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An outbreak of dengue virus (DENV) type 2 Cosmopolitan genotype in Israeli travellers returning from the Seychelles, April 2017

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Dengue virus infection was diagnosed in six Israeli travellers returning from the Seychelles in April 2017. Phylogenetic analysis identified identical sequences belonging to the Cosmopolitan genotype of dengue virus type 2 in all samples sequenced, thus providing evidence for a probable dengue type 2 outbreak in the Seychelles. This report further demonstrates the role of travellers as sentinels for arboviral infections, especially in countries with limited diagnostic capabilities.

Because of the high number of mosquito species and especially the abundance of *Aedes albopictus* mosquitoes [1], the Seychelles remain under the threat of outbreaks, particularly arboviruses [2]. However, only limited data are available on the types and molecular characteristics of arboviruses circulating in the region. In this report we investigated an outbreak of dengue virus (DENV) infection in six Israeli travellers returning from the Seychelles, a popular tourist destination located in the Indian ocean east of the East African coast with a population of roughly 90,000 inhabitants.

Dengue diagnosis in Israeli travellers

Nine Israeli citizens who had travelled to the Seychelles islands reported a febrile illness which started 1–2 days after returning to Israel. They were part of a group of 32 travellers visiting a different island every day between 13 and 22 April 2017. The islands visited, in consecutive order, were: Mahe, Curieuse, Aride, Praslin, La Digue and Moyenne.

In six of the febrile travellers, dengue fever was confirmed, in four of them by both quantitative (q) RT-PCR [3] or PCR [4] and DENV IgM and IgG antibody capture ELISA (Panbio, Brisbane, Australia), in one only by qRT-PCR, and in one only by positive dengue virus NS1 antigen (Panbio, Brisbane, Australia) and IgM and IgG serology (Table). Since DENV RNA in serum can only be detected for a short time after symptom onset [5-8], DENV RNA in urine and whole blood was tested for samples obtained more than 10 days post symptom onset (Table). qRT-PCR of DENV-1–4 [3] or PCR [4] demonstrated that all five PCR-positve cases had DENV type 2. The remaining three febrile patients did not present to our clinics and therefore were not tested for dengue.

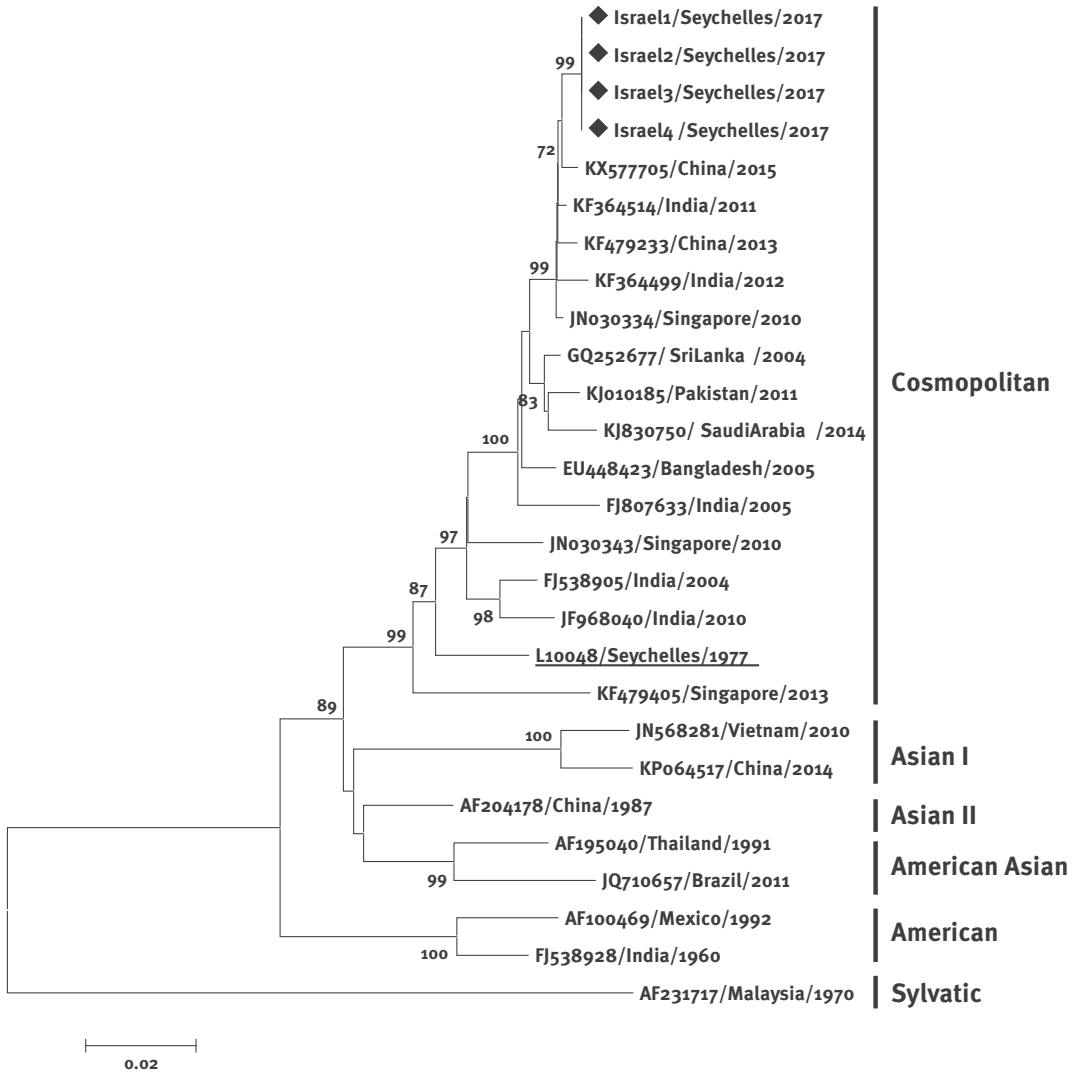
Three additional members of this group of travellers suffered from upper respiratory symptoms (one of them was only febrile). They were examined in our clinic, tested for DENV infection (by qRT-PCR, NS1 and serology) and found to be negative. We were not able to test the remaining asymptomatic travellers.

Genetic relationship

From the four RNA samples positive for DENV in qRT-PCR, 2,450 nt spanning the capsid, pre-membrane (prM), membrane (M) and envelope (E) genes were amplified [4]. The raw sequence data were analysed and trimmed to generate a 1,429-nt consensus sequence of the E gene, using Sequencher 5.4 (GeneCodes, Ann Arbor, Michigan), and the DENV E gene sequences were aligned with 23 DENV type 2 E gene sequences obtained from the GenBank database. The accession numbers for reference sequences are specified in the Figure. Phylogenetic analysis was conducted using a neighbour-joining algorithm in MEGA, version 6 [9], with 1,000 replicates for bootstrap testing. A neighbour-joining phylogenetic tree based on the aligned sequence data showed that all four DENV 2 sequences from Israeli travellers were identical and clustered with the Cosmopolitan genotype. Most importantly, strains belonging to lineage I of the Cosmopolitan genotype

Figure

Phylogenetic analysis of the envelope gene sequence of DENV type 2 from Israeli travellers, Seychelles, April 2017 (n = 4)



The analysis was conducted on a nucleotide sequence of the genes encoding the E protein (1,429 bp), using the neighbour-joining method implemented in MEGA 6.0 software. The robustness of branching pattern was tested by 1,000 bootstrap replications. The percentage of successful bootstrap replicates is indicated at nodes, showing only values of>70%. The bar denotes 0.02 nucleotide substitutions per site. DENV strains sequenced in this study are marked by black diamonds. Reference strains are indicated by accession number and place and year of isolation. The DENV type 2 strain isolated in 1977 in the Seychelles is underlined.

from China, India and Singapore were found to cluster with the strains of the present study (Figure).

Discussion

DENV is currently a leading cause of illness and death in the tropics and subtropics with as many as 400 million people infected yearly [10]. However, because it occurs in many areas with limited diagnostic resources and co-circulates with other arboviruses with similar clinical manifestations, travellers are often the most sensitive sentinels for arboviral outbreaks. Here we report a dengue outbreak in the Seychelles that was revealed by six Israeli travellers diagnosed with acute DENV infection, demonstrating the power of such passive arbovirus surveillance.

Mahe is the largest and most populated island where most of the previous outbreaks in the Seychelles have been reported [11]. Assuming that DENV exposure happened there would result in an incubation time of 10–12 days, which is longer than what is usual for dengue (4–7 days) [12]. Therefore, and because all travellers had disease onset almost on the same day, a simultaneous exposure on one of the other islands is more plausible. The immediate result of this study was an alert through ProMED [13] assuming that this would also alert the Seychelles public health authorities of the possibility of an outbreak on one of the smaller islands.

Table

Clinical and diagnostic data of dengue virus-infected Israeli travellers, Seychelles, April 2017 (n = 6)

Patient	Sex/age group	Main symptoms (description)	RT-PCR result (Ct), sample type	Serum sample			
				Time from onset (days)	NS1 early antigen	IgM	IgG
1	F/50–59	Fever + Rash	Pos (26.7), urine	14	Neg	Pos	Pos
2	M/70–79	Fever + Pruritus	Pos (26), urine	16	Neg	Pos	Pos
3	M/60–69	Fever	Pos (31), urine	15	Neg	Pos	Pos
4	F/70–79	Fever + Rash	Neg, serum	10	Pos	Pos	Pos
5	F/70–79	Fever + Rash	Pos (25.6), serum	6	ND	ND	ND
6	M/80–89	Fever + Rash	Pos[a], whole blood	15	Neg	Pos	Pos

F: female; M: male; ND: not done; Neg: negative; Pos: positive.

[a] Only PCR was performed and therefore no Ct value is available.

Although DENV RNA was identified in five patients, NS1 antigen was not detected. This is not surprising, since all NS1-negative samples had been obtained more than 12 days after symptom onset and it has been demonstrated previously that samples from dengue patients do not test positive for NS1 later than 12 days post symptom onset [14]. While detection of DENV RNA in serum is also limited to a short time (<1 week) after symptom onset, RNA in urine and whole blood can be detected for a longer period, similarly to other flaviviruses such as West Nile and Zika virus [5-8]. Indeed, in our patients, urine and whole blood samples obtained more than 14 days post symptom onset were positive for DENV RNA.

During the past 5 years, two outbreaks of dengue or dengue-like symptoms have been documented in the Seychelles and both DENV types 1 and 2 have been identified [11]. However, DENV was isolated only once in 1977 (GenBank accession number: L10048) and found to be type 2 [15]. Phylogenetic analysis performed in 1999 identified that this 1977 DENV strain was most similar to dengue 2 strains isolated in Delhi, India in 1996 [16]. Our data demonstrate that the 1977 isolate similarly to the 2017 isolates, belonged to the Cosmopolitan genotype. It is tempting to speculate that the Cosmopolitan genotype has continued to circulate in the Seychelles from the 1970s until now. However, because the phylogeny differed considerably between the current and former DENV-2 strains from the Seychelles and similar cosmopolitan strains were identified in China in 2013 and 2015 and in India in 2011–12, we hypothesise that the DENV responsible for the current outbreak was imported from a country endemic for Cosmopolitan DENV-2.

Conclusion

This study identified a DENV outbreak in the Seychelles. The diagnosis of six individuals with DENV infection among 32 Israeli travellers suggests that there is considerable circulation of DENV in mosquitoes in the Seychelles. Future monitoring of DENV circulation in mosquitoes in this area should reveal the extent of penetration of DENV and other arboviruses in the Seychelles and will contribute considerably to the epidemiological characterisation of these zoonotic viruses.

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Conflict of interest

None declared.

Authors' contributions

Y.L. contributed to the study design, performed the molecular experiments, analysed the data, wrote and edited the manuscript, D.W and O.H contributed to the study design and edited the manuscript, E.S coordinated the work, analysed the data, contributed to the study design and provided critical review of the manuscript.

References

1. Le Goff G, Boussès P, Julienne S, Brengues C, Rahola N, Rocamora G, et al. The mosquitoes (Diptera: Culidae) of Seychelles: taxonomy, ecology, vectorial importance, and identification keys. Parasit Vectors. 2012;5(1):207. DOI: 10.1186/1756-3305-5-207 PMID: 22999320
2. Bovet P, Gedeon J, Louange M, Durasnel P, Aubry P, Gauzere BA. [Health situation and issues in the Seychelles in 2012.]Med Sante Trop. 2013;23(3):256-66. French.
3. Waggoner JJ, Abeynayake J, Sahoo MK, Gresh L, Tellez Y, Gonzalez K, et al. Comparison of the FDA-approved CDC DENV-1-4 real-time reverse transcription-PCR with a laboratory-developed assay for dengue virus detection and serotyping. J Clin Microbiol. 2013;51(10):3418-20. DOI: 10.1128/JCM.01359-13 PMID: 23903549
4. Cruz CD, Torre A, Troncos G, Lambrechts L, Leguia M. Targeted full-genome amplification and sequencing of dengue virus types 1-4 from South America.J Virol Methods. 2016;235:158-67. DOI: 10.1016/j.jviromet.2016.06.001 PMID: 27334982
5. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine.Emerg Infect Dis. 2015;21(1):84-6. DOI: 10.3201/eid2101.140894 PMID: 25530324
6. Hirayama T, Mizuno Y, Takeshita N, Kotaki A, Tajima S, Omatsu T, et al. Detection of dengue virus genome in urine by real-time reverse transcriptase PCR: a laboratory diagnostic method useful after disappearance of the genome in serum. J

Clin Microbiol. 2012;50(6):2047-52. DOI: 10.1128/JCM.06557-11 PMID: 22442323

7. Lustig Y, Mannasse B, Koren R, Katz-Likvornik S, Hindiyeh M, Mandelboim M, et al. Superiority of West Nile Virus RNA Detection in Whole Blood for Diagnosis of Acute Infection. J Clin Microbiol. 2016;54(9):2294-7. DOI: 10.1128/JCM.01283-16 PMID: 27335150
8. Lustig Y, Mendelson E, Paran N, Melamed S, Schwartz E. Detection of Zika virus RNA in whole blood of imported Zika virus disease cases up to 2 months after symptom onset, Israel, December 2015 to April 2016. Euro Surveill. 2016;21(26):30269. DOI: 10.2807/1560-7917.ES.2016.21.26.30269 PMID: 27386894
9. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.Mol Biol Evol. 2013;30(12):2725-9. DOI: 10.1093/molbev/mst197 PMID: 24132122
10. Ayukekbong JA, Oyero OG, Nnukwu SE, Mesumbe HN, Fobisong CN. Value of routine dengue diagnosis in endemic countries. World J Virol. 2017;6(1):9-16. DOI: 10.5501/wjv.v6.i1.9 PMID: 28239567
11. Sandin A. Emergency plan of action final report Seychelles: dengue outbreak. Geneva: International Federation of Red Cross and Red Crescent Societies; 2016. Available from: http://reliefweb.int/sites/reliefweb.int/files/resources/MDRSC004fnr.pdf
12. Wilder-Smith A, Schwartz E. Dengue in travelers.N Engl J Med. 2005;353(9):924-32. DOI: 10.1056/NEJMra041927 PMID: 16135837
13. ProMED-mail. Dengue/DHF update (06): Israel ex Seychelles. Archive Number 20170531.5073985. 30 May 2017. Available from: 31.05.17. http://www.promedmail.org/direct.php?id=20170531.5073985
14. Fuchs I, Bin H, Schlezinger S, Schwartz E. NS1 antigen testing for the diagnosis of dengue in returned Israeli travelers.J Med Virol. 2014;86(12):2005-10. DOI: 10.1002/jmv.23879 PMID: 24390973
15. Lewis JA, Chang GJ, Lanciotti RS, Kinney RM, Mayer LW, Trent DW. Phylogenetic relationships of dengue-2 viruses.Virology. 1993;197(1):216-24. DOI: 10.1006/viro.1993.1582 PMID: 8212556
16. Singh UB, Maitra A, Broor S, Rai A, Pasha ST, Seth P. Partial nucleotide sequencing and molecular evolution of epidemic causing Dengue 2 strains.J Infect Dis. 1999;180(4):959-65. DOI: 10.1086/315043 PMID: 10479118

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Co-circulation of multiple subtypes of enterovirus A71 (EV- A71) genotype C, including novel recombinants characterised by use of whole genome sequencing (WGS), Denmark 2016

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In Europe, enterovirus A71 (EV-A71) has primarily been associated with sporadic cases of neurological disease. The recent emergence of new genotypes and larger outbreaks with severely ill patients demonstrates a potential for the spread of new, highly pathogenic EV-A71 strains. Detection and characterisation of these new emerging EV variants is challenging as standard EV assays may not be adequate, necessitating the use of whole genome analysis.

