance on developing advice Uncertainty tables
Stage 5	Implementation	A checklist of key points to address in risk communication
Stage 6	Monitoring and evaluation	A protocol for review and audit

Tools required for this stage:

- A protocol for rapid searching for relevant peer-reviewed and grey literature,
- Checklists and templates for rapid appraisal of the evidence,
- An international database on disease incidents and outbreak reports,
- A rapid risk assessment procedure and tool.

Stage 4: Developing advice

Guidance will need to recognise explicitly the situational context and the population groups to which it is applied, but should seek to follow agreed EBM principles as embodied in, for example, the guidelines evaluation tool AGREE II (appraisal of guidelines for research and evaluation) [17]. The grading of recommendations applicability, development and evaluation (GRADE) instrument was developed to evaluate and make explicit the steps from evidence to recommendations about treatments of diseases, but these principles also apply when a public health decision is to be made under time constraints [18]. An essential part of developing advice is to state clearly what are the options for interventions and the expected relative merits of different options, as well as openness in dealing with uncertainty [19]. Following the principles of EBM under pressure of time will usually reveal a higher level of uncertainty about the conclusions and recommendations than medium- or long-term risk assessments. We are aware that it is difficult, especially for public health agencies, to translate scientific uncertainty into policy advice [20]. Stakeholders expect certainty and clear answers. However, we also believe that scientific uncertainty should be included in the assessment and the decision-making process as information, not ignored [21].

The working group considered the added value to communicable disease incident control of integrating principles from the discipline of risk analysis, as embodied, for example, in the Codex Alimentarius [22]. If we consider the Public health decision making process as a predictive model, uncertainties can arise both from the potential errors associated with the structure of the model (such as the context of the outbreak, modes of transmission and potential control measures for new infections) and from uncertainties in the values of the model parameters (incomplete data or measurement errors) [23]. These uncertainties are an integral part of scientific judgment and should be reflected in communication with policy makers and the public.

Tools required:

- Guidance on developing advice, including assessment of the quality of evidence;
- Uncertainty tables addressing uncertainties arising directly from the data and from the model/ process used to capture and interpret the data.

Stage 5: Implementation

For effective implementation, advice must be framed by requirements of the target groups. Public perception and communication of risk must therefore be considered. Various governments and international organisations have published guidelines on risk communication which embrace the need for consistent, credible and high-quality information to be shared with the public [24,25]. In acute scenarios, the rapidly changing picture and accumulation of intelligence needs to be explained, and caveats about interim advice clearly admitted.

Tools required:

- A checklist of key points to address in risk communication.

Stage 6: Monitoring and evaluation

The last stage is monitoring the implementation of control measures. It is increasingly recognised by public health agencies that they should have in place systems for learning lessons from incidents and continuously improving performance [26]. Therefore, incidents should be reviewed systematically to identify the lessons for better management of future incidents, and to identify new knowledge about the causative agent and the risks to the population. This would be aided by the use of standardised audit tools [27] and protocols [28] that should be followed to give a rapid but systematic approach to identifying lessons within a framework of organisational accountability.

Tools required:

- Protocols for review and audit of lessons to be learned from of incidents.

