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Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections

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We present a rigorously validated and highly sensitive confirmatory real-time RT-PCR assay (*1A* assay) that can be used in combination with the previously reported *upE* assay. Two additional RT-PCR assays for sequencing are described, targeting the *RdRp* gene (*RdRpSeq* assay) and *N* gene (*NSeq* assay), where an insertion/deletion polymorphism might exist among different hCoV-EMC strains. Finally, a simplified and biologically safe protocol for detection of antibody response by immunofluorescence microscopy was developed using convalescent patient serum.

Introduction

A novel human coronavirus, hCoV-EMC, has recently emerged in the Middle East region [1-3]. The virus has caused severe acute respiratory infection (SARI) in at least nine patients to date. Latest reports from the World Health Organization (WHO) suggest that infections have occurred since April 2012, as hCoV-EMC was found retrospectively in two patients from a group of 11 epidemiologically linked cases of SARI in Jordan, eight of whom were healthcare workers [4].

We have recently presented methods for the rapid detection of hCoV-EMC by real-time reverse transcription polymerase chain reaction (RT-PCR) [2]. One of these protocols, the *upE* gene assay, has been used as a first-line diagnostic assay for all human cases to date. More than 100 laboratories worldwide have since been equipped with positive-control material necessary to conduct the *upE* assay. We also presented a confirmatory RT-PCR assay targeting the open reading frame *(ORF) 1b* gene, with slightly lower sensitivity than the *upE* assay.

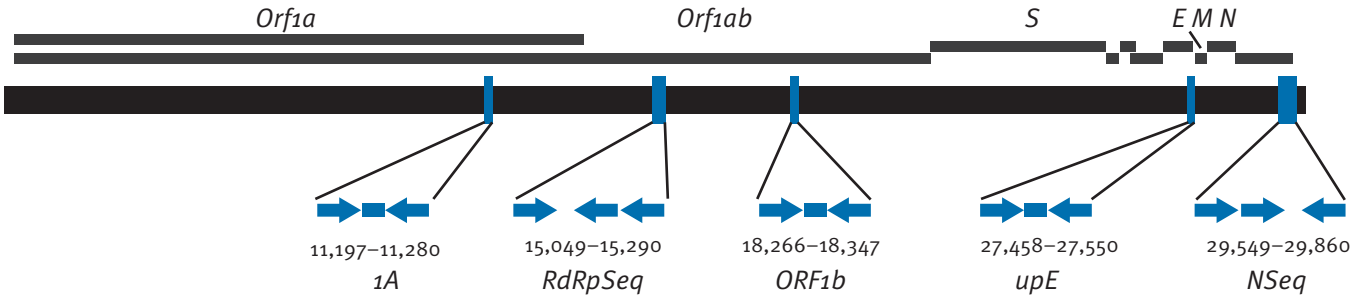
In view of the growing knowledge of the epidemiology of hCoV-EMC infections, WHO is continuously updating its guidelines for laboratory testing. During an expert consultation on 28 November 2012, it was concluded that first-line screening should involve the *upE* assay [2]. Confirmatory testing can involve any appropriately validated RT-PCR assay for alternative targets within the viral genome, followed by sequencing of at least a portion of one viral gene that can then be compared with hCoV-EMC sequences deposited in GenBank.

Recent investigations into a cluster of cases in Saudi Arabia have revealed the possibility that the virus may not be detected by RT-PCR in all patients with symptoms and proven epidemiological linkage [5]. From our previous experience during the severe acute respiratory syndrome (SARS) epidemic in 2003, such issues were predicted to occur when testing by RT-PCR alone [2]. In SARS patients, in particular those seen more than 10 days after symptom onset, serological testing by immunofluorescence assay (IFA) has been successfully used to complement RT-PCR findings [6,7].

On 22 November 2012, German health authorities were notified of a patient who had been treated for SARI in a hospital in Essen, Germany [5]. On the basis of clinical samples from this case, we present here a set of validated assays for the confirmation of cases of hCoV-EMC infection, including a confirmatory real-time RT-PCR assay in the *ORF1a* gene, two sequencing amplicons in the RNA-dependent RNA polymerase (*RdRp*) and nucleocapsid *(N)* protein genes, as well as a straightforward methodology for biologically safe immunofluorescence testing.

Figure 1

RT-PCR target regions for screening, confirmation and sequencing of novel human coronavirus (hCoV-EMC)



N: nucleocapsid; *Orf*: open reading frame; *RdRp*: RNA-dependent RNA polymerase; RT-PCR: reverse transcription-polymerase chain reaction.

The figure shows the relative positions of amplicon targets presented in this study, as well as in [2]. Primers are represented by arrows, probes as blue bars. Numbers below amplicon symbols are genome positions according to the hCoV-EMC/2012 prototype genome presented in [1].

The *1A* assay is the confirmatory real-time RT-PCR test presented in this study (target in the *ORF1a* gene). The *RdRpSeq* assay is a hemi-nested sequencing amplicon presented in this study (target in the *RdRp* gene). The *ORF1b* assay is a confirmatory real-time RT-PCR presented in [2]. The *upE* assay is a real-time RT-PCR assay recommended for first-line screening as presented in [2] (target upstrem of *E* gene). The *NSeq* assay is a hemi-nested sequencing amplicon presented in this study (target in *N* gene).