Enterovirus A71 (EV-A71) has been detected in Denmark in relatively low numbers since 2001. Of the different genotypes of EV-A71, only B5, C1, C2, and C4 have been identified [1,2]. All genotypes have been either associated with neurological symptoms, or milder hand, foot, and mouth disease (HFMD) in Denmark. EV-A71 subtype C4a, commonly circulating in Asia, has been identified as the cause of extensive outbreaks of HFMD with neurological complications and fatalities [3,4]. During a pilot project validating next generation sequencing (NGS) technology for full genome typing of EVs at the National World Health Organization (WHO) Reference Laboratory for Poliovirus at Statens Serum Institut (SSI), a novel EV-A71 variant was identified. This variant was shown to be genetically closely related to a recently published novel EV-A71 genotype C1 recombinant variant identified in Germany [5,6]. Re-analysis of sequence data and full genome re-sequencing of selected samples from the Danish EV surveillance database revealed additional viruses phylogenetically closely related to this new variant.

Laboratory analyses

Samples included in the analysis were collected through the Danish EV surveillance system [7]. They were genotyped using VP2 and/or VP1 PCR assays [8,9] and sequenced as described previously [2]. All 20 EV-A71 cases from 2016, as well as additional 12 historical cases previously subtyped as C1 were analysed. NGS was carried out directly on clinical sample material, as described previously [10], for a subset of samples (n = 10) and one sample was characterised from cultured material obtained as part of another study. The Illumina MiSeq platform was used to generate the sequencing data that were analysed using the CLC genomics workbench. Consensus sequences were exported as fasta files, and aligned with all available full-length EV-A71 genomes downloaded from the National Center for Biotechnology Information (NCBI) GenBank using SSE v1.3[11]. Phylogenetic analysis was carried out using MEGA 6 [12], maximum likelihood with a general time reversible model, gamma distribution and invariable sites, and 1,000 bootstrap replications. Bootscanning analysis was also carried out using SimPlot v3.5.1 [13]. Sequences have been submitted to GenBank, accession numbers pending.

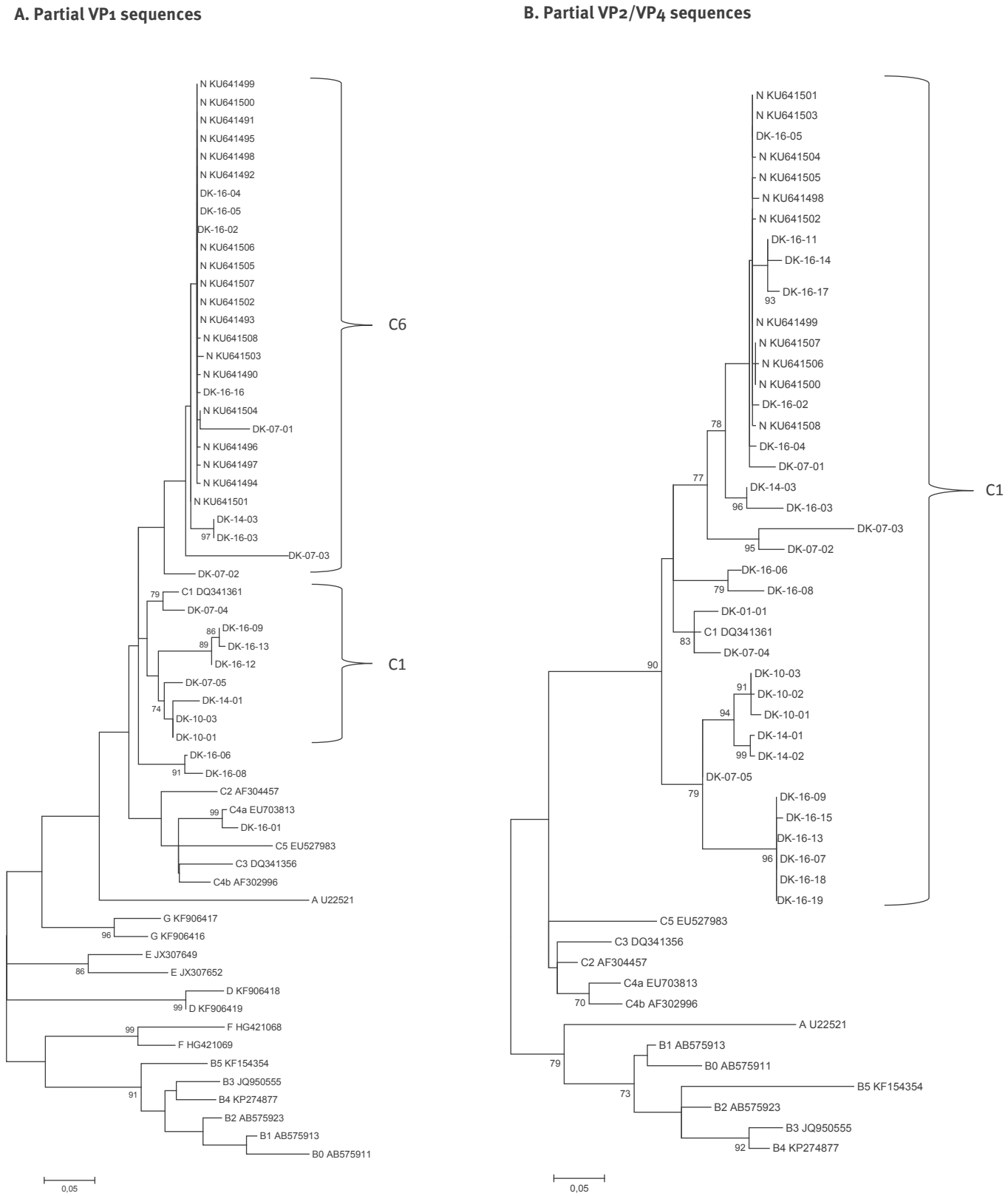
Enterovirus A71 genotype C cases 2001–2016

The most common sample material available for the 32 EV-71 RNA-positive patients was stool (n = 22) followed by vesicular fluid (n = 6). Other sample materials were urine, cerebrospinal fluid (CSF), respiratory secretion and biopsy. (Table 1).

Patient ages ranged from 7 days to 36 years with a median of 3 months and inter-quartile ranges of 2 and 12 months, respectively, and 11 of 32 patients were females. Clinical information was available for 23 of 32 cases, collected as part of the enhanced EV surveillance system in place [7]. Six cases had HFMD, 11 had gastrointestinal symptoms, 14 had fever, five had

Figure 1

Phylogenetic analysis of enterovirus A71 genotype C cases, Denmark, 2001–2016

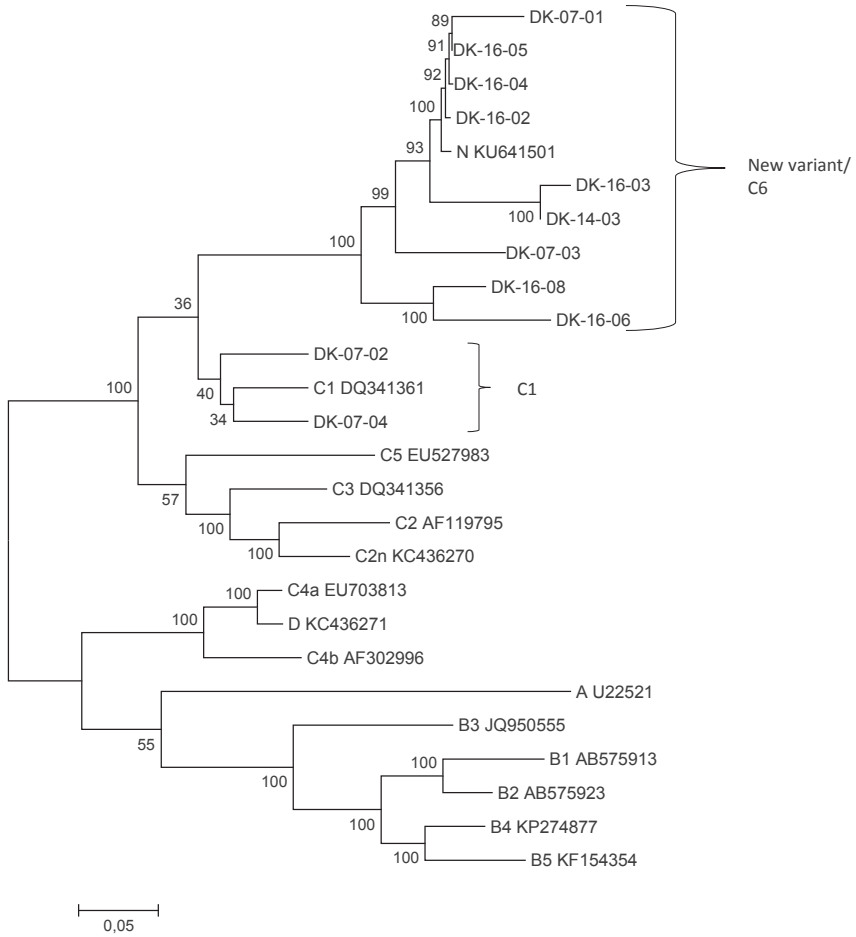


German new variant strains are identified with a capital N as a prefix, Danish strains are identified by the case ID as described in Table 1 prefixed by DK. Genotypes C1 and C6 are marked with brackets. Reference sequences for other genotypes are prefixed by the genotype, followed by the GenBank accession number.

Phylogenetic analysis was carried out using MEGA 6 [12], maximum likelihood with a general time reversible model, gamma distribution and invariable sites and 1,000 bootstrap replications. The bootstrap support is indicated at nodes, showing only values of›70%. The bar denotes 0.05 nt substitutions per site.

Figure 2

Phylogenetic analysis of near-complete genome sequences, enterovirus A71 genotype C cases, Denmark, 2001–2016



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Phylogenetic analysis was carried out using MEGA 6 [12], maximum likelihood with a general time reversible model, gamma distribution and invariable sites, and 1,000 bootstrap replications. The bootstrap support is indicated at nodes. The bar denotes 0.05 nt substitutions per site.

central nervous system involvement (Guillain–Barré syndrome, meningitis, encephalitis), and there was one death. The death and one of the sepsis cases were associated with a bacterial co-infection (*Clostridium difficile* and group B *Streptococcus*, respectively). Hospital admission data was available for 13 cases, and the hospitalisation ranged from less than one day to 10 days (average 3.6 days, SD 2.8).

Phylogenetic analysis

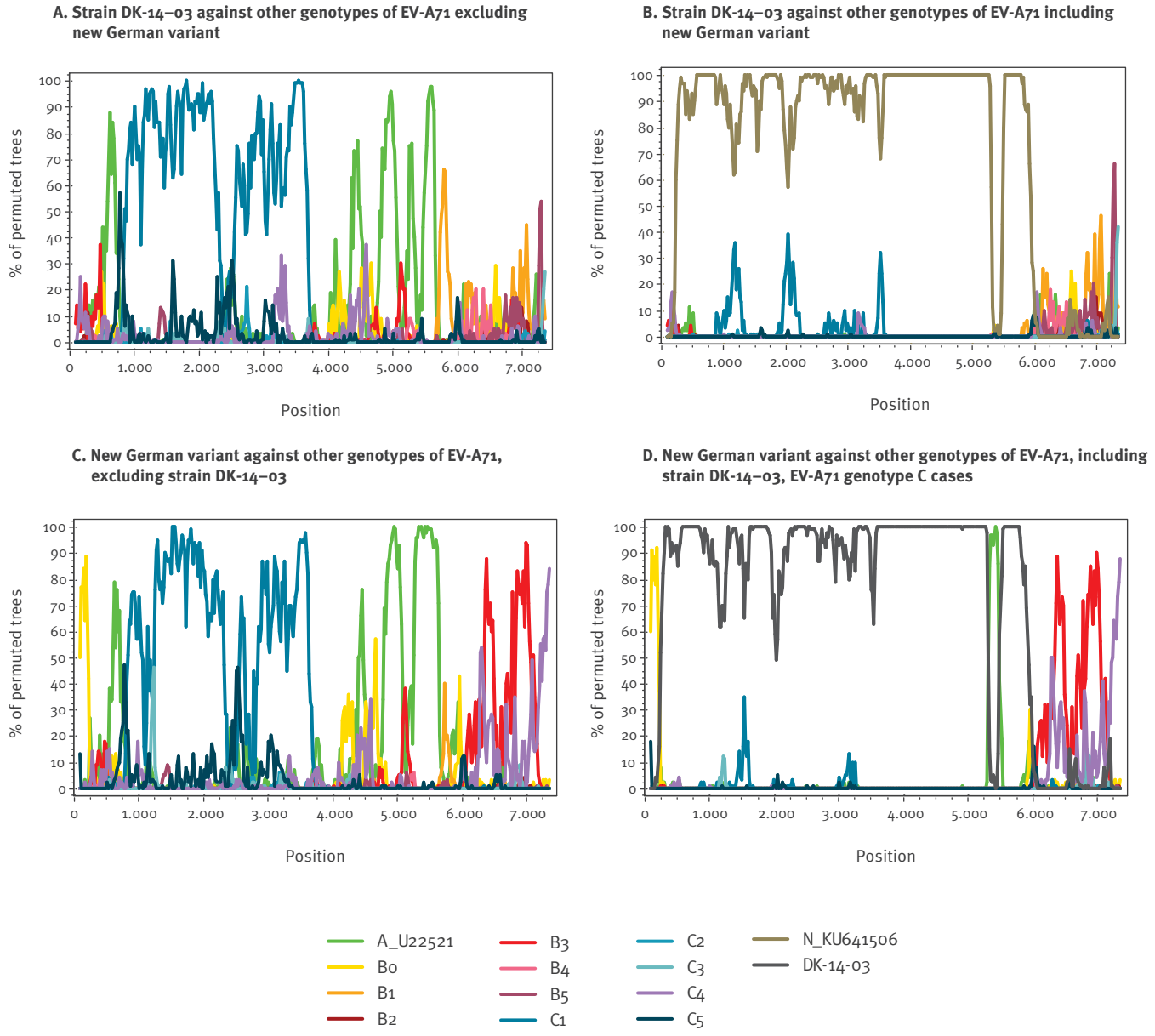
Phylogenetic analysis of partial VP2/VP4 and /or VP1 sequence data showed that 17 samples could be characterised as belonging to EV-A71 genotype C1 and 13 samples were closer to a new variant recently identified in our laboratory. Moreover, one EV-A71 genotype C2 and one C4 were identified (Figure 1). NGS data was obtained for a total of 11 strains, nine were found to belong to the new genotype, and two were C1 (Figure 2).