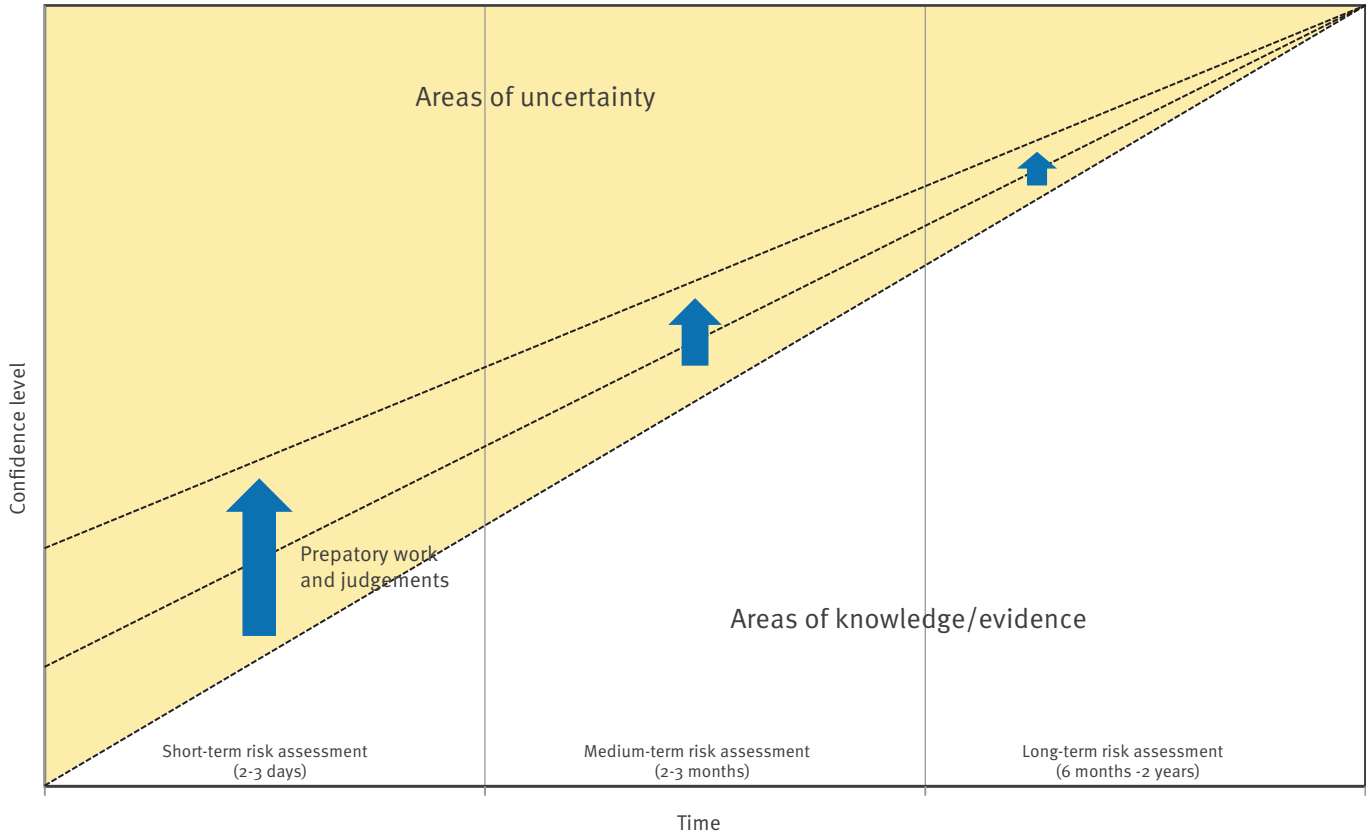
Discussion

The validity, credibility and success of public policy and risk management of public health threats are increasingly being seen as dependent upon the use of the best available scientific evidence developed through a transparent and open process [1]. To this end, a working group set up by ECDC has assessed the potential value of a more widespread use of strategies from evidence-based medicine in communicable disease control.

The EBM movement started as an application of epidemiological and public health principles in clinical practice; the application to public health threats is a more recent trend [28]. We recognise that there are important distinctions between evidence-based strategies applied to the review and appraisal in clinical medicine and the reality of public health policy making and communicable disease control, not least the lack of a strong evidence base and the pressure of time. In the sister discipline of risk analysis it is also increasingly being recognised that public health decision making is generally a result of a more complex interaction of the best available evidence from research and other epidemiological sources, with judgements made on needs,

Figure 2

Conceptual model of the relationship between uncertainty and time in risk assessments



resources, local circumstances, and ethical, legal and societal implications [29].

We see considerable merit in an integrative approach bringing risk analysis methods together with the epidemiological principles of EBM. For example, the EU Scientific Committee for New and Emerging Health Risks uses the expression 'lines of evidence' to characterise different sources and levels of evidence and information [29]. They consider lines of evidence that lie at the bottom of the EBM hierarchy. The highest levels of evidence from systematic reviews of randomised trials are seldom available in acute communicable disease incidents and advice has to be derived from observational studies underpinned by microbiological and virological principles. Sometimes advice has to be based on analogy and modelling, using laboratory research, animal experiments and mathematical modelling of outbreak data. When empirical data in an outbreak emerge, they first appear in expert committee papers and conference presentations, well before peer-reviewed publication, making it difficult to identify that knowledge systematically and quickly. But as with higher-level forms of evidence, the quality of such studies, their collation and interpretation should be guided by EBM methods. This demands the application of rigorous, standardised and systematic ways of handling evidence so that the risk of bias is minimised and assumptions are made explicit.

The application of risk analysis methods is particularly important when dealing with the uncertainties implicit in rapid decision making. It is important to acknowledge that the level of confidence in the conclusions reached is typically inversely related to the time that has passed since the start of the event (Figure 2).