Methods

RT-PCR assays for the screening and confirmation of infections with hCoV-EMC

Figure 1 provides a summary of the target regions on the viral genome for screening, confirmation and sequence determination. Documentation on sources of materials used is provided in the Acknowledgements section.

RNA preparation

The procedures for RNA preparation have been described previously [2].

Confirmatory real-time RT-PCR assay in *ORF 1a* (*1A* assay)

A 25 µl reaction was set up containing 5 µl of RNA, 12.5 µl of 2 X reaction buffer from the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each dNTP and 3.2 mM $MgSO_4$), 1 µl of reverse transcriptase/Taq mixture from the kit, 0.4 µl of a 50 mM $MgCl_2$ solution (Invitrogen – not provided with the kit), 1 µg of non-acetylated bovine serum albumin (Sigma), 400 nM of primers EMC-*Orf1a*-Fwd (CCACTACTCCCATTTCGTCAG) and EMC-*Orf1a*-Rev (CAGTATGTGTAGTGCGCATATAAGCA), as well as 200 nM of probe EMC*Orf1a*-Prb (6-carboxyfluorescein (FAM)-TTGCAAATTGGCTTGCCCCCACT -6-carboxy-N,N,N,N´-tetramethylrhodamine (TAMRA)). Thermal cycling was performed at 55 °C for 20 min for the RT, followed by 95 °C for 3 min and then 45 cycles of 95 °C for 15 s, 58 °C for 30 s.

RT-PCR for generating amplicons for sequencing the *RdRp* gene target (*RdRpSeq* assay)

For the first round, a 25 µl reaction was set up containing 5 µl of RNA, 12.5 µl of 2 X reaction buffer from the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each dNTP and 3.2 mM $MgSO_4$), 1 µl of reverse transcriptase/Taq mixture from the kit, 0.4 µl of a 50 mM $MgSO_4$ solution (Invitrogen – not provided with the kit), 1 µg of non-acetylated bovine serum albumin (Sigma), 400 nM of each primer RdRpSeq-Fwd (TGC TAT WAG TGC TAA GAA TAG RGC; R=A/G, W=A/T) and RdRpSeq-Rev (GCA TWG CNC WGT CAC ACT TAG G; W=A/T, N=A/C/T/G). Thermal cycling was performed at 50 °C for 20 min, followed by 95 °C for 3 min and then 45 cycles of 95 °C for 15 s, 56 °C for 15 s and 72 °C for 30 s, with a terminal elongation step of 72 °C for 2 min.

In cases where no amplification products were obtained with the RT-PCR assay, a 50 µl second-round reaction was set up containing 1 µl of reaction mixture from the first round, 5 µl of 10 X reaction buffer provided with the Platinum Taq Polymerase Kit (Invitrogen), 2 µl of a 50 mM $MgCl_2$ solution (provided with the kit), 200 µM of each dNTP, 400 nM concentrations of each second round primer RdRpSeq-Fwd (the same as in the first round) and RdRpSeq-Rnest (CAC TTA GGR TAR TCC CAW CCC A) and 0.2 µl of Platinum Taq from the kit. Thermal cycling was performed at 95 °C for 3 min and 45 cycles of 95 °C for 15 s, 56 °C for 15 s and 72 °C for 30 s, followed by a 2 min extension step at 72 °C.

RT-PCR for sequencing in the *N* gene (*NSeq* assay)

The assay employed the same conditions as the *RdRpSeq* assay, except that the primer sequences were *NSeq*-Fwd (CCT TCG GTA CAG TGG AGC CA) and *NSeq*-Rev (GAT GGG GTT GCC AAA CAC AAA C) for the first round and *NSeq*-Fnest (TGA CCC AAA GAA TCC CAA CTA C) and *NSeq*-Rev (the same as in the first round) for the second round. The second round was only done if no product was visible by agarose gel electrophoresis after the first round.

Virus quantification by real-time RT-PCR using in-vitro transcribed RNA

In-vitro transcribed RNA was prepared as described previously [2]. Serial 10-fold dilutions of this RNA were amplified in parallel with samples in a Roche LightCycler 480II after entering the known RNA concentrations of standards in the quantification module of the operation software. Virus concentrations in terms of genome copies per ml of original sample were extrapolated using a conversion factor of 85.7, as explained previously [2].

Virus growth, infection and titration

Virus stocks of the clinical isolate hCoV-EMC/2012 (kindly provided by Ron Fouchier [1]) were grown on African green monkey kidney (Vero B4) cells. Cells were infected at a multiplicity of infection (MOI) of 0.01 and supernatants were harvested two days post infection. Titres were determined by plaque assay on Vero B4 cells as described previously [8].

hCoV-EMC antibody detection assays

Two IFAs have been developed.

(i) Conventional IFA

Vero cells were seeded onto glass coverslips in 24-well plates, grown to subconfluence, and infected at an MOI of 0.5. After 24 hours, cell monolayers were fixed with acetone [9].