In the VP1 and near-full genome phylogenetic analyses, the new variant forms a clade separately from other C1 viruses. The new recombinant strain was identified in a sample dating back to 2007. In the WGS three clades were seen: (i) one containing Danish strains from 2007 and 2016 as well as strains from Germany, (ii) one with single Danish strains from 2014 and 2016, and (iii) one with two Danish strains from 2016. There was no difference between the genotypes regarding the clinical symptoms of the cases (Table 1). In the VP2 analysis it was not possible to clearly distinguish between C1 and the new variant.

Bootscanning analysis showed that Danish new variants were nearly identical to the German new variants over the majority of the genome. However, there was no similarity in the 3Dpol region, where both the German and the Danish strains differed from all published subtypes (Figure 3). The German strains appear to have a

Figure 3

Bootscanning and SimPlot analysis of novel enterovirus A71 genotype C cases DK-14–03 (Denmark 2014) and KU641506 (Germany 2015)



DK: Denmark; EV: enterovirus.

mosaic genome, more closely related to different genotypes in different parts of the genome. When analysed without comparison to the German strain, the Danish variant also showed a similar mosaic genome (Figure 3). Both strains appear to be closest to C1 in the VP1 region of the genome.

Discussion and conclusions

This study documents the circulation of new emerging EV-A71 genotype C variants associated with neurologic symptoms, as well as HFMD, in the Danish population. Novel EV-A71 variants were detected with WGS, and could be traced back to the first detection in the Danish population in 2007. Phylogenetically, a higher level of genetic variation was seen among the Danish strains as compared with the previously reported German EV-A71 C1 variant strains [5,6] suggesting that these novel variants have arisen from genetic reassortment of EV-A71 viruses over a prolonged period of time.

Three EV-A71 C variant clades were identified in Denmark one of which contains Danish EV-A71 C strains from 2007 and 2016 as well as the German 2015 strains. The phylogenetic analyses in this study show that the new variant EV-A71 sub-genotype C viruses form a clade separate from the C1 viruses, and the

Table

Clinical description of enterovirus A71 genotype C cases, Denmark, 2001–2016 (n = 32)

Year of detection–case ID	Age	Sample material	Sequence type[a]	EV-A71 genotype	Symptoms	Hospital admission
01–01	1–2 years	Unknown	VP2	C1	Guillain–Barré syndrome	Unknown
07–01	5–10 years	Vesicular fluid	VP2,WGS	C6	Vesicles	Unknown
07–02	<6 months	Stool	VP1,VP2,WGS	C1	Unknown	Unknown
07–03	<6 months	Stool	VP1,VP2,WGS	C6	Unknown	Unknown
07–04	1–2 years	Stool	VP1,VP2,WGS	C1	Diarrhoea for 3 weeks after travelling	Unknown
07–05	<6 months	Stool and urine	VP1,VP2	C1	Meningitis, fever, abdominal pain	Unknown
10–01	35–40 years	Vesicular fluid	VP1,VP2	C1	HFMD– rash, vesicles	<1 day
10–02	0–2 months	Stool	VP2	C1	Sepsis – fever>38.5, diarrhoea, vomiting blood	5 days
10–03	0–2 months	Stool and CSF	VP1,VP2	C1	Meningitis	Unknown
14–01	25–30 years	Vesicular fluid	VP1,VP2	C1	HFMD – rash, vesicles	No
14–02	1–2 years	Respiratory secretion	VP2	C1	Respiratory symptoms	Unknown
14–03	<6 months	Unknown	VP1,VP2,WGS	C6	Fever>38.5 °C, diarrhoea, vomiting	1 day
16–01	<6 months	Small intestine biopsy	VP1	C4	Sudden death, also *Clostridium difficile* infection	Unknown
16–02	<6 months	Stool	VP1,VP2,WGS	C6	Fever>38.5 °C, diarrhoea, rash	1 day
16–03	1–2 years	Vesicular fluid	VP2,WGS	C6	HFMD – rash, vesicles	no
16–04	<6 months	Stool	VP1,VP2,WGS	C6	Fever>38.5 °C, diarrhoea, rash	<1 day
16–05	6–11 months	Stool	VP1,VP2,WGS	C6	HFMD – fever>38.5 °C, rash, vesicles, vomiting	6 days
16–06	<6 months	Stool	VP1,VP2,WGS	C new/C6 [b]	HFMD – fever>38.5 °C, rash, vesicles, respiratory symptoms	4 days
16–07	<6 months	Stool	VP2	C1	Encephalitis – fever>38.5 °C, abnormal sensitivity to stimuli, diarrhoea, rash, respiratory symptoms, sepsis-like symptoms	5 days
16–08	4–5 years	Stool	VP2,WGS	C new/C6 [b]	Meningitis –neck stiffness, affected consciousness, fever, rash, vomiting	5 days
16–09	<6 months	Stool	VP1,VP2	C1	Unknown	Unknown
16–10	1–2 years	Stool	VP1	C1	Unknown	Unknown
16–11	1–2 years	Stool	VP2	C1/C6 [c]	Fever, rash, vesicles, diarrhoea	Unknown
16–12	<6 months	Stool	VP1	C1	Fever>38.5 °C, rash	4 days
16–13	<6 months	Stool	VP1,VP2	C1	Unknown	Unknown
16–14	<6 months	Stool	VP2	C1/C6 [c]	Fever>38.5 °C, group B *Streptococcus* sepsis	10 days
16–15	<6 months	Stool	VP2	C1	Fever	7 days
16–16	<6 months	Stool	VP1	C6	Fever>38.5 °C, vomiting, sepsis	4 days
16–17	<6 months	Stool	VP2	C1/C6 [c]	Unknown	Unknown
16–18	1–2 years	Vesicular fluid	VP2	C1	Unknown	Unknown
16–19	25–30 years	Vesicular fluid	VP2	C1	Unknown	Unknown
16–20	1–2 years	Stool	VP2	C2	Unknown	Unknown

HFMD: hand, foot, and mouth disease.

[a] VP1: sequence was obtained from VP1 PCR; VP2: sequence was obtained from VP2 PCR; WGS: sequence was obtained from whole genome sequencing.

[b] Grouping with C6 in near-complete genome analysis, but not with C6 or C1 in VP1 analysis. [c] Grouping with C6 when analysing the VP1 region, but no complete genome sequence available and therefore not possible to confidently assign as C6.

authors propose that these new variants are seen as a new genotype, C6, rather than a lineage of C1.

Both German and Danish new variants appear to be recombinant forms, with 3Dpol regions of separate origins. This was previously described for the German strains [6]. In fact, both the German and Danish strains appear to be mosaic, a result of several recombination events throughout the genome. The co-circulation of multiple genotypes of EV-A71 in one country during a single season/year, as demonstrated in this study, provides the environment for the appearance of future novel recombinant variants.

EV-A71 genotype C4 subtypes associated with more severe clinical outcomes than other EV-A71 genotypes and subtypes have previously been described [3,4]. The new C variant was described as emerging in 2015 in Germany and associated with rhomboencephalitis/brainstem encephalitis and severe neurological and cardiopulmonary complications [5,6,14]. However, the collection of samples for the EV surveillance system, as in the case of Denmark and Germany, may introduce a bias in this regard. The new EV-A71 genotype C variant identified in Denmark in 2016, was associated with both neurological symptoms and HFMD, illustrating the ability of EVs to cause a wide range of symptoms with rare cases of severe complications.

New emerging EVs have already demonstrated their potential to cause devastating epidemics such as the major EV-A71 epidemics in Asia and South Pacific Region. There is therefore a need to detect and monitor these viruses closely. In addition to detection and reporting of an emerging new EV-A71 recombinant virus, a proposed genotype C6, this study demonstrates important challenges in detection as well as characterisation of emerging EV infections. Current state-of-the-art EV PCR-based methods continuously need to undergo evaluation to ensure that primers for diagnostics, as well as typing, maintain the ability to detect and fully classify new EV variants beyond the (sero)type level. PCR-based typing relies on the amplification of short genome fragments, and as a consequence may not only result in missed detection of new genotypes, but also in misclassification due to a lack of appropriate reference sequences. Furthermore, timely and public sharing of whole EV genome sequence data are essential for detection of new variants.

The possible severity of EV-A71 infections together with the continuing evolution and appearance of new EV-A71 genotypes, as well as other emerging EV causing neurological disease, underscores the importance and relevance to prioritise strengthening of EV surveillance globally.

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Conflict of interest

None declared.

Authors' contributions

SOI: conceptualised the study in collaboration with THF, was involved with the design of the study, drafted the manuscript and conducted the phylogenetic analyses.

AGN: carried out part of the pilot study of next generation sequencing of enteroviruses, identified the first novel recombinant, and has critically reviewed the manuscript.

RATR: was involved with the design of the study, and has contributed to the writing of the manuscript.

MWP: conducted the laboratory characterisation of enteroviruses, and has critically reviewed the manuscript.

PEA: responsible for the collection of clinical information for the Danish enterovirus surveillance system, and has critically reviewed the manuscript.

TKF: conceptualised the study in collaboration with SOI, and has contributed to the writing of the manuscript.

References

1. Badran SA, Midgley S, Andersen P, Böttiger B. Clinical and virological features of enterovirus 71 infections in Denmark, 2005 to 2008.Scand J Infect Dis. 2011;43(8):642-8. DOI: 10.3109/00365548.2011.577094 PMID: 21526904
2. Fischer TK, Nielsen AY, Sydenham TV, Andersen PH, Andersen B, Midgley SE. Emergence of enterovirus 71 C4a in Denmark, 2009 to 2013.Euro Surveill. 2014;19(38):20911. DOI: 10.2807/1560-7917.ES2014.19.38.20911 PMID: 25306878
3. Zhang Y, Tan X, Cui A, Mao N, Xu S, Zhu Z, et al. Complete genome analysis of the C4 subgenotype strains of enterovirus 71: predominant recombination C4 viruses persistently circulating in China for 14 years. PLoS One. 2013;8(2):e56341. DOI: 10.1371/journal.pone.0056341 PMID: 23441179
4. Liu Y, Fu C, Wu S, Chen X, Shi Y, Zhou B, et al. A novel finding for enterovirus virulence from the capsid protein VP1 of EV71 circulating in mainland China. Virus Genes. 2014;48(2):260-72. DOI: 10.1007/s11262-014-1035-2 PMID: 24442718
5. Böttcher S, Obermeier PE, Neubauer K, Diedrich S, Laboratory Network for Enterovirus Diagnostics. Recombinant Enterovirus A71 Subgenogroup C1 Strains, Germany, 2015.Emerg Infect Dis. 2016;22(10):1843-6. DOI: 10.3201/eid2210.160357 PMID: 27439117
6. Karrasch M, Fischer E, Scholten M, Sauerbrei A, Henke A, Renz DM, et al. A severe pediatric infection with a novel enterovirus A71 strain, Thuringia, Germany. J Clin Virol. 2016;84:90-5. DOI: 10.1016/j.jcv.2016.09.007 PMID: 27771495
7. Condell O, Midgley S, Christiansen CB, Chen M, Chen Nielsen X, Ellermann-Eriksen S, et al. Evaluation of the enterovirus laboratory surveillance system in Denmark, 2010 to 2013. Euro Surveill. 2016;21(18):30218. DOI: 10.2807/1560-7917.ES.2016.21.18.30218 PMID: 27173593
8. Nasri D, Bouslama L, Omar S, Saoudin H, Bourlet T, Aouni M, et al. Typing of human enterovirus by partial sequencing of VP2. J Clin Microbiol. 2007;45(8):2370-9. DOI: 10.1128/JCM.00093-07 PMID: 17537940
9. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens.J Clin

Microbiol. 2006;44(8):2698-704. DOI: 10.1128/JCM.00542-06 PMID: 16891480

10. Fonager J, Stegger M, Rasmussen LD, Poulsen MW, Rønn J, Andersen PS, et al. A universal primer-independent next-generation sequencing approach for investigations of norovirus outbreaks and novel variants. Sci Rep. 2017;7(1):813. DOI: 10.1038/s41598-017-00926-x PMID: 28400558
11. Simmonds P. SSE: a nucleotide and amino acid sequence analysis platform.BMC Res Notes. 2012;5(1):50. DOI: 10.1186/1756-0500-5-50 PMID: 22264264
12. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.Mol Biol Evol. 2013;30(12):2725-9. DOI: 10.1093/molbev/mst197 PMID: 24132122
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
14. Antona D, Kossorotoff M, Schuffenecker I, Mirand A, Leruez-Ville M, Bassi C, et al. Severe paediatric conditions linked with EV-A71 and EV-D68, France, May to October 2016. Euro Surveill. 2016;21(46):30402. DOI: 10.2807/1560-7917.ES.2016.21.46.30402 PMID: 27918268

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Hepatitis E virus infection in Europe: surveillance and descriptive epidemiology of confirmed cases, 2005 to 2015

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Hepatitis E virus (HEV) is an under-recognised cause of acute hepatitis in high-income countries. The purpose of this study was to provide an overview of testing, diagnosis, surveillance activities, and data on confirmed cases in the European Union/European Economic Area (EU/EEA). A semi-structured survey was developed and sent to 31 EU/EEA countries in February 2016, 30 responded. Twenty of these countries reported that they have specific surveillance systems for HEV infection. Applied specific case definition for HEV infection varied widely across countries. The number of reported cases has increased from 514 cases per year in 2005 to 5,617 in 2015, with most infections being locally acquired. This increase could not be explained by additional countries implementing surveillance for HEV infections over time. Hospitalisations increased from less than 100 in 2005 to more than 1,100 in 2015 and 28 fatal cases were reported over the study period. EU/EEA countries are at different stages in their surveillance, testing schemes and policy response to the emergence of HEV infection in humans. The available data demonstrated a Europe-wide increase in cases. Standardised case definitions and testing policies would allow a better understanding of the epidemiology of HEV as an emerging cause of liver-related morbidity.