The confidence level which can be achieved for short-term risk assessments is largely dependent upon the preparatory work done. "*Constraints, uncertainties and assumptions having an impact on the risk assessment should be explicitly considered at each step in the risk assessment and documented in a transparent manner. Expressions of uncertainty or variability in risk estimates may be qualitative or quantitative, but should be quantified to the extent that is scientifically achievable*" [22]. The applicability and relevance of standard EBM methods increases with time as the outbreak investigations proceed, but at any particular time there is also the necessity to consider the application of the

precautionary principle, and to be clear that lack of evidence of harm is not interpreted as evidence for no harm [30]. The principles of EBM, working rigorously, systematically and transparently and according to best available evidence, should apply at all times.

Next steps

In order to improve the management of outbreaks of communicable disease across Europe, the working group developed a conceptual framework and a potential set of tools and checklists that need to be developed to deal with the twin pressures of timeliness of risk assessment and lack of evidence. We hypothesise that these tools would improve outbreak management and thereby reduce the human and resource costs of outbreaks. They would also provide a clear auditable trail of decision making that would allow continuous learning from outbreaks. We envisage that the tools described above, collected together with worked examples in the format of a work book, could provide a uniform, consistent methodology for health protection practitioners. The international health protection community should work together to take this agenda forward and in particular identify leadership and responsibilities for developing the tools and for setting up and managing the archives and databases identified as a necessary part of EBM applied to outbreak control. Led by the Robert Koch Institute and based on a tender from ECDC, a multidisciplinary team has started to develop and pilot a systematic, transparent and comprehensive evidence assessment framework for rating the evidence and strength of recommendations in the area of infectious disease prevention and control.