(ii) Rapid, biologically safe IFA

Vero B4 cells in flasks were infected at an MOI of 0.01 and harvested two days post infection. Infected cells were mixed with non-infected Vero B4 cells (ratio 1:1) and spotted on glass slides by dispensing and immediately aspirating the cell suspension. The concentration of the cell suspension was 10e7 cells per ml in medium. The time between dispensing and back-aspiration was 2 seconds. About 6 wells could be loaded with the content of one 50 µl pipette tip. It was important for the success of cell spotting that the IFA slides used for the procedure should have undergone aggressive cleaning and autoclaving before use. After drying, the slides were fixed and virus inactivated with 4% paraformaldehyde for 30 minutes. Slides were immersed into ice-cold acetone/methanol (ratio 1:1) to permeabilise the cells. In the assay, patient sera (25 µl per dilution) were subjected to serial dilution in sample buffer (Euroimmun AG, Lübeck, Germany) starting at 1:40 and applied at 25 µl per well. As a positive control, a macaque-anti-hCoV-EMC (day 14 post infection), provided by author B. H. was used in a 1:20 dilution. Slides were incubated at 37 °C for 1 hour (rapid slides) or at room temperature for 30 minutes (conventional coverslips) and washed three times with phosphate-buffered saline (PBS)-Tween (0.1%) for 5 minutes. The secondary antibody was a goat-anti human Cy2-labelled immunoglobulin G conjugate. After incubation at 37 °C (spotted slides) or room temperature (conventional coverslips) for 30 minutes, they were washed three times with PBS-Tween for 5 minutes, rinsed with water and mounted with DAPI ProLong mounting medium (Life Technologies).

Recombinant assays for confirmatory IFA and western blot analysis

The hCoV-EMC/2012 spike (*S*) and *N* genes were amplified from cDNA. For PCR amplification of FLAG-tagged *N* and *S* and subsequent cloning into a pCG1 vector (kindly provided by Georg Herrler, TIHO, Hannover), the following primers were used: 2c-nhCoV-SflagN-BamHI-F (TACGGATCCGCCACCATGGATTACAAGGATGACGATGACAA GGGAGGCATACACTCAGTGTTTCTACTGATGT), 2c-nhCoV-S-SalI-R (AGCGTCGACTTAGTGAACATGAAC CTTATGCGG), 2c-nhCoV-NflagN-BamHI-F (TACGGATCCGCCACCATGGATTACAAGGATGACGATG ACAAGGGAGGCGCATCCCCTGCTGCACCTCGT) and 2c-nhCoV-N-XbaI-R (AGCTCTAGACTAATCAGTGTTAACATCAATCATTG).

For IFA, Vero B4 cells were transfected in suspension using 0.5 µg of plasmid DNA and the FuGENE HD protocol (Roche, Basel, Switzerland). Transfected cells were seeded into a 24-well plate containing glass coverslips. After 24 hours, cells were fixed with 4% paraformaldehyde, washed twice with PBS-Tween and permeabilised with PBS containing 0.1% Triton X-100. For western blot analysis of recombinant spike and nucleocapsid proteins, transfections were performed similarly but in six-well plates with HEK-293T cells using 2 µg of plasmid DNA. After 24 hours post-transfection, cells were washed three times with ice-cold PBS and harvested for western blot analysis. Cell lysis was performed with RIPA lysis buffer containing Protease Inhibitor Cocktail III (Calbiochem, San Diego, United States), 5mM DTT and nuclease (25 U/ml). Lysates from untransfected HEK-293T cells were used as controls. Patient serum was serially diluted 1:100 to 1:8,000 in PBS-Tween with 1% milk powder. Blot strips were incubated for 1.5 hours at room temperature. The secondary antibody, a horseradish peroxidase-conjugated goat-anti human immunoglobulin, was applied (1:20,000 in PBS-Tween with 1% milk powder). Detection was performed by using SuperSignal West Pico Chemiluminescence Substrate (Pierce Biotechnology).

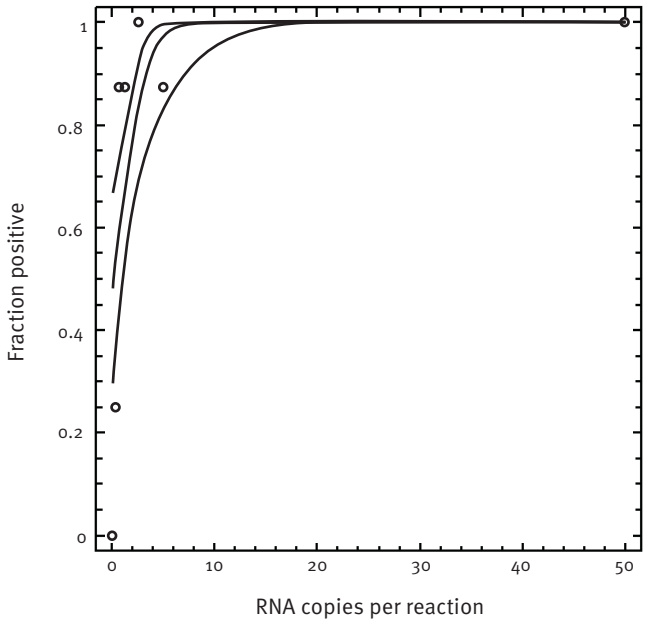
Results

1A assay

The *1A* RT-PCR assay is directed to the *Orf1a* gene: this was optimised for sensitivity by testing several

Figure 2

Technical limit of detection for the *1A* assay, novel human coronavirus (hCoV-EMC)



The *1A* assay is the confirmatory real-time RT-PCR test presented in this study (target in *ORF1a*).