Introduction

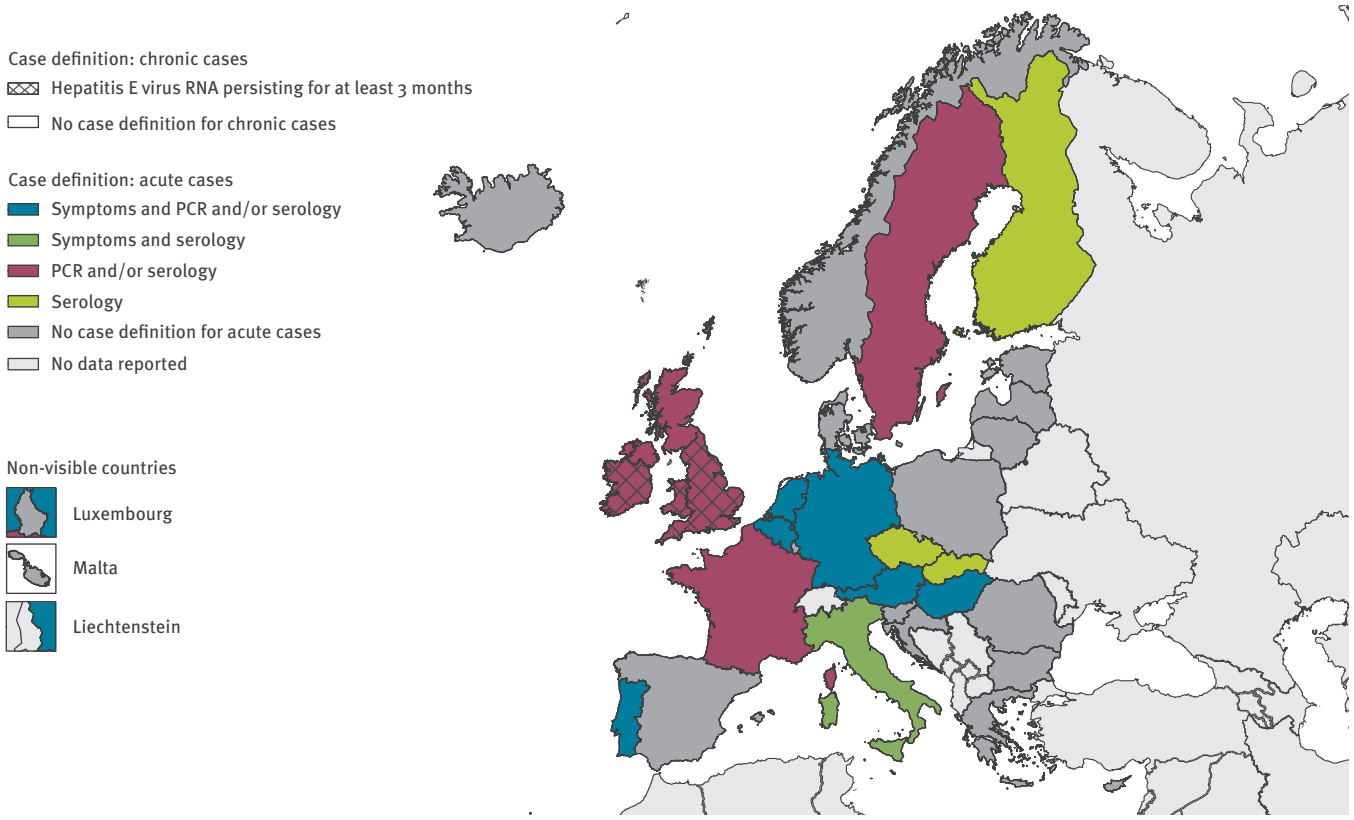
Hepatitis E virus (HEV) infection is one of the leading causes of acute viral hepatitis worldwide with four different genotypes (1–4) responsible for most human infections. HEV genotype 3 predominates in high-income countries, including those in Europe. Transmission of this genotype is usually zoonotic and has been linked to the consumption of pork products, and in some instances, shellfish [1-3]. Infection, which is most often asymptomatic, may cause an acute self-limiting hepatitis, with symptomatic infection more commonly reported among men older than 50 years of age [4,5]. Reports of chronic infection among immunocompromised people or those with pre-existing liver disease have been described [4].

There is evidence that HEV is an under-recognised pathogen in high-income countries, and that the incidence of confirmed cases has been steadily increasing over the last decade [6-10]. Although population studies have shown stable or decreasing seroprevalence rates [11,12], some countries have reported a consistently high seroprevalence and proportion of HEV-RNA-positive blood donors [13-16]. A systematic review conducted by the World Health Organization (WHO) reported HEV seroprevalence (as denoted by presence of IgG antibody) to range from 0.03% to 52% among the general population and blood donors in the WHO European Region, with the highest prevalence reported from studies in blood donors from France and the Netherlands [17]. Evidence of current HEV infection in up to 77% of patients presenting with symptomatic acute hepatitis suggests that the virus could be a considerable cause of liver morbidity in European countries [17]. However, the use of different serological test systems significantly influences the estimates of seroprevalence [18].

Europe-wide surveillance is not in place for hepatitis E, and the European Centre for Disease Prevention and Control (ECDC) has launched a number of activities

Figure 1

Type of applied case definition for confirmed cases of hepatitis E virus infection in 20 European Union/European Economic Area (EU/EEA) countries, 2015



PCR: polymerase chain reaction; RNA: ribonucleic acid.

Administrative boundaries from EuroGraphics. Map produced on 8 June 2017.

that aim to better understand the current epidemiology, as well as the national monitoring systems for HEV infection within European Union/European Economic Area (EU/EEA) countries. A recent study involving HEV experts from 17 countries documented an increase in reported cases in nearly all EU/EEA countries that provided data, with France, Germany, England and Wales, and the Netherlands reporting more hepatitis E than hepatitis A notifications. The study also highlighted a lack of consistency in the reporting of cases, and wide variation in surveillance activity and case definitions [7]. The current study builds on this work to conduct a wider assessment of testing, diagnosis, surveillance activities and confirmed cases of HEV infection in the EU/EEA in order to inform the Europe-wide response to this emerging infection.

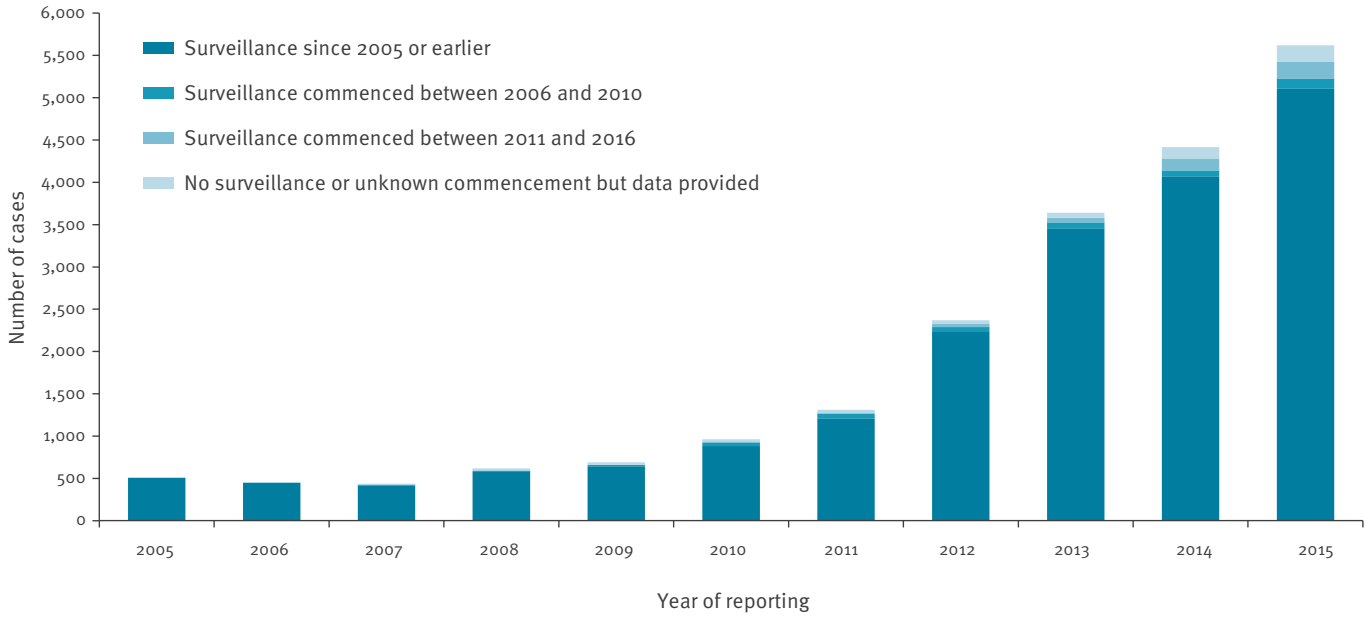
Methods

A semi-structured survey of EU/EEA countries was conducted during 2015/16. The survey was divided into four sections (surveillance, testing and diagnosis, data on diagnosed cases, and transfusion-associated HEV infections) to allow separate country-level respondents for each topic area with a total of 24 open and closed questions [19]. A pilot survey was conducted at a meeting of the ECDC HEV expert group in December 2015, and a revised survey was subsequently circulated by email to nominated representatives of ECDC's networks, the National Focal Points for Food- and Waterborne Diseases and Zoonoses as well as for Microbiology and Surveillance, in February 2016. Respondents were given three weeks to complete the survey (which could be completed electronically), and email and phone reminders were used to follow up with those who had not responded.

Returned survey data were manually extracted into Excel and double-checked. Any unclear responses were checked by email communication with the respective experts. Analyses were conducted in Excel and STATA version 13. The United Kingdom (UK) provided three separate responses (from England and Wales, Scotland, and Northern Ireland) which were considered as a single country response for all quantitative analyses and listed separately for qualitative analyses. A descriptive analysis was performed. The proportion of hospitalised cases was calculated using data exclusively from countries reporting hospital data, with the number of total cases as denominator. The Eurostat

Figure 2

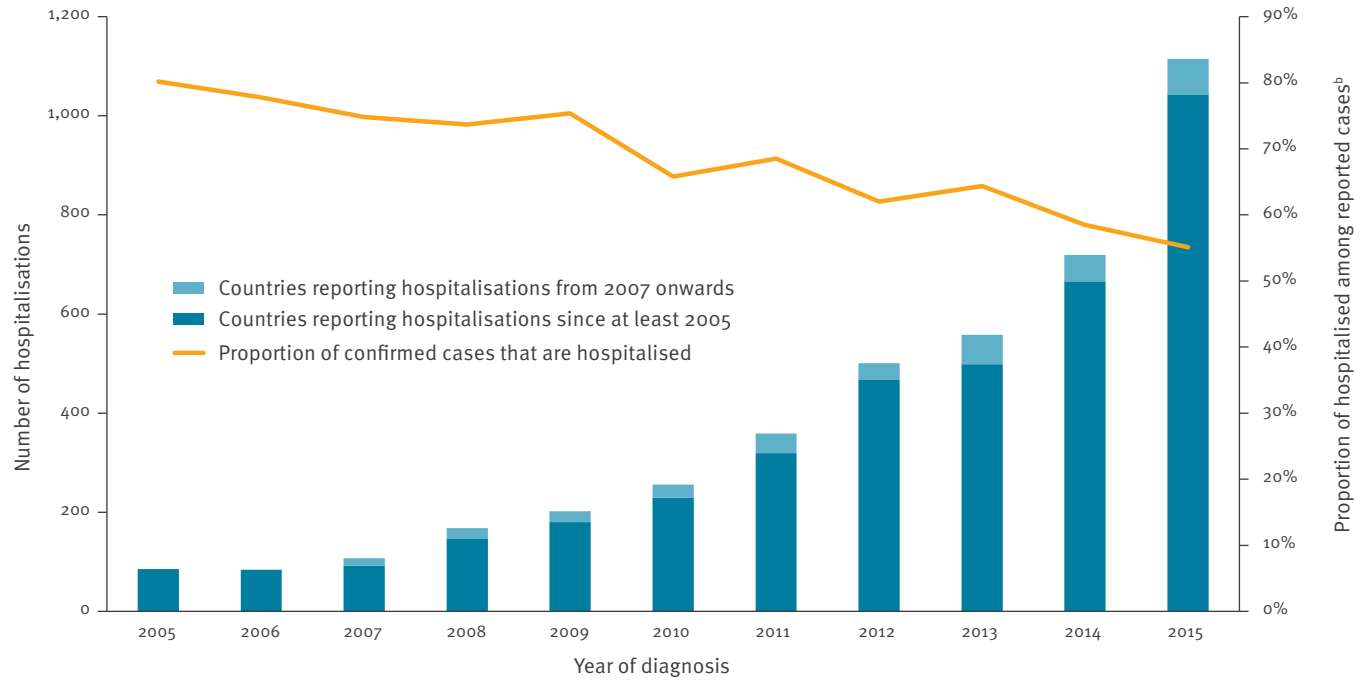
Reported number of cases of hepatitis E virus infection by year of notification and year of surveillance commencement, 22 European Union/European Economic Area (EU/EEA) countries, 2005–2015[a]



[a] Data available for the following countries (year of commencement of HEV surveillance/year since cases reported during study period 2005–2015): Austria (1980/2012), Belgium (2010/2010), Bulgaria (no surveillance/2015), Croatia (2009/2009), Cyprus (no surveillance/2009), Czech Republic (1996/2005), Estonia (1997/2005), Finland (1995/2005), France (2002/2005), Germany (2001/2005), Hungary (1993/2005), Italy (2007/2007), Latvia (commencement unknown/2007), the Netherlands (2012/2012), Norway (no surveillance/2005), Poland (no surveillance/2014), Portugal (commencement unknown/2014), Slovakia (2007/2005), Slovenia (1995/2005), Spain (commencement unknown/2006), Sweden (1993/2005), and UK (England and Wales (2003/2005), Scotland (2000/2005) and Northern Ireland (unknown/2005).

Figure 3

Number and proportion of hospitalisations among reported cases of hepatitis E virus infection, 14 European Union/European Economic Area (EU/EEA) countries, 2005–2015a

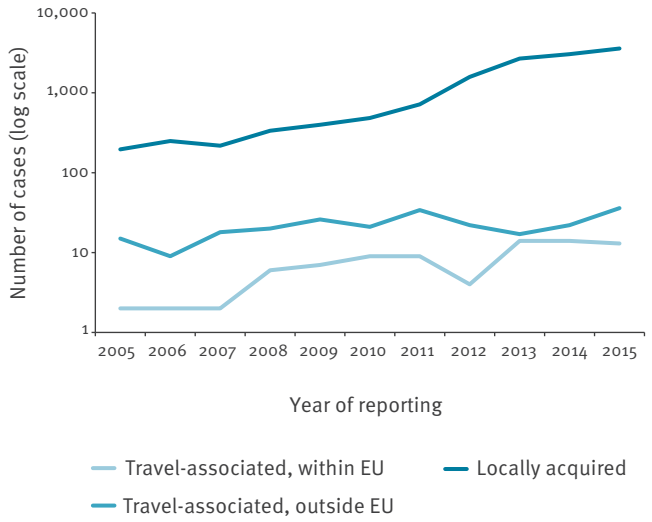


[a] Data available for (year of first report): Austria (2012), Belgium (2010), Croatia (2012), Czech Republic (2005), Estonia (2012), Germany (2005), Hungary (2005), Italy (2007), Latvia (2007), Poland (2014), Portugal (2015), Slovakia (2005), Slovenia (2005) and the UK (Northern Ireland) (2013).