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References

1. Hine D. The 2009 influenza pandemic. An independent review of the UK response to the 2009 influenza pandemic. London: Cabinet Office; 2010. Available from: http://www.dhsspsni.gov.uk/the2009influenzapandemic_acc.pdf
2. European Centre for Disease Prevention and Control (ECDC). Evidence-based methodologies for public health. Technical Report. Stockholm: ECDC; 2011. Available from: http://www.ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=738
3. European Centre for Disease Prevention and Control (ECDC). Guide to public health measures to reduce the impact of influenza pandemics in Europe: 'The ECDC Menu'. Stockholm: ECDC; 2009. Available from: http://ecdc.europa.eu/en/publications/publications/0906_ter_public_health_measures_for_influenza_pandemics.pdf
4. European Centre for Disease Prevention and Control (ECDC). Risk assessment of Q fever. Technical Report. Stockholm: ECDC; 2010. Available from: http://www.ecdc.europa.eu/en/publications/Publications/1005_TER_Risk_Assessment_Qfever.pdf
5. Buehler JW, Hopkins RS, Overhage JM, Sosin DM, Tong V. Framework for evaluating public health surveillance systems for early detection of outbreaks. MMWR Recomm Rep. 2004;53(RR-5):1-11. PMid:15129191
6. Voirin N, Barnet B, Netzger MH, Vanhems P. Hospital-acquired influenza: a synthesis using the outbreak reports and intervention studies of nosocomial infection (ORION) statement. J Hosp Infect. 2009;71(1):1-14. http://dx.doi.org/10.1016/j.jhin.2008.08.013 Mid:18952319
7. Heijne JC, Teunis P, Morroy G, Wijkmans C, Oostveen S, Dinzer E, et al. Enhanced hygiene measures and norovirus transmission during an outbreak. Emerg Infect Dis. 2009;15(1):24-30. http://dx.doi.org/10.3201/1501.080299 PMid:19116045 PMCid:2660689
8. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzscher PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370(9596):1453-7. http://dx.doi.org/10.1016/S0140-6736(07)61602-X
9. Des Jarlais DC, Lyles C, Crepaz N, TREND Group. Improving the reporting quality of non-randomised evaluations of behavioural and public health interventions. The TREND statement. Am J Public Health. 2004;94(3):361-6. http://dx.doi.org/10.2105/AJPH.94.3.361 PMid:14998794 PMCid:1448256
10. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. JAMA. 2000;283(15):2008-12. http://dx.doi.org/10.1001/jama.283.15.2008 PMid:10789670
11. Stone SP, Cooper BS, Kibbler CC, Cookson BD, Roberts JA, Medley GF, et al. The ORION Statement: guidelines for transparent reporting of outbreak reports and intervention studies of nosocomial infection. J Antimicrob Chemother. 2007;59(5):833-40. http://dx.doi.org/10.1093/jac/dkm055 PMid:17387116
12. Palmer SR, Brown D, Morgan D. Early qualitative risk assessment of the emerging zoonotic potential of animal diseases. BMJ. 2005;331(7527):1256-60. http://dx.doi.org/10.1136/bmj.331.7527.1256 PMid:16308389 PMCid:1289329
13. Cox A, Guglielmetti P, Coulombier D. Assessing the impact of the 2009 H1N1 influenza pandemic on reporting of other threats through the Early Warning and Response System. Euro Surveill. 2009;14(45):pii=19397. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19397 PMid:19941788
14. Paquet C, Coulombier D, Kaiser R, Ciotti M. Epidemic Intelligence: a new framework for strengthening disease surveillance in Europe. Euro Surveill. 2006;11(12): pii=665. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=665
15. Jackson R, Ameratunga S, Broad J, Connor J, Lethaby A, Robb G, et al. l. The GATE frame: critical appraisal with pictures. Evid Based Med. 2006;11(2):35-8. http://dx.doi.org/10.1136/ebm.11.2.35 PMid:17213070
16. European Centre for Disease Prevention and Control (ECDC). Operational guidance on rapid risk assessment methodology. Stockholm: ECDC;2011. Available from: http://ecdc.europa.eu/en/publications/Publications/1108_TED_Risk_Assessment_Methodology_Guidance.pdf
17. Brouwers M, Kho ME, Browman GP, Burgers JS, Cluzeau F, Feder G, et al. AGREE II: advancing guideline development, reporting and evaluation in health care. CMAJ. 2010;182(18):E839-42. http://dx.doi.org/10.1503/cmaj.090449 PMid:20603348 PMCid:3001530
18. Guyatt GH, Oxman AD, Schunemann HJ, Tugwell P, Knottnerus A. GRADE guidelines: A new series of articles in the Journal of Clinical Epidemiology. J Clin Epidemiol. 2011;64(4):380–2. http://dx.doi.org/10.1016/j.jclinepi.2010.09.011 PMid:21185693
19. The Parliamentary Office of Science and Technology. Handling uncertainty in scientific advice. Postnote. 2004;220. Available from: http://www.parliament.uk/documents/post/postpn220.pdf
20. Kelly M, Morgan A, Ellis S, Younger T, Huntley J, Swann C. Evidence based public health: A review of the experience of the National Institute of Health and Clinical Excellence (NICE) of developing public health guidance in England. Soc Sci Med. 2010;71(6):1056-62. http://dx.doi.org/10.1016/j.socscimed.2010.06.032 PMid:20678836
21. Forland F, De Carvalho Gomes H, Nokleby H, Escriva A, Coulombier D, Giesecke J, et al. Applicability of evidence-based practice in public health: risk assessment on Q fever under an ongoing outbreak. Euro Surveill. 2012;17(3):pii=20060. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20060 PMid:22297099
22. Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Food Standards Programme. Codex Alimentarius Commission: procedural manual. Section III. 15th ed. Rome: FAO, WHO; 2005. Available from: ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_15e.pdf
23. Droguett EL, Mosleh A. Bayesian methodology for model uncertainty using model performance data. Risk Anal. 2008;28(5):1457-76. http://dx.doi.org/10.1111/j.1539-6924.2008.01117.x PMid:18793282
24. Cabinet Office. Communicating Risk. Available from: https://update.cabinetoffice.gov.uk/sites/default/files/resources/communicating-risk-guidance.pdf
25. National Institute for Public Health and the Environment (RIVM)/ Environmental Assessment Agency (MNP) Guidance for uncertainty assessment and communication: detailed guidance. Utrecht: Utrecht University; 2003. Available from: http://www.nusap.net/downloads/detailedguidance.pdf
26. Late Lessons from early warnings: the precautionary principle 1896–2000. Environmental issue report no 22. Copenhagen: European Environmental Agency, Copenhagen, 2001. Available from: www.eea.europa.eu/publications/environmental_issue_report_2001_22/Issue_Report_No_22.pdf
27. Kipping RR, Hamilton S, Roderick M, Alexander K. Developing audit standards required for outbreaks of communicable diseases – lessons from a mumps outbreak. J Public Health. 2006;28(4):347-50. http://dx.doi.org/10.1093/pubmed/fdl065 PMid:17065178
28. Brownson RC, Fielding JE, Maylahn CM. Evidence-based public health: a fundamental concept for public health practice. Annu Rev Public Health. 2009;30:175-201. http://dx.doi.org/10.1146/annurev.publhealth.031308.100134 PMid:19296775
29. European Commission. The Scientific Committees on Consumer Safety (SCCS), Health and Environmental Risks (SCHER), Emerging and Newly Identified Health Risks (SCENIHR). Rules of procedure. Brussels: DG Health and Consumers. Available from: http://ec.europa.eu/health/ph_risk/documents/rules_procedure_en.pdf
30. Rutter M. Identifying the environmental causes of disease: how should we decide what to believe and when to take action? London: Academy of Medical Sciences, 2007. Available from: http://www.acmedsci.ac.uk/download.php?file=/images/publication/119615475058.pdf