Probit regression analysis using results from parallel runs of the *1A* assay containing very low concentrations of in-vitro transcribed hCoV-EMC RNA (between 50 and 0.3 average copies per reaction, 16 parallel determinations per datum point).

different candidate primers. The assay was compared with the *upE* assay by testing dilution series of the cell culture supernatant containing hCoV-EMC. There was complete concordance of the endpoints of the two assays. A total of 40 reactions using water instead of RNA were performed, in order to exclude any artificial signals due to irregular primer-/probe hybridisations. In-vitro transcribed RNA was generated for the peri-amplicon region of the *1A* assay and used for parallel end-point dilution testing and probit regression analysis. The target concentration at which >95% of *1A* assays can be expected to yield positive results was 4.1 RNA copies per reaction tube, i.e. a sensitivity equivalent to that of the *upE* assay ([2] and Figure 2). To exclude the possibility of false-positive results, human coronaviruses 229E, NL63, OC43, as well as SARS-CoV were tested in form of cell-culture supernatants in both assays (Table). A total of 42 clinical samples known to contain other respiratory viruses were tested as well, eight of which contained human coronaviruses including the unculturable hCoV-HKU1: all samples yielded negative results (Table).

For a final comparison of sensitivity, the *upE*, *ORF1b*, and *1A* assays were applied in parallel reactions to test a bronchoalveolar lavage sample from the patient treated in Essen, Germany. This sample had a very low RNA concentration of 360 copies per ml as determined with the *upE* assay using in-vitro transcribed RNA as the quantification standard [2]. The *upE* and *1A* assays consistently detected RNA in this sample in repeated tests. The concentration determined by the *1A* assay was between 66.5 and 100 copies per ml, reflecting

Table

Summary of experiments to determine sensitivity and cross-reactivity, novel human coronavirus (hCoV-EMC)

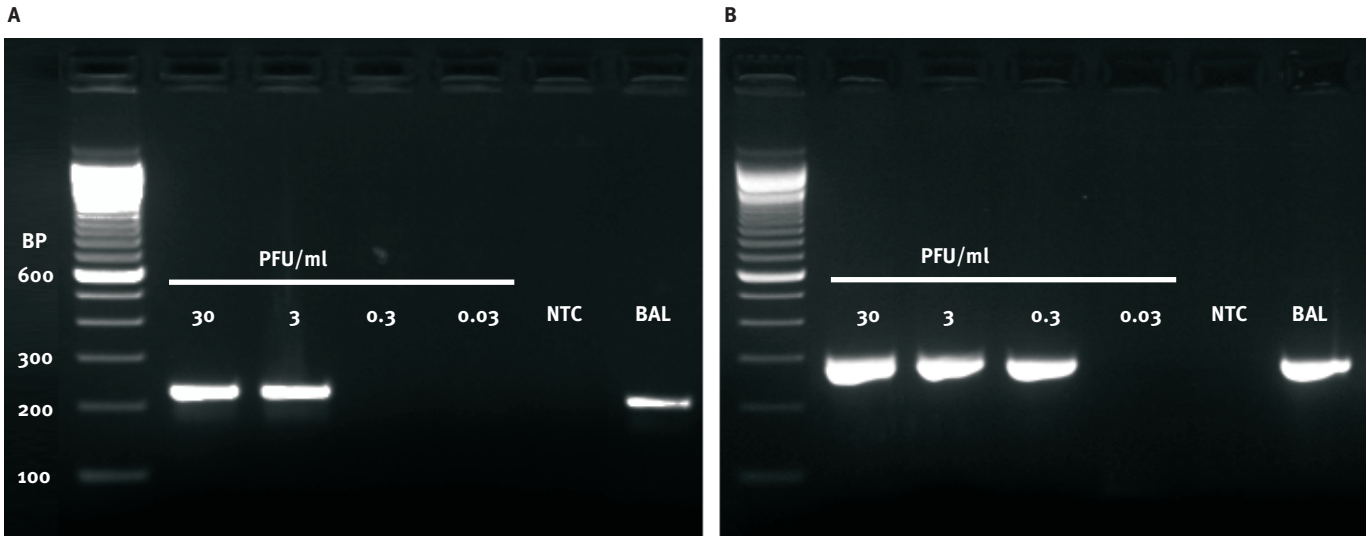
Experiment	*ORF1b* assay
Technical limit of detection[a]	4.1 RNA copies/reaction (95% CI: 2.8– 9.5)
Cross-reactivity with hCoV-229E	No reactivity with virus stock containing 10^5 PFU/ml (3×10^9 RNA copies/ml)
Cross-reactivity with hCoV-NL63	No reactivity with virus stock containing 10^6 PFU/ml (4×10^9 RNA copies/ml)
Cross-reactivity with hCoV-OC43	No reactivity with virus stock containing 10^4 PFU/ml (1×10^8 RNA copies/ml)
Cross-reactivity with SARS-CoV	No reactivity with virus stock containing 3×10^6 PFU/ml (5×10^{10} RNA copies/ml)
Cross-reactivity with clinical samples containing respiratory viruses	No reactivity with 42 samples containing the following viruses: hCoV-HKU1 (n=3 samples); hCoV-OC43 (n=1); hCoV-NL63 (n=3); hCoV-229E (n=1); human rhinovirus (n=2); enterovirus (n=4); human parechovirus (n=3); human metapneumovirus (n=4); respiratory syncytial virus (n=3); parainfluenza virus 1, 2, 3, 4 (n=7); influenza A virus (n=5); influenza B virus (n=2); adenovirus (n=4)

PFU: plaque-forming units.