[b] Proportion of hospitalisations only included denominator data from countries reporting both number of reported cases and number of hospitalised cases.

Figure 4

Reported cases of hepatitis E virus infection by year of notification and travel history, 15 European Union/ European Economic Area (EU/EEA) countries, 2005–2015[a]



[a] Data available for: Austria, Croatia, Czech Republic, Estonia, France, Hungary, Italy, Latvia, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden and the UK (England, Northern Ireland and Wales).

population database from 2015 was used as source of population denominator data [20].

Results

All EU/EEA countries with the exception of Liechtenstein responded to the survey, giving a response rate of 30 out of 31. The data provided from countries that conduct surveillance was comprehensive, detailed and of high quality.

Hepatitis E surveillance systems

Of 30 responding countries, 20 had specific surveillance systems for HEV infection. The remaining 10 countries had more generic viral hepatitis syndromic surveillance with no plans to develop any specific surveillance for HEV infection. Of the 20 EU/EEA countries with specific systems for HEV infection, 15 had national surveillance systems, three had laboratory surveillance through national reference laboratories with unknown coverage, one had blood service surveillance with unknown coverage, and one had a sentinel surveillance system covering approximately half of the population.

Thirteen of 20 surveillance systems specific for HEV infection had a case definition for confirmed cases. There was wide variation in the type of case definition used: seven used laboratory and clinical definitions, and six used a laboratory definition only (Figure 1). Most surveillance systems collected demographic information as well as the date of notification and onset of disease, whereas morbidity and mortality data were collected less frequently (Table 1). Two countries' surveillance systems (Ireland and the UK (England and Wales)) differentiated between acute and chronic HEV infection: acute infection was defined as both HEV-IgM-positive and IgG-positive, or HEV-RNA-positive, and chronic infection was defined as HEV RNA persisting for at least 3 months.

Hepatitis E testing and diagnosis

Twenty-six countries conducted HEV testing in their own countries, with two countries sending samples abroad for testing and two reporting their testing availability as unknown. In 12 countries, there were less than five laboratories able to conduct diagnostic tests for HEV. Laboratories in 20 countries used more than one type of test (Table 2). For HEV antibody testing in acute cases, IgM ELISA was used in 21 countries and IgM Western Blot in 11. Of the 22 countries using any IgM antibody test, 20 also used IgG ELISA and nine IgG Western Blot for the detection of IgG antibodies. Nineteen countries reported that they were able to conduct HEV RNA testing; all used serum/plasma and 12 also used stool specimens. Seventeen countries reported that HEV sequencing was conducted for epidemiological investigations (15 countries), for research purposes (14 countries) or as routine practice (10 countries). Ten countries provided information on why a laboratory might conduct a test for HEV. Two countries had a laboratory policy on testing for HEV: (i) UK (Scotland), where some laboratories automatically test for HEV if alanine transaminase (ALT) was ≥100 U/L, and (ii) Ireland, where an HEV test is automatically conducted if a test for hepatitis A virus (HAV) is requested. The remaining countries reported that HEV testing was conducted only at the request of a clinician.

Confirmed cases of hepatitis E virus infection

Twenty-two countries covering 469 million people (i.e. 91% of the EU/EEA population of 514 million people) provided data on confirmed cases of hepatitis E (Figure 2). The total number of reported cases increased from 514 cases in 2005 to 5,617 cases in 2015, with 21,018 cases overall during the 11-year period studied (Figure 2). A total of 93% (19,531/21,018) of cases were accounted for by countries that have had hepatitis E-specific surveillance since at least 2005, and 80% (16,810/21,018) of cases were reported from just three countries (Germany, France, and the UK), which comprise 41% of the EU/EEA population. In 16 countries providing data on the age of reported cases, the overall proportion of those aged more than 50 years increased from 30–47% during 2005–2008 to 60–61% during 2013–2015. In 17 countries providing data on the sex of reported cases, the proportion of male cases remained stable over 2005–2015, ranging from 61% in 2005 and 2015 to 69% in 2006.

Severity associated with hepatitis E virus infection

Data from 14 countries reporting on the total number of hospitalisations related to HEV infection showed an increase from 85 hospitalised cases in 2005 to

Table 1

Variables collected by specific surveillance systems for hepatitis E virus infection in 20 European Union/European Economic Area (EU/EEA) countries, 2015

Countries collecting data on hepatitis E cases via their surveillance systems	Variables
Majority (>14 countries)	Unique patient identifier, date of notification, source of notification, date of birth, sex, date of onset of disease
Some (6–14 countries)	Date of diagnosis, cluster link, occupation, pregnancy, clinical symptoms, travel, food history, contact with animals, hospitalisation, death
Few (<6 countries)	Ethnicity, migration status, alcohol consumption, medication, immunosuppressive medication or condition, other medical conditions, past transfusion/transplantation

1,115 in 2015 (Figure 3). Most of this increase could be accounted for by four of the five countries that have conducted reporting on hospitalisation status since at least 2005. Over the same time period, the proportion of cases being hospitalised has decreased from 80% (85/106 cases) to 55% (1,115/2,023 cases). Twelve countries provided data on deaths associated with HEV infection, with five countries recording these data since 2005. In total, 28 fatal cases were reported by five countries (Austria, Czech Republic, Germany, Hungary and Italy), increasing from 0–2 cases per year during 2005–2011 to 4–8 cases per year during 2012–2015.

Travel history of diagnosed cases

Fifteen countries provided data on travel history. Of hepatitis E cases reported in these countries during the period 2005–2015, 87% (13,511/15,525) were autochthonous (defined as locally acquired within the reporting country). The proportion of known autochthonous cases increased from 45–73% per year during 2006–2011 to 89–97% per year during 2012–2015. This suggests that the considerable increase in confirmed cases observed after 2011 (Figures 2 and 4) can largely be accounted for by locally acquired infection. A very small number of cases per year (9–36 cases) were known to be associated with travel outside of the EU/EEA.

Discussion

This study assessed testing policy and practice, laboratory diagnosis, and surveillance systems and activities for HEV infection in 31 EU/EEA countries. Thirty of the countries responded to the survey, providing the most comprehensive picture to date of HEV as an emerging infection among humans in Europe. The 10-fold increase in the number of cases reported between 2005 and 2015, more than 21,000 confirmed cases overall and a total of 28 deaths, underline the relevance and emerging nature of this zoonotic infection in Europe.

The survey findings demonstrate a varied response to hepatitis E across EU/EEA countries; while two thirds of countries have established surveillance systems, a third of countries have no specific surveillance and no plans to develop any. For those with existing surveillance, nearly one third do not apply a case definition for HEV infection nationally. Only 19 countries reported that they were able to conduct HEV RNA testing in-country, although there may be testing within hospitals or private settings that the national public health institutes or survey respondents are unaware of. In most countries, it was not possible to ascertain the criteria for referral or for HEV test requests. While there may be an increasing awareness of locally acquired HEV infections, there is still concern that within parts of the clinical community, HEV is not considered as a possible cause of hepatitis unless there is a recent history of travel. It is therefore likely that the infection remains under-diagnosed. There also appears to be a gap in knowledge around the testing protocols used in outpatient and hospital settings within countries, which should be investigated further to understand when, who, and why patients are tested for HEV.

Twenty-two countries (comprising more than 90% of the total EU/EEA population) were able to provide data on confirmed cases for at least some of the period 2005–2015. These data demonstrate that the number of reported cases has been increasing year-on-year, with a more than threefold increase between 2011 and 2015. The majority of cases were reported by three countries (the UK, France and Germany) that have had surveillance systems in place since at least 2005. A small number of countries reported changes to surveillance systems during the study period; for example, Belgium created a national reference centre for HEV in 2011 and Portugal commenced web-based notification in 2014. However, there were no changes that could have contributed substantially to the increase in the number of confirmed cases. A greater awareness of and increased testing for HEV, particularly of patients presenting with acute hepatitis by general practitioners in outpatient settings, is likely to be one of the contributing factors e.g. through the inclusion of HEV in some standard diagnostic protocols. The rise in the number of symptomatic cases might also be related to the switch in virus subtypes that has been observed in some EU/EEA countries [7,21].

The concurrent increase in number of hospitalisations (a measure of increased HEV infection severity) and

Table 2a

Types of tests used to detect hepatitis E virus infection in 28 European Union/European Economic Area (EU/EEA) countries, 2015

Country	IgM ELISA			IgM Western blot			IgG ELISA			IgG Western blot			PCR Serum			PCR Stool			Other
	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	EM
Belgium	Y	N	Wantai	N	N	–	Y	N	Wantai	N	N	–	Y	Y	RealStar HEV RT-PCR, Altona	Y	–	In-house PCR	N
Bulgaria	Y	–	–	–	–	–	Y	–	–	–	–	–	–	–	–	–	–	–	–
Croatia	Y	N	recomWell HEV IgM, Mikrogen	Y	–	recomLine HEV IgG/ IgM, Mikrogen	Y	–	recomWell HEV IgG, Mikrogen	Y	–	recomLine HEV IgG/ IgM, Mikrogen	Y	–	[25,26]	N	–	–	N
Cyprus	Y	N	Radim no. KH3 IW	N	N	–	Y	N	Radim no. KH1 IW	N	N	–	N	N	–	N	–	–	N
Czech Republic	Y	N	–	N	N	–	Y	N	–	N	N	–	Y	N	–	Y	N	–	N
Denmark	Y	–	–	N		–	Y		–	N	–	–	Y		–	Y	–	–	N
Estonia	Y	N	recomWell HEV IgM, Mikrogen	Y	N	recomLine HEV IgG/ IgM, Mikrogen	Y	N	recomWell HEV IgG Mikrogen	Y	N	recomLine HEV IgG/ IgM, Mikrogen	Y	N	In-house PCR	N	N	–	N
Finland	Y	N	recomWell HEV IgM, Mikrogen	N	N	–	Y	N	recomWell HEV IgG, Mikrogen	N	N	–	Y	–	–	N	–	–	N
France	N	N	–	N	N	–	N	N	–	N	N	–	Y	Y	–	Y	Y	–	N
Germany	Y	N	recomWell HEV IgM, Mikrogen; HEV IgM, Euroimmun	Y	N	recomLine HEV IgG/ IgM, Mikrogen	Y	N	recomWell HEV IgG, Mikrogen; HEV IgG, Euroimmun	Y	N	recomLine HEV IgG/ IgM, Mikrogen	Y	Y	In-house PCR; RealStar HEV RT-PCR, Altona; Mikrogen; Ceeram; Roche	Y	Y	In-house PCR; RealStar HEV RT-PCR, Altona; Mikrogen; Ceeram; Roche	Y
Greece	N		–	N			N		–	N		–	N		–	N		–	N
Hungary	Y	N	Mikrogen; DiaPro	Y	N	Mikrogen	N	N	–	N	N	–	Y	Y	In-house PCR	Y	Y	In-house PCR	N
Iceland	N	N	–	N	N	–	N	N	–	N	N	–	N	N	–	N	N		N

–: not available; ELISA: enzyme-linked immunosorbent assay; EM: electron microscope; E + W: England and Wales; HEV: hepatitis E virus; IgG: Immunoglobulin G; IgM: Immunoglobulin M; N: no; NI: Northern Ireland; PCR: polymerase chain reaction; qPCR: quantitative polymerase chain reaction; RNA: ribonucleic acid; RT-PCR: reverse transcriptase-polymerase chain reaction; S: Scotland; Y: yes.

[a] Assay type and brand name are separated by comma, brand names are separated by semicolon. Brand names: EIAgen Adaltis (Guidonia Montecelio, Italy); Altona diagnostics (Hamburg, Germany); Ceeram (La Chapelle sur Erdre, France); DiaPro Diagnostic BioProbes (Sesto San Giovanni, Italy); DSI Diagnostic System Italy (Saronno, Italy); Euroimmun (Lübeck, Germany); Fortress diagnostics (Antrim, the United Kingdom); Genome Diagnostics (Nijmegen, the Netherlands); Mikrogen (Neuried, Germany); Radim (Rome, Italy); Roche (Penzberg, Germany); Wantai (Beijing, China).

Table 2b

Types of tests used to detect hepatitis E virus infection in 28 European Union/European Economic Area (EU/EEA) countries, 2015

Country	IgM ELISA			IgM Western blot			IgG ELISA			IgG Western blot			PCR Serum			PCR Stool			Other
	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	Acute	Chronic	Test[a]	EM
Ireland	Y	N	Fortress	N	N	–	Y	N	Fortress	N	N	–	Y	Y	RealStar HEV RT-PCR, Altona	Y	N	RealStar HEV RT-PCR, Altona	N
Italy	Y	N	Wantai	N	N	–	Y	N	Wantai	N	N	–	Y	N	RealStar HEV RT-PCR, Altona	N	N		N
Latvia	Y	–	recomWell HEV IgM, Mikrogen; HEV IgM Kit, EIAgen Adaltis	N	–	–	Y	–	recomWell HEV IgG, Mikrogen; HEV IgG Kit, EIAgen Adaltis	N	–	–	Y	–	Geno-Sen's HEV Real Time PCR Kit, Genome Diagnostics	N	–	–	N
Lithuania	N	–	–	Y	–	Mikrogen	N	–	–	Y		Mikrogen	N	N	–	N	N	–	N
Luxembourg	Y	N	Mikrogen	N	–	–	Y	N	Mikrogen	N	–	–	N	–	–	N	–	–	N
The Netherlands	N	N	–	Y	N	–	N	N	–	N	N	–	Y	Y	–	Y	Y	–	N
Norway	Y	Y	IgM, Wantai	N	N	–	Y	Y	IgG, Wantai	N	N	–	Y	Y	RealStar HEV RT-PCR, Altona	N	N	–	N
Poland	Y	–	–	Y		–	Y	–	–	Y	–	–	N	–	–	N	–	–	N
Portugal	Y	–	–	N		–	Y	–	–	N	–	–	Y	–	–	Y	–	–	N
Romania	N	N	–	N	N	–	N	N	–	N	N	–	N	N	–	N	N	–	N
Slovakia	Y	N	–	Y	N	–	Y	N	–	Y	N	–	–	–	–	–	–	–	–
Slovenia	N	Y	–	Y	Y	–	N	Y	–	Y	Y		Y	Y	–	Y	Y	–	–
Spain	Y	Y	DSI	Y	Y	Mikrogen	Y	Y	DSI	Y	Y	Mikrogen	Y	Y	In-house PCR	Y	Y	In-house PCR	N
Sweden	Y	Y	Mikrogen; DiaPro; Wantai	N	N	–	Y	Y	Mikrogen; DiaPro; Wantai	N	N	–	Y	Y	In-house PCR	Y	Y	In-house PCR	N
UK - E+W	Y	Y	Wantai; Mikrogen	N	N	–	Y	Y	Wantai; Mikrogen	N	N	–	Y	Y	In-house qPCR; commercial assays	Y	Y	In-house qPCR	N
UK - S	N	N	Mikrogen	N	N	–	N	N	Mikrogen	N	N	–	Y	Y	In-house PCR	Y	N	In-house PCR	N
UK - NI	N	–	–	N	–	–	N	–	–	N	–	–	N		–	N	–	–	N

–: not available; ELISA: enzyme-linked immunosorbent assay; EM: electron microscope; E + W: England and Wales; HEV: hepatitis E virus; IgG: Immunoglobulin G; IgM: Immunoglobulin M; N: no; NI: Northern Ireland; PCR: polymerase chain reaction; qPCR: quantitative polymerase chain reaction; RNA: ribonucleic acid; RT-PCR: reverse transcriptase-polymerase chain reaction; S: Scotland; Y: yes.