[a] Defined as the novel human coronavirus (hCoV-EMC) RNA concentration at which >95% of parallel tests will return positive results.

Figure 3

Comparison of *RdRpSeq* and *NSeq* assays, novel human coronavirus (hCoV-EMC)



BAL: bronchoalveolar lavage; BP: base pairs; N: nucleocapsid; NTC: No template control; RdRp: RNA-dependent RNA polymerase; PFU: plaque-forming units; RT-PCR: reverse transcription-polymerase chain reaction.

RT-PCR amplification of sequencing fragments within the *RdRp* gene (panel A, *RdRpSeq* assay) and *N* gene (panel B, *NSeq* assay). Cell culture stock solutions of hCoV-EMC were diluted to the virus concentrations specified (in PFU per ml), of which 50 µl were extracted using the Qiagen Viral RNA mini kit and tested with both assays. The *NSeq* assay is more sensitive than the *RdRpSeq* assay. Both assays detected virus in a BAL sample from the Essen, Germany, patient.

slightly lower target abundance in the non-structural gene RNA, as observed previously for SARS-CoV [10]. Critically, the *ORF1b* assay presented in [2] did not detect virus in this sample.

RdRpSeq and *NSeq* assays

Two different RT-PCRs to produce amplicons for sequencing were designed. One amplicon was from the *RdRp* gene, a common target for CoV detection and a genome region where sequences for most coronaviruses are available (*RdRpSeq* assay, Figure 1). The assay was designed to provide broad detection of *Betacoronavirus* clade C sequences including hCoV-EMC as well as related viruses from animal sources such as bats (unpublished observations). The other amplicon was from a highly specific fragment within the hCoV-EMC *N* gene (*NSeq* assay, Figure 1). This region was chosen because it comprised a two amino acid (6 nt) deletion in the corresponding sequence published from a patient treated in London, United Kingdom [11]. As shown in Figure 3, both amplicons were sensitive enough to detect cell culture-derived virus at very low concentrations. Both assays also yielded amplification products from the bronchoalveolar lavage sample from the Essen patient, in spite of its very low RNA concentration. Sequencing results are shown in Figure 4.

hCoV-EMC antibody detection

Finally, slides for immunofluorescence microscopy were produced following two different common protocols. While the first method, growing cells on coverslips, provides better cell morphology, the second is commonly used to circumvent the necessity to optimise infection dose and duration, and to obtain slides with no infectious virus, to meet the biosafety requirements for shipment. For the first (conventional) protocol, Vero cells were seeded on microscope coverslips and infected with virus in situ. Infection conditions had been previously optimised to ensure infection of about 30% of cells in a series of experiments. For the second option, Vero cells were infected in conventional cell culture and mixed with an equivalent quantity of uninfected cells, after which they were spotted on glass microscope slides and further inactivated with paraformaldehyde. Both types of slides were stained with serum of a cynomolgus macaque infected with hCoV-EMC or with serum from the Essen patient. Figure 5, panel A, shows a typical coronavirus cytoplasmic fine-to-medium granular fluorescence with pronounced perinuclear accumulation, sparing the nucleus on the coverslip culture. The same result was also achieved with the convalescent serum from an experimentally infected cynomolgus macaque, suggesting that this

Figure 4

Sequence alignments comparing the results of *RdRpSeq* and *Nseq* sequencing assays, novel human coronavirus (hCoV-EMC) and sequence obtained from a patient from Essen, Germany

A

```
                          5          15         25         35         45
                          |          |          |          |          |
hCoV-EMC                  TCGCACTGTT GCAGGCGTGT CCATACTTAG CACAATGACT AATCGCCAGT
London sequence           .......... .......... .......... .......... ..........
Essen  sequence           .......... .......... .......... .......... ..........

                          55         65         75         85         95
                          |          |          |          |          |
hCoV-EMC                  ACCATCAGAA AATGCTTAAG TCCATGGCTG CAACTCGTGG AGCGACTTGC
London sequence           .......... .......... .......... .......... ..........
Essen  sequence           .......... .......... .......... .......... ..........

                          105        115        125        135        145
                          |          |          |          |          |
hCoV-EMC                  GTCATTGGTA CTACAAAGTT CTACGGTGGC TGGGATTTCA TGCTTAAAAC
London sequence           .......... .......... ...T...... .......... ..........
Essen  sequence           .......... .......... ...T...... .......... ..........

                          155        165        175
                          |          |          |
hCoV-EMC                  ATTGTACAAA GATGTTGATA ATCCGCATCT TA
London sequence           .......... .......... .......... ..
Essen  sequence           .......... .......... .......... ..
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B

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                          5          15         25         35         45
                          |          |          |          |          |
hCoV-EMC                  AATAAGTGGT TGGAGCTTCT TGAGCAAAAT ATTGATGCCT ACAAAACCTT
London sequence           .......... .......... .......... .......... ..........
Essen  sequence           .......... .......... .......... .......... ..........

                          55         65         75         85         95
                          |          |          |          |          |
hCoV-EMC                  CCCTAAGAAG GAAAAGAAAC AAAAGGCACC AAAAGAAGAA TCAACAGACC
London sequence           .......... .......... .......... .......... ..........
Essen  sequence           .......... .......... .......... .......... ..........

                          105        115        125        135        145
                          |          |          |          |          |
hCoV-EMC                  AAATGTCTGA ACCTCCAAAG GAGCAGCGTG TGCAAGGTAG CATCACTCAG
London sequence           .......... ......T... .....T.... .........- ----......
Essen  sequence           .......... ......T... .....G.... .........A GCATC.....

                          155        165        175        185        195
                          |          |          |          |          |
hCoV-EMC                  CGCACTCGCA CCCGTCCAAG TGTTCAGCCT GGTCCAATGA TTGATGTTAA
London sequence           .......... .......... .......... .......... ..........
Essen  sequence           .......... .......... .......... .......... ..........

                          205        215        225        235
                          |          |          |          |
hCoV-EMC                  CACTGATTAG TGTCACTCAA AGTAACAAGA TCGCGGCAAT C
London sequence           .......... .......... .......... .......... .
Essen  sequence           .......... .......... .......... .......... .
```

Panel A. Results from the *RdRpSeq* assay on the Essen patient.
Panel B. Results of the *Nseq* assay.
Dots represent identitical nucleotides, hyphens represent sequence gaps.

can be used as a valid positive control in absence of available patient material. Figure 5, panel B, shows results from two convalescent sera of the patient, taken about four weeks apart, on simplified biologically safe slides. As expected, the fluorescence pattern was less well differentiated compared with slides infected and tested in situ. However, a very clear cytoplasmic perinuclear pattern is discernible, suggesting those slides will be appropriate for diagnostic application in spite of their simpler production and safer handling.

Sera from a limited number of German blood donors were tested by this IFA assay, with no relevant false-positive findings in a non-exposed population. However, much more validation is needed, because antibodies against betacoronaviruses are generally known to cross-react within the genus. Sera from patients with a high antibody titre against any other human coronavirus such as OC43 or HKU1 may well lead to false-positive results if tested by IFA alone. We propose to use this IFA only for patients with a very clear epidemiological linkage, ideally presenting positive results with a first-line assay such as *upE*. Paired sera should be investigated wherever possible.

As shown in Figure 5, panel C, IFA reactivity was also demonstrated in cells overexpressing recombinant S or N proteins. Anti-S and anti-N antibodies were also confirmed by western blot.

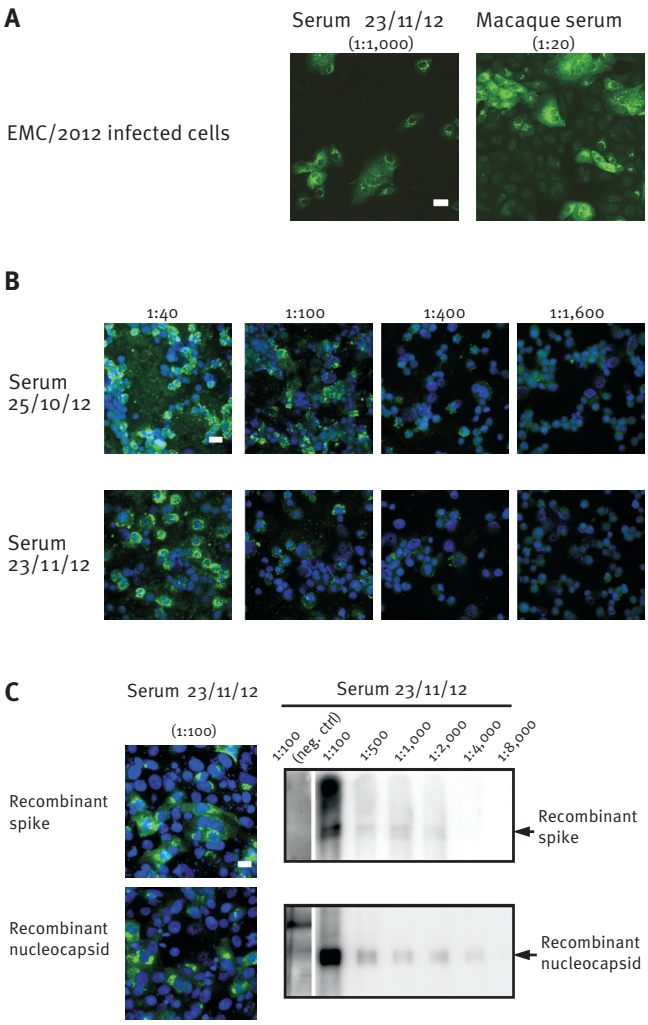
Discussion

Here we present nucleic acid-based and serological assays for the confirmation of hCoV-EMC infections. The current strategy and recommendations by WHO require reference laboratories to be involved in cases where first-line screening has provided positive results. However, with the potential occurrence of more cases of hCoV-EMC infection, the demand for confirmatory testing might grow in a way that it could overwhelm the capacity of reference laboratories. The major challenge in setting up confirmatory methodology will be the validation of tests. Technical studies can be tedious and clinical validation is hard to achieve if no patient samples are at hand. The documentation here of proven methodology is presented with those laboratories in mind that will have to provide diagnostic testing and additional reference services in the future, but cannot rely on their own validation studies.