[a] Assay type and brand name are separated by comma, brand names are separated by semicolon. Brand names: EIAgen Adaltis (Guidonia Montecelio, Italy); Altona diagnostics (Hamburg, Germany); Ceeram (La Chapelle sur Erdre, France); DiaPro Diagnostic BioProbes (Sesto San Giovanni, Italy); DSI Diagnostic System Italy (Saronno, Italy); Euroimmun (Lübeck, Germany); Fortress diagnostics (Antrim, the United Kingdom); Genome Diagnostics (Nijmegen, the Netherlands); Mikrogen (Neuried, Germany); Radim (Rome, Italy); Roche (Penzberg, Germany); Wantai (Beijing, China).

gradual decrease in proportion of total cases hospitalised between 2005 and 2015 may suggest better outpatient diagnosis and an increase in the sensitivity of the diagnostic system, i.e. a change in testing practice from hospitals to community settings. For cases where clinical information was recorded, every second case was admitted to hospital and there were two to eight deaths associated with HEV infection annually during 2011–2015. These figures appear high for a disease that has previously been considered to be a mild self-limiting infection [22]. However, a testing bias and overestimation of the hospitalisation rate can be assumed because of a higher likelihood that cases of severe liver disease or other comorbidities are tested for HEV. Notably, only four countries were able to provide data on morbidity status since 2005 and only five were able to provide data on case fatality since 2005, although nearly half of the EU/EEA countries indicated that they are able to collect this information as of 2015. More data are needed to address the lack of knowledge in this area.

As our results show, the number and proportion of autochthonous HEV infections has been increasing since 2005. Only a very small number of cases were known to be related to travel outside of the EU/EEA. Although genotype data were not requested from countries, it is known that autochthonous cases tend to be due to HEV genotype 3, the same genotype present in European pigs and consumed pork products; travel-related cases would be expected to be infected with genotype 1 or 4 [2-5]. Lately, there appears to have been a change in circulating subtypes in Europe. For example, the number of subtype 3c recently exceeded the numbers of subtypes 3e, f and g in humans in England and Wales [21]. Such changes may be contributing to the emergence of HEV as an important infection in humans. Changes in the pattern of food preparation and consumption may also be implicated. A case–control study in England and Wales identified pork sausages and ham purchased in supermarkets as possible sources of infection, raising concerns about whether current practice in preparing these products is sufficient to prevent HEV infection [23]. More studies among humans, animal populations and on food are essential to informing on possible sources of HEV infection.

The main limitations of this study are the variation in the denominator data for reported cases of hepatitis E (most countries were only able to provide a subset of demographic information or a limited number of years of data) and the merging of data on reported cases of hepatitis E despite the different case definitions and testing approaches used by EU/EEA countries. These limitations have in part been addressed by showing data by year of surveillance introduction and by limiting the denominator to cases where the relevant information was known. The varying number of countries providing data inconsistently over the study period prompted us not to perform any trend analysis to avoid further introduction of bias. In the future these limitations could be addressed by standardisation of surveillance systems for HEV infection, harmonisation of the case definition as well as testing algorithms across EU/EEA countries. This would enable better assessment of the burden of disease due to HEV in Europe and facilitate a better understanding of the actual number of performed tests or population coverage as a denominator, thus avoiding diagnostic and surveillance artefacts.

In summary, EU/EEA countries are at different stages in their surveillance and testing capacity for HEV, and there is no standardised European case definition. For the time period 2005–2015, at least 22 countries were able to report on cases infected with HEV, either through formal surveillance or existing systems of laboratory notifications. WHO's global health sector strategy on viral hepatitis asks countries to establish surveillance for viral hepatitis, particularly in blood donors [24]. Our study describes the availability of general hepatitis E surveillance in most EU/EEA countries. These data demonstrate a Europe-wide increase in reported cases, as well as a high number of reported HEV-related hospitalisations and a total of 28 deaths, between 2005 and 2015. They also provide a better understanding of the epidemiology and burden of HEV as an emerging cause of liver-related morbidity in EU/EEA countries.

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Conflict of interest

No conflict of interest declared.

Authors' contributions

All authors contributed to the research article and approved the final version.

Contributions of authors: EJA contributed to study design, analysed the data and drafted the manuscript. EC, MF, BS and SI provided country data, were involved in data analysis, contributed to writing, and reviewed and approved the manuscript. LT contributed to study design and provided expert review of the manuscript. JT supported the survey process and reviewed the manuscript. CA provided lead supervision of the project, analysed the data and provided expert review of the manuscript. The country experts provided their national data and expert review of the manuscript.

References

1. Said B, Ijaz S, Kafatos G, Booth L, Thomas HL, Walsh A, et al. Hepatitis E outbreak on cruise ship. Emerg Infect Dis. 2009;15(11):1738-44. DOI: 10.3201/eid1511.091094 PMID: 19891860
2. Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis. 2010;202(6):825-34. DOI: 10.1086/655898 PMID: 20695796
3. Riveiro-Barciela M, Mínguez B, Gironés R, Rodriguez-Frías F, Quer J, Buti M. Phylogenetic demonstration of hepatitis E infection transmitted by pork meat ingestion.J Clin Gastroenterol. 2015;49(2):165-8. DOI: 10.1097/MCG.0000000000000113 PMID: 24637729
4. Nelson KE, Kmush B, Labrique AB. The epidemiology of hepatitis E virus infections in developed countries and among immunocompromised patients.Expert Rev Anti Infect Ther. 2011;9(12):1133-48. DOI: 10.1586/eri.11.138 PMID: 22114964
5. Lapa D, Capobianchi MR, Garbuglia AR. Epidemiology of Hepatitis E Virus in European Countries.Int J Mol Sci. 2015;16(10):25711-43. DOI: 10.3390/ijms161025711 PMID: 26516843
6. Koot H, Hogema BM, Koot M, Molier M, Zaaijer HL. Frequent hepatitis E in the Netherlands without traveling or immunosuppression.J Clin Virol. 2015;62:38-40. DOI: 10.1016/j.jcv.2014.11.020 PMID: 25542468
7. Adlhoch C, Avellon A, Baylis SA, Ciccaglione AR, Couturier E, de Sousa R, et al. Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. J Clin Virol. 2016;82:9-16. DOI: 10.1016/j.jcv.2016.06.010 PMID: 27393938
8. Ijaz S, Vyse AJ, Morgan D, Pebody RG, Tedder RS, Brown D. Indigenous hepatitis E virus infection in England: more common than it seems.J Clin Virol. 2009;44(4):272-6. DOI: 10.1016/j.jcv.2009.01.005 PMID: 19217345
9. Pischke S, Behrendt P, Bock CT, Jilg W, Manns MP, Wedemeyer H. Hepatitis E in Germany--an under-reported infectious disease.Dtsch Arztebl Int. 2014;111(35-36):577-83.PMID: 25249359
10. Chalupa P, Vasickova P, Pavlik I, Holub M. Endemic hepatitis E in the Czech Republic.Clin Infect Dis. 2014;58(4):509-16. DOI: 10.1093/cid/cit782 PMID: 24280093
11. Holm DK, Moessner BK, Engle RE, Zaaijer HL, Georgsen J, Purcell RH, et al. Declining prevalence of hepatitis E antibodies among Danish blood donors. Transfusion. 2015;55(7):1662-7. DOI: 10.1111/trf.13028 PMID: 25819381
12. Wenzel JJ, Sichler M, Schemmerer M, Behrens G, Leitzmann MF, Jilg W. Decline in hepatitis E virus antibody prevalence in southeastern Germany, 1996-2011.Hepatology. 2014;60(4):1180-6. DOI: 10.1002/hep.27244 PMID: 24912687

13. Hogema BM, Molier M, Slot E, Zaaijer HL. Past and present of hepatitis E in the Netherlands.Transfusion. 2014;54(12):3092-6. DOI: 10.1111/trf.12733 PMID: 24889277
14. Tedder RS, Tettmar KI, Brailsford SR, Said B, Ushiro-Lumb I, Kitchen A, et al. Virology, serology, and demography of hepatitis E viremic blood donors in South East England. Transfusion. 2016;56(6pt2):1529-36. DOI: 10.1111/trf.13498 PMID: 26841005
15. Mansuy JM, Legrand-Abravanel F, Calot JP, Peron JM, Alric L, Agudo S, et al. High prevalence of anti-hepatitis E virus antibodies in blood donors from South West France. J Med Virol. 2008;80(2):289-93. DOI: 10.1002/jmv.21056 PMID: 18098159
16. Lucarelli C, Spada E, Taliani G, Chionne P, Madonna E, Marcantonio C, et al. High prevalence of anti-hepatitis E virus antibodies among blood donors in central Italy, February to March 2014. Euro Surveill. 2016;21(30):30299. DOI: 10.2807/1560-7917.ES.2016.21.30.30299 PMID: 27494608
17. World Health Organization (WHO). A Systematic Review on Hepatitis E Virus Globally. Geneva: WHO; 2014. Available from: http://www.who.int/immunization/sage/meetings/2014/october/7_summary_HEV_systematic_review.pdf
18. Hartl J, Otto B, Madden RG, Webb G, Woolson KL, Kriston L, et al. Hepatitis E Seroprevalence in Europe: A Meta-Analysis. Viruses. 2016;8(8):211. DOI: 10.3390/v8080211 PMID: 27509518
19. European Centre for Disease Prevention and Control (ECDC). Hepatitis E in the EU/EEA, 2005–2015.Stockholm: ECDC;Forthcoming.
20. Eurostat. Population (Demography, Migration and Projections), Population data, Database, 2015. Luxembourg: Eurostat. [Accessed 7 Oct 2016]. Available from: http://ec.europa.eu/eurostat/web/population-demography-migration-projections/population-data/database
21. Ijaz S, Said B, Boxall E, Smit E, Morgan D, Tedder RS. Indigenous hepatitis E in England and Wales from 2003 to 2012: evidence of an emerging novel phylotype of viruses.J Infect Dis. 2014;209(8):1212-8. DOI: 10.1093/infdis/jit652 PMID: 24273173
22. Sayed IM, Vercouter AS, Abdelwahab SF, Vercauteren K, Meuleman P. Is hepatitis E virus an emerging problem in industrialized countries?Hepatology. 2015;62(6):1883-92. DOI: 10.1002/hep.27990 PMID: 26175182
23. Said B, Ijaz S, Chand MA, Kafatos G, Tedder R, Morgan D. Hepatitis E virus in England and Wales: indigenous infection is associated with the consumption of processed pork products.Epidemiol Infect. 2014;142(7):1467-75. DOI: 10.1017/S0950268813002318 PMID: 24054519
24. World Health Organization (WHO). Global health sector strategy on viral hepatitis 2016–2021. Geneva: WHO. [Accessed 8 Jun 2017]. Available from: http://apps.who.int/iris/bitstream/10665/246177/1/WHO-HIV-2016.06-eng.pdf?ua=1
25. van der Poel WHM, Pavio N, van der Goot J, van Es M, Martin M, Engel B. Development and validation of a genotype 3 recombinant protein-based immunoassay for hepatitis E virus serology in swine.Braz J Med Biol Res. 2014;47(4):334-9. DOI: 10.1590/1414-431X20133249 PMID: 24676472
26. Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus.J Virol Methods. 2006;131(1):65-71. DOI: 10.1016/j.jviromet.2005.07.004 PMID: 16125257

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Research article

Dot map cartograms for detection of infectious disease outbreaks: an application to Q fever, the Netherlands and pertussis, Germany

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Geographical mapping of infectious diseases is an important tool for detecting and characterising outbreaks. Two common mapping methods, dot maps and incidence maps, have important shortcomings. The former does not represent population density and can compromise case privacy, and the latter relies on pre-defined administrative boundaries. We propose a method that overcomes these limitations: dot map cartograms. These create a point pattern of cases while reshaping spatial units, such that spatial area becomes proportional to population size. We compared these dot map cartograms with standard dot maps and incidence maps on four criteria, using two example datasets. Dot map cartograms were able to illustrate both incidence and absolute numbers of cases (criterion 1): they revealed potential source locations (Q fever, the Netherlands) and clusters with high incidence (pertussis, Germany). Unlike incidence maps, they were insensitive to choices regarding spatial scale (criterion 2). Dot map cartograms ensured the privacy of cases (criterion 3) by spatial distortion; however, this occurred at the expense of recognition of locations (criterion 4). We demonstrate that dot map cartograms are a valuable method for detection and visualisation of infectious disease outbreaks, which facilitates informed and appropriate actions by public health professionals, to investigate and control outbreaks.

Introduction

Here we propose a method of mapping infectious disease data, the dot map cartogram, which displays the geographical locations of reported cases from routine surveillance or outbreak investigations, such that public health experts can visualise both absolute numbers and spatial trends in incidence of infection. The method is developed to address two major causes of misinterpretation in commonly used maps: masking patterns of disease by not taking into account the population density distribution, and masking patterns by categorisation of information (across space and incidence of disease). With the dot map cartogram, we address the problem, raised by a recent systematic review [1], that spatial methods are underutilised and used in only ca 0.4% of all published outbreak investigations.

The two most frequently used map types for spatial outbreak data are the dot map and choropleth incidence map [1]. In a dot map, every case is represented by a point on the map, showing absolute numbers of cases. Illustrating absolute numbers of cases reveals the size of the outbreak and identifies areas without cases. Dot maps do not account for the underlying geographical distribution of the population. As populations are usually heterogeneously distributed, important variations in incidence of infection can be masked. Another drawback of dot maps is that they may reveal too much information about the location of specific cases, by which the privacy of a case might be violated.