The *1A* real-time RT-PCR assay provides the same sensitivity as the *upE* first-line assay, and should provide consistent results in case of truly positive patients. It should be mentioned that the *ORF1b* assay along with the *upE* assay can also serve as a highly robust confirmatory test [2]. However, patients may be seen at times when they excrete small amounts of virus, e.g. very early or very late after symptom onset [6]. Moreover, samples may be diluted due to clinical processes such as lavage, as exemplified by the case investigated here. In such instances, confirmatory assays must have the same sensitivity as the first-line

Figure 5

Examples of serological assays, novel human coronavirus (hCoV-EMC)



Panel A. Conventional immunofluorsecence assay (IFA) using cells grown and infected on coverslips. The patient serum from the later time point (23/11/12) was tested positive in a 1:1,000 dilution. As control, a serum of an hCoV-EMC/2012 infected macaque (taken 14 days post infection) was applied.

Panel B. Rapid/biologically safe immunofluorescence assay (IFA) slides. Mixed infected and non-infected Vero cells incubated with serially diluted sera from an hCoV-EMC-infected patient taken at two different time points post infection.

Panel C. IFA using Vero cells expressing recombinant spike and nucleocapsid proteins, as well as western blot against lysates from the same transfected cells.

Bars represent 20 µm.

test. Such high sensitivity is achieved by the *1A* assay, providing an appropriate complement to the *upE* assay proposed previously [2].

While real-time RT-PCR products can be sequenced, the shortness of their fragments makes DNA preparation inefficient and limits the length of useful sequence information. We present here two different sequencing amplicons (*RdRpSeq* and *NSeq* assays) that will yield reasonably large fragments even from samples containing very low virus concentration. We are not proposing to preferentially use either of those two assays, as both have different properties that suggest using them in combination. The *RdRpSeq* assay provides sequencing results that can be compared with a large database of cognate sequences, as it is commonly used for typing coronaviruses. The amplicon overlaps to a large extent with that proposed earlier by Vijgen et al. for pan-coronavirus detection, ensuring good comparability between laboratory results from different groups [12]. The primers of the *RdRpSeq* assay are highly conserved and will cross-react with other betacoronaviruses including hCoV-OC43 or -HKU1. Critically, this amplicon should not be used for screening if not connected with subsequent sequence analysis, as false-positive results are possible in patients infected with other human coronaviruses. In contrast, the *NSeq* assay provides highly sensitive and specific detection for hCoV-EMC, enabling a sequence-based confirmation even for cases that present with very low virus concentration. Here it is interesting to note that a sequence presented from a patient treated in London has a deletion in the amplified fragment. We should not draw early conclusions on virus diversity from these limited data, but it will be interesting to sequence and compare the *NSeq* fragment from more viruses in the future, in order to determine whether lineages with and without the deletion might have formed already. The *NSeq* assay might be used as a tool for provisional strain classification in the future.

For the augmentation of confirmatory testing by serology, IFA, ideally in paired sera taken several days apart, proved highly robust during the SARS epidemic [6,7]. In contrast to EIA, IFA provides additional criteria for result interpretation via the localisation of signals within cells. False-positive reactivity can thus be circumvented. The data presented here are intended as reference for those laboratories willing to confirm cases of hCoV-EMC infection by IFA. We have shown in this single patient that antibodies were detectable by IFA at a time when the patient still presented severe disease and the virus was not yet eliminated from respiratory secretions as detectable by RT-PCR (case report to be presented elsewhere). As in many SARS patients, the antibody titre was in the medium range, below 1:1,000, even in convalescence [6]. In SARS patients, IFA seroconversions usually began to show from day 10 of symptoms onward, while virus RNA could not be detected by RT-PCR in respiratory secretions starting from day 15 onward [6,7].

It is important to mention that IFA slides contain virus-infected cells which in theory could retain infectious virus. However, it has been shown in a meticulous investigation of SARS-CoV that acetone fixation of IFA slides results in the reduction of infectivity to undetectable levels. The extent of reduction of infectivity was at least 6.55 log 10 infectious virus doses [9] (greater reductions could not be measured by the assay applied). In the rapid and biologically safe IFA procedure we presented here, further reduction of any conceivable residues of infectivity was achieved by combining acetone fixation with paraformaldehyde treatment. This treatment was shown to confer efficient reduction on SARS-CoV [9] and is also effective against other enveloped RNA viruses [13]. No residual infectivity should exist in the rapid and biologically safe IFA slides described here.