In a choropleth map, a quantitative attribute is displayed per spatial unit. For example, ordinal classes of incidence may be displayed by municipality, with the areas shaded according to their incidence value and a range of shading classes [2]. Choropleth maps are mostly used to display surveillance data. They are less suitable to display outbreak data when the interest is in exact locations of cases. Although this type of map gives a quick overview of where there are more cases than expected based on the background population, it has a major limitation: The appearance of this map is heavily influenced by arbitrary choices with regard to the classification system for the ordinal classes and the spatial unit used (the latter is also referred to as the Modifiable Areal Unit Problem, MAUP [3]). Because the data are categorised both across the quantitative attribute and across the geographical units, this can lead to a loss of information by masking important

internal patterns and variations. Therefore, this type of map can be misleading. This is especially problematic when mapping rare events, which is often the case with infectious diseases.

Here we combine the advantages of both maps into one map type which we call dot map cartogram. This map type uses a diffusion-based algorithm [4], which creates contiguous or value-by-area cartograms. In these value-by-area cartograms, regions are enlarged or reduced relative to the number of individuals they contain. We apply this principle to traditional dot maps: we deform the map contours based on population size and simultaneously deform the dot pattern. In this way, a dense point pattern in a big city will become more dispersed, whereas a dense point pattern in a rural area will become even more dense. A similar technique called density-equalising map projection (DEMP) has been pioneered before to describe the spatial distribution of cryptosporidiosis among AIDS patients in San Francisco [5]. This earlier study used a different density-equalising algorithm, and application was limited to local outbreak data. This method has the advantages that the density of the dots reflects the incidence, the number of dots represent the absolute number of cases, and arbitrary choices for scale of spatial unit and classification system are avoided.

In this study, we assessed whether the advantages of the dot map cartogram outweigh the potential disadvantages. One potential drawback is that the dot map cartogram may reveal too much information with regard to the privacy of the cases. Another potential drawback is that the spatial distortion makes it hard to recognise or localise the map topology. We created dot map cartograms using real life data and compared them to the traditional choropleth and dot maps regarding four criteria: (i) ability to show both absolute numbers and incidence of the disease, (ii) sensitivity to choices regarding spatial scale and classification system, (iii) ability to ensure the privacy of individual cases, and (iv) ability to recognise locations. The comparison was applied to the mapping of a point-source outbreak and to the mapping of the occurrence of a human-to-human transmissible disease.

Methods

Data

As an example of outbreak mapping to locate a source, we used data from the Q fever outbreak in the Netherlands in 2009. Q fever case reports in the Netherlands communicable disease notification system (Osiris) include the 4-digit postal code of residence. Cases with date of onset of illness in 2009 (n = 1,740) were extracted for this analysis. Population data and shapefiles (a data format which stores geometrical locations and metadata such as population size per geometrical location) on different spatial levels (4-digit postal code and municipality level) were accessed via Statistics Netherlands [5].

As an example of a human-to-human transmissible disease, we used pertussis notifications in Germany in 2015. Data on pertussis cases was made available by the Robert Koch Institute through the SurvStat@RKI 2.0 web portal [6], in which the district of residence is registered for every case. We extracted data on laboratory-confirmed pertussis cases with a date of diagnosis in 2015 (n = 9,017). The most recent population data and shapefiles for district and federal state level boundaries (2013) were accessed through the open data portal of the Federal Agency for Cartography and Geodesy Germany [7]. We assumed that the population size per district or federal state in 2015 was sufficiently similar to that in 2013.

Geographical representation

Dot map

In a dot map, every case is represented by a dot on their location of residence. As this would reveal the exact locations of the cases and harm their privacy, we chose to use a proxy location by assigning a random point location in the spatial unit of residence (4-digit postal code for the Netherlands and district for Germany) to every case. The 4-digit postal code areas in the Netherlands have a mean population of 4,119 persons (mean surface: 8.6 km2), and the districts in Germany have a mean population of 200,914 persons (mean surface: 888.7 km2). For the map of the Netherlands we have used the national RD ('rijksdriehoeksstelsel')-based projection and for Germany we used the Universal Transverse Mercator (UTM) 32 projection. Both are conformal map projections in which local angles are preserved and shapes are represented accurately and without distortion for small areas.

Choropleth incidence map

In a choropleth incidence map, disease incidence is displayed per spatial unit, using ordinal classes. We chose two spatial unit levels for each country: one at the same level as the patient data (spatial unit of residence as described above) and the other one level higher (municipality in the Netherlands or federal state in Germany). In addition, we categorised incidence into five ordinal classes, using two separate classification systems: the Jenks' natural breaks algorithm [8] which seeks to reduce the variance within classes and maximise the variance between classes, and the quantile method in which equal numbers of spatial units are placed into each class [8]. The colour schemes for the incidence classes were based on previously established map colour palettes [9].

Dot map cartogram

Basic principle

We created cartograms by reshaping the spatial units such that their area was proportional to their population by applying the Gastner-Newman diffusion-based algorithm [4] without changing the underlying map

topology [10]. The principle is that once the areas have been scaled to be proportional to their population, then population density is by definition the same for each area of visually the same size on the cartogram. To create dot map cartograms, the point patterns of cases (as described in the dot map section) were simultaneously transformed in the reshape process of the spatial units so that the number of cases per unit area reflected the incidence. In addition, to provide points of reference to interpret the dot map cartograms, capital municipality, provincial or state boundaries were simultaneously transformed, and an inset was added with the undistorted map. We used ScapeToad 1.2 software [11] to reshape the spatial units and the point patterns. From this programme, the transformed layers were exported as shapefiles. The exported files were imported into R statistical software to create the maps. The main R package used to create the maps was *ggplot2*. The R code for the construction of the dot map cartogram is provided elsewhere (https://github.com/lsoetens/DotMapCartogram).

Cartogram quality

As we used spatial units with a population size at a certain spatial level to deform the original map, the MAUP problem was inherited. However, the consequences of this problem can be reduced by relying on an objective measure to assess which spatial level we could best use for our transformations. We assessed the cartogram quality by the objective measures, i.e. the average and maximum normalised cartographic error [10]. The cartographic error is the most commonly used measure for distortion in the value-by-area realisation. We assumed an input map *M* that is partitioned into *n* regions with polygonal boundaries. For each region *v*, *a(v)* denotes the area of *v* in *M* and the weight *w(v)* is the desired area for the region based on the background population. The diffusion-based algorithm constructed the cartogram *Mo*, that is a deformation of *M*, in which *o(v)* is the observed area of region *v*. The average normalised cartographic error *e* was calculated as:

Formula 1

$$e = \frac{1}{n} \sum_{v \in V} \frac{|o(v) - w(v)|}{\max\{o(v), w(v)\}}$$

with a range of [0,1]

and the maximum normalised cartographic error *x* was calculated as:

Formula 2

$$x = \max\left(\frac{|o(v) - w(v)|}{\max\{o(v), w(v)\}}\right)$$

with a range of [0,1]

No guidelines exist as to what constitutes acceptable cartogram quality; to be consistent with the values measured in a previous publication [10], we aimed for $e < 0.1$ and $x < 0.4$ in our cartograms. The performance of the algorithm, and subsequently the cartogram quality, depends on the distribution of the population density in the input file and the grid size on which the computation of the population density is based. Large discrepancies in the population density between regions impair the algorithm performance, while a finer grid will produce a higher quality cartogram, but may introduce distortion. We started with an input map at the same spatial unit of residence as the case data (i.e. 4-digit postal code for the Netherlands and district level for Germany) and assessed the average and maximum error of the cartograms. For the Netherlands, cartogram error was not satisfactory and we changed to the municipality level and accepted a maximum normalised cartogram error of $x > 0.4$; this was due to the very small population size of the municipalities on the West Frisian Barrier Islands. These areas should be smaller than presented in the created maps, but would no longer be visible. We used a 512 × 512 grid, which gave sufficient results; a higher-resolution grid of 1,024 × 1,024 is possible, but requires substantially greater computation times.

User requirements

To create the dot map cartograms, no specific geographical information system skills are needed. The only requirement is some basic knowledge of the R statistical software to be able to run the provided code (https://github.com/lsoetens/DotMapCartogram). Required software includes R studio and ScapeToad [11]. Both programmes are open source programmes, which can be downloaded for free. A detailed manual for creating the dot map cartograms is provided along with the code (https://github.com/lsoetens/DotMapCartogram).

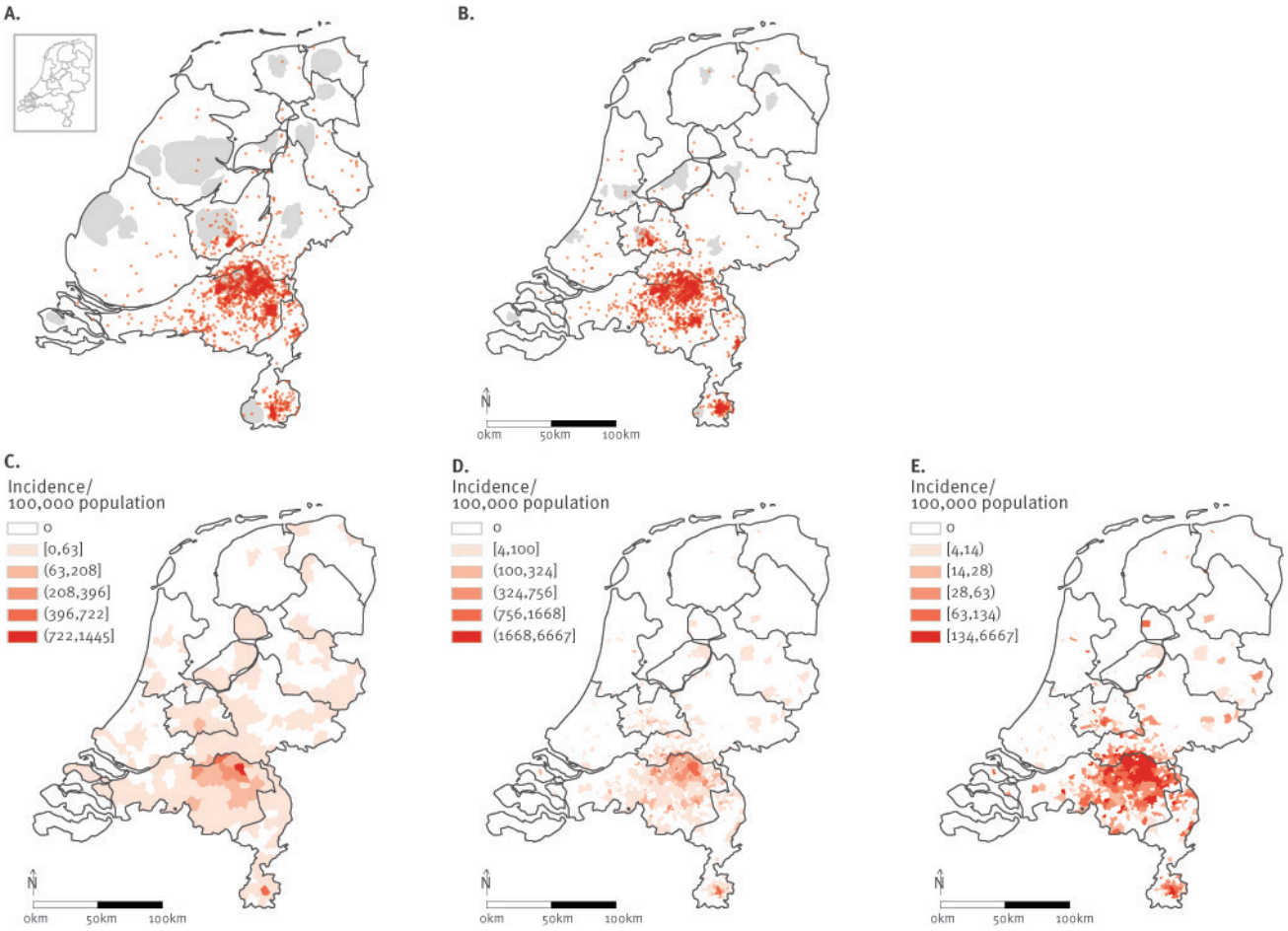
Results

Geographical representation of point-source outbreaks: Q fever in the Netherlands

The maps of Q fever cases are compared in Figure 1. The dot map cartogram (Figure 1A) is based on the

Figure 1

Spatial mapping of the Q fever outbreak in the Netherlands, 2009



A. Dot map cartogram. One red dot represents one case, shaded grey areas indicate the national and provincial capital municipalities. The geographical administrative units displayed as underlying layer are distorted as a result of building the cartograms.

B. Dot map. One red dot represents one case, shaded grey areas indicate the national and provincial capital municipalities.

C. Choropleth incidence map by municipality and Jenks' natural breaks classification system.

D. Choropleth incidence map by 4-digit postal code and Jenks' natural breaks classification system.

E. Choropleth incidence map by 4-digit postal code and the quantile classification system.

population size per municipality in the Netherlands in 2009 ($e = 0.08$, sd = 0.09; $x = 0.78$). It is compared with the dot map (Figure 1B) and the choropleth incidence maps (Figures 1C-E).

Ability to show both absolute numbers and incidence

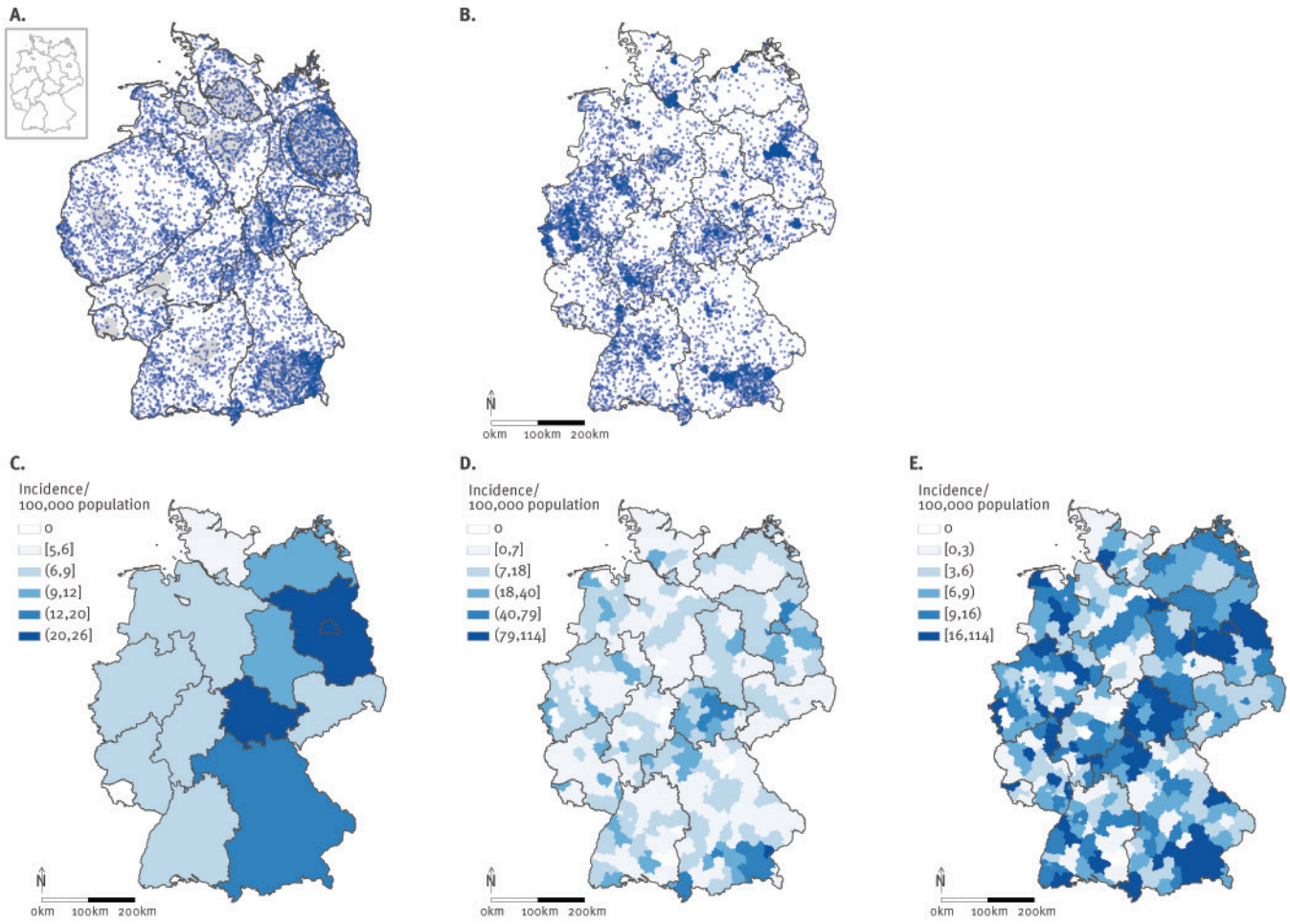
The dot map cartogram is able to show both absolute numbers and incidence. Incidence is readily inferred from the choropleth incidence maps, but the exact size of the outbreak in absolute numbers is not apparent. The dot map showed a clear clustering pattern with several hotspots, but whether this is related to the population density (incidence) cannot be inferred from the dot map.