We have also shown that there is good correlation between IFA results and western blot against the two major structural proteins, S and N. Western blotting might therefore be an option as a confirmatory diagnostic for serology. However, in absence of data from a considerably larger number of patients, care must be taken in interpreting the results from western blot alone, as SARS patients were found to vary in their immune responses against single proteins in western blot [14,15]. Not only western blot but also neutralisation tests should be evaluated for their capacity to afford a highly specific confirmation of serological results [7]. This is of particular importance because it is unknown to what extent hCoV-EMC antibodies cross-react with those against common human coronaviruses such as OC43 and HKU1. In the present study, we have not investigated cross-reactivity in a larger group of patients, as this requires meticulous counter-testing and selection of samples with high titres against other human coronaviruses, as well as confirmation by additional methods such as differential virus neutralisation tests. The serological data presented here should be regarded as suggestions for confirmatory testing of epidemiologically linked individuals, or of cases under investigation due to positive results in first-line tests.

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Oligonucleotides can be ordered from stock at Tib-Molbiol, Berlin (www.tib-molbiol.de). Limited numbers of IFA slides as well as in-vitro transcribed control RNA for the *upE* and *1A* assays can be acquired from author C. D. through the European Virus Archive platform (www.european-virus-archive.com), funded by the European Commission under contract number 228292. Further information and assay updates can be obtained from www.virology-bonn.de.

References

1. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-20.
2. Corman V, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill, 2012;17(39):pii=20285. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20285
3. Bermingham A, Chand M, Brown C, Aarons E, Tong C, Langrish C et al. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill. 2012; 17(40): pii=20290. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20290
4. ProMED mail. Novel coronavirus - Eastern Mediterranean: WHO, Jordan, confirmed, request for information . Archive Number: 20121130.1432498. Available from: http://www.promedmail.org/direct.php?id=20121130.1432498
5. ProMED mail. Novel coronavirus - Saudi Arabia (18): WHO, new cases, cluster, fatality. Archive Number: 20121123.1421664. Available from: http://www.promedmail.org/direct.php?id=20121123.1421664
6. Herzog P, Drosten C, Müller MA. Plaque assay for human coronavirus NL63 using human colon carcinoma cells. Virol J. 2008;5:138.
7. Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS coronavirus. Med Microbiol Immunol. 2005; 194(1-2): 1-6.
8. Kraus AA, Priemer C, Heider H, Kruger DH, Ulrich R, et al. Inactivation of Hantaan virus-containing samples for subsequent investigations outside biosafety level 3 facilities. Intervirology. 2005; 48(4): 255-61.
9. Drosten C Chiu LL, Panning M, Leong HN, Preiser W, Tam JS et al. Evaluation of advanced reverse transcription-PCR assays and an alternative PCR target region for detection of severe acute respiratory syndrome-associated coronavirus. J Clin Microbiol. 2004;42(5): 2043-7.
10. Health Protection Agency (HPA). Genetic sequence information for scientists about the novel coronavirus 2012. London: HPA; 2012. [Accessed 4 Nov 2012]. Available from: http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/NovelCoronavirus2012/respPartialgeneticsequenceofnovelcoronavirus/
11. Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet. 2003; 361(9371): 1767-72.
12. Vijgen L, Moës E, Keyaerts E, Li S, Van Ranst M, et al. A pancoronavirus RT-PCR assay for detection of all known coronaviruses. Methods Mol Biol. 2008; 454: 3-12.
13. Peiris JS, Yuen KY, Osterhaus AD, Stöhr K, et al. The severe acute respiratory syndrome. N Engl J Med. 2003; 349(25): 2431-41.
14. He Q, Chong KH, Chng HH, Leung B, Ling AE, Wei T, et al. Development of a Western blot assay for detection of antibodies against coronavirus causing severe acute respiratory syndrome. Clin Diagn Lab Immunol. 2004; 11(2): 417-22.
15. Tan YJ, Goh PY, Fielding BC, Shen S, Chou CF, Fu JL, et al. Profiles of antibody responses against severe acute respiratory syndrome coronavirus recombinant proteins and their potential use as diagnostic markers. Clin Diagn Lab Immunol. 2004; 11(2): 362-71.

Laboratory capability for molecular detection and confirmation of novel coronavirus in Europe, November 2012

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A rapid survey by the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) Regional Office for Europe ascertained the availability of national reference laboratory testing for a recently detected novel coronavirus as of 28 November 2012. Screening by internal quality controlled *upE*-RT-PCR assay was available in 23/46 of responding countries in the WHO European Region, of which 19/30 in European Union (EU) and European Economic Area (EEA) countries. Confirmation of positive screened samples by either *ORF1b*-RT-PCR, or other target RT-PCR assays with sequence analysis or whole-genome sequence analysis was available in 22/46 responding countries of which 18/30 in EU/EEA countries.

In September 2012, a novel coronavirus was first characterised by genome sequencing at the Erasmus Medical Center (EMC) of a viral isolate from a patient in Saudi Arabia with severe pneumonia [1-2]. This virus belongs to the genus beta-coronavirus and is closely related to some bat coronaviruses. Since then, a total of nine confirmed cases of human infection with the novel coronavirus have been reported to public health authorities and WHO [3-6]. These patients developed a severe respiratory disease in Saudi Arabia, Qatar and Jordan over the period April to November 2012 and five had a fatal outcome [1-6]. Two patients were referred to Europe for specialised care [1-6].

Coronaviruses are membrane enveloped viruses with large RNA genomes and a distinctive surface crown causing respiratory and enteric infections in humans and animals. In 2003, zoonotic transmission