Sensitivity to choices regarding spatial scale and classification system

The dot map cartogram and dot map do not suffer from arbitrary classification issues. The choropleth incidence maps are sensitive to this; different spatial scale and classification systems result in highly variable maps. When only one of those maps is used, the geography of the outbreak could be misinterpreted. One would conclude that the outbreak is more widespread

Figure 2

Spatial mapping of endemic pertussis in Germany, 2015



A. Dot map cartogram. One blue dot represents one case, shaded grey areas indicate the national and federal capital municipalities. The geographical administrative units displayed as underlying layer are distorted as a result of building the cartograms.

B. Dot map. One blue dot represents one case, shaded grey areas indicate the national and federal capital municipalities.

C. Choropleth incidence map by federal state and Jenks' natural breaks classification system.

D. Choropleth incidence map by district and Jenks' natural breaks classification system.

E. Choropleth incidence map by district and the quantile classification system.

(Figure 1C) or more intense (Figure 1E) than is illustrated in Figure 1D.

Ability to ensure the privacy of cases

The dot map compromises case privacy by revealing exact locations of cases, while the choropleth incidence map protects privacy by aggregating case information. The dot map cartogram meets this criterion partially by deforming the underlying region.

Ability to recognise locations

The dot map cartogram is harder to read, and specific locations are difficult to recognise. This can be improved by providing locations of major towns or district boundaries. The choropleth incidence map and dot map outperform the dot map cartogram on this criterion.

Geographical representation of a human-to-human transmissible disease: pertussis in Germany

The maps for pertussis cases are compared in Figure 2. Figure 2A depicts the dot map cartogram based on the population size by district in Germany in 2013 ($e = 0.07$, sd = 0.06; $x = 0.29$); this map is contrasted with the

corresponding dot map (Figure 2B) and choropleth incidence maps (Figures 2C-E).

Ability to show both absolute numbers and incidence

A much larger number of cases are illustrated in this example than for the Q fever data. This is immediately apparent from the dot map and the dot map cartogram, but less so from the choropleth incidence map. The clustering patterns on the dot map in several large cities and for example in the Ruhr district (in the west of Germany) disappear into an almost random pattern in the dot map cartogram, indicating that the clustering patterns on the dot map can be explained by the underlying population density in those areas. In contrast, in the south-east of Germany near Munich, a clustering pattern is present in both the dot map and the dot map cartogram, indicating that this cluster is not attributable to population density but reflects a higher incidence of pertussis notifications. Therefore, in this situation, both incidence and absolute number of cases are important in interpreting the map: they reveal the size of the problem and provide clustering of cases not attributable to the population density.

Sensitivity to choices regarding spatial scale and classification system

The choropleth incidence maps showed that the choice of classification system or spatial level results in highly variable maps. In this comparison, Figure 2D, based on the Jenks' natural breaks classification system and the district level, is comparable to the dot map cartogram, showing the same areas with high incidence; however, there is no way to determine a priori which classification system and spatial level would result in the 'right' map.

Ability to secure the privacy of cases

All maps meet this criterion. In this dataset, the dot map does not compromise privacy because of the frequency of occurrence of the disease. As before, the dot map cartogram protects case privacy due to the deformation of the underlying regions, and the choropleth incidence map because information is aggregated.

Ability to recognise locations

The choropleth incidence map and dot map outperform the dot map cartogram on this criterion. Adding reference points in the dot map cartogram, such as the federal state and federal state municipality boundaries, can help in recognising locations.

Discussion

We have proposed the dot map cartogram for displaying spatial infectious disease data and illustrating both incidence and absolute numbers of cases. A similar technique has been suggested before [12], but to our knowledge it was never used to study clustering of point patterns at a national level in infectious disease epidemiology. We compared the dot map cartogram to the frequently used choropleth incidence map and the dot map [1]. The main advantages of the dot map cartogram over the other two is that it is able to simultaneously reveal epidemic patterns adjusted for the population distribution, and to unmask patterns that are hidden by aggregation and categorisation of information. The visual distortion of the dot map cartogram is a barrier to pinpointing the location of a case: this is a benefit in the field of infectious diseases because case privacy should be ensured in presenting surveillance data. In addition, dot map cartograms illustrate areas without cases, which are harder to discern by the use of choropleth incidence maps.

We did not address map types other than dot maps and incidence maps for this comparison. Smooth incidence maps are an alternative to the choropleth incidence maps, in which the incidence is smoothed across the spatial units. This technique was applied to the data from a previous study for the Q fever outbreak in the Netherlands in 2009 [13]. In illustrating hotspots, the dot map cartogram is comparable to the smooth incidence map. The advantage of the smooth incidence map is that it permits identification of the exact location of hot spots, since the map is not deformed. However, smooth incidence maps do not reveal areas without cases and it is hard to discern the absolute number of cases and the true scope and size of the outbreak. Many more mapping techniques exist, such as map types based on other interpolation techniques (such as inverse distance weighting, kriging and trend surface fitting [2]), incidence based value-by-area cartograms [14,15], and many others. Assessment of all available methods was not within the scope of this paper.

We used the Gastner and Newman diffusion-based algorithm [4] to construct the cartogram. Alternative algorithms are available to construct contiguous cartograms [16-26]. We chose the Gastner and Newman algorithm because it performs well compared to the alternative algorithms in terms of quantitative measures such as the cartographic error, shape error and topology error [10], and qualitative measures such as subjective preferences and task performance [27]. Recent studies have proposed quantitative [10] and qualitative [27] measures for cartogram generation techniques. The development of standardised performance measures will allow objective ranking and selecting of cartogram algorithms.

With the demonstration of dot map cartograms, we provide public health professionals with an alternative spatial method for outbreak analysis. Firstly, we expect that dot map cartograms minimise misinterpretation of the data. Secondly, as demonstrated in this study, dot map cartograms protect the privacy of cases. Thirdly, dot map cartograms do not require expensive specialised GIS software, which facilitates use in settings with limited resources. The only requirements to construct dot map cartograms are free software, case reports with location data and access to population data as shapefiles. As there is a general trend for governments

to make administrative population data publicly available, the latter is available for most countries. Finally, our method can easily be extended with information on covariates relevant for mapping: a relevant example in infectious disease surveillance or research is to colour the case dots for a specific attribute, such as age group, sex or vaccination status. Introducing other layers of detail or attributes further broadens the utility of this spatial method for use in infectious disease research and surveillance. However, as the technique is graphical rather than geographical, it does not replace a geographical information system explaining the impact of various geographical factors on the spread of a certain infectious disease.

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Conflict of interest

None declared.

Authors' contributions

Authors LS, SH, and JW contributed to the design of the study. LS led on the data analysis and drafting of the manuscript supported by SH and JW. All authors commented on drafts of the manuscript and approved the final version.

References

1. Smith CM, Le Comber SC, Fry H, Bull M, Leach S, Hayward AC. Spatial methods for infectious disease outbreak investigations: systematic literature review.Euro Surveill. 2015;20(39):30026.
2. Heywood I, Cornelius S, Carver S. An introduction to geographical information systems. New Jersey: Prentice-Hall; 2011. ISBN: 9780273722595.
3. Openshaw S. Ecological fallacies and the analysis of areal census data.Environ Plann A. 1984;16(1):17-31. DOI: 10.1068/a160017 PMID: 12265900
4. Gastner MT, Newman ME. From The Cover: Diffusion-based method for producing density-equalizing maps.Proc Natl Acad Sci USA. 2004;101(20):7499-504. DOI: 10.1073/pnas.0400280101 PMID: 15136719
5. Wijk- en buurtkaart 2008. [District and neighborhood maps 2008]. Den Haag: Statistics Netherlands. [Accessed: 20 Jan 2016]. Dutch. Available from: http://www.cbs.nl/nl-NL/menu/themas/dossiers/nederland-regionaal/publicaties/geografische-data/archief/2009/2010-wijk-en-buurtkaart-2008.htm
6. SurvStat@RKI 2.0. Berlin: Robert Koch Institute. [Accessed: 3 Feb 2016]. Available from: https://survstat.rki.de/
7. Open Data - Freie Daten und Dienste des BKG. [Open data – free data and services of the BKG]. Leipzig: Federal Agency for Cartography and Geodesy (BKG). [Accessed: 15 Nov 2015]. German. Available from: http://www.geodatenzentrum.de/geodaten/gdz_rahmen.gdz_div?gdz_spr=deu&gdz_akt_zeile=5&gdz_anz_zeile=0&gdz_user_id=0
8. Brewer CA, Pickle L. Evaluation of methods for classifying epidemiological data on choropleth maps in series.Ann Assoc Am Geogr. 2002;92(4):662-81. DOI: 10.1111/1467-8306.00310
9. Harrower M, Brewer CA. ColorBrewer.org: An online tool for selecting colour schemes for maps.Cartogr J. 2003;40(1):27-37. DOI: 10.1179/000870403235002042
10. Alam MJ, Kobourov SG, Veeramoni S. Quantitative Measures for Cartogram Generation Techniques.Comput Graph Forum. 2015;34(3):351-60. DOI: 10.1111/cgf.12647
11. Andrieu D, Kaiser C, Ourednik A. ScapeToad: not just one metric. [Accessed: 20 Sep 2015]. Lausanne: Choros Laboratory, Available from: http://scapetoad.choros.ch/
12. Khalakdina A, Selvin S, Merrill DW, Erdmann CA, Colford JM. Analysis of the spatial distribution of cryptosporidiosis in AIDS patients in San Francisco using density equalizing map projections (DEMP).Int J Hyg Environ Health. 2003;206(6):553-61. DOI: 10.1078/1438-4639-00245 PMID: 14626902
13. van der Hoek W, van de Kassteele J, Bom B, de Bruin A, Dijkstra F, Schimmer B, et al. Smooth incidence maps give valuable insight into Q fever outbreaks in The Netherlands. Geospat Health. 2012;7(1):127-34. DOI: 10.4081/gh.2012.111 PMID: 23242690
14. Dodd PJ, Sismanidis C, Seddon JA. Global burden of drug-resistant tuberculosis in children: a mathematical modelling study.Lancet Infect Dis. 2016;16(10):1193-201. DOI: 10.1016/S1473-3099(16)30132-3 PMID: 27342768
15. Dorling D, Barford A, Newman M. WORLDMAPPER: the world as you've never seen it before.IEEE Trans Vis Comput Graph. 2006;12(5):757-64. DOI: 10.1109/TVCG.2006.202 PMID: 17080797
16. Tobler WR. Democratic representation and apportionment: a continous transformation useful for districting.Ann N Y Acad Sci. 1973;219:215-20. DOI: 10.1111/j.1749-6632.1973.tb41401.x PMID: 4518429
17. Dougenik JA, Chrisman NR, Niemeyer DR. An algorithm to construct continuous area cartograms.Prof Geogr. 1985;37(1):75-81. DOI: 10.1111/j.0033-0124.1985.00075.x
18. Gusein-Zade SM, Tikunov VS. A new technique for constructing continuous cartograms.Cartogr Geogr Inf Syst. 1993;20(3):167-73. DOI: 10.1559/152304093782637424
19. Keim DA, North SC, Panse C. CartoDraw: a fast algorithm for generating contiguous cartograms.IEEE Trans Vis Comput Graph. 2004;10(1):95-110. DOI: 10.1109/TVCG.2004.1260761 PMID: 15382701
20. Keim DA, Panse C, North SC. Medial-axis-based cartograms. IEEE Comput Graph Appl. 2005;25(3):60-8. DOI: 10.1109/MCG.2005.64 PMID: 15943089
21. Kämper JH, Kobourov SG, Nöllenburg M. Circular-arc cartograms. 2013 IEEE Pacific Visualization Symposium (PacificVis). Sydney; 2013. http://dx.doi.org/10.1109/PacificVis.2013.6596121
22. Dorling D. Area cartograms: their use and creation. In: The map reader: theories of mapping practice and cartographic representation. Dodge M, Kitchin R, Perkins C, eds. Chichester: John Wiley & Sons; 2011. p. 252-60. http://dx.doi.org/10.1002/9780470979587.ch33
23. Edelsbrunner H, Waupotitsch R. A combinatorial approach to cartograms.J. Comput. Geom.1997;7(5-6):343-60.
24. House DH, Kocmoud CJ. Continuous cartogram construction. Proceedings of the IEEE Visualization. Research Triangle Park; 18-23 Oct 1998. p. 197-204. https://dx.doi.org/10.1109/VISUAL.1998.745303
25. Schulman J, Selvin S, Merrill DW. Density equalized map projections: a method for analysing clustering around a fixed point.Stat Med. 1988;7(4):491-505. DOI: 10.1002/sim.4780070406 PMID: 3368676
26. Selvin S, Merrill D, Schulman J, Sacks S, Bedell L, Wong L. Transformations of maps to investigate clusters of disease.Soc Sci Med. 1988;26(2):215-21.
27. Nusrat S, Alam MJ, Kobourov SG. Evaluating cartogram effectiveness. IEEE Trans Vis Comput Graph. 2016. https://dx.doi.org/10.1109/TVCG.2016.2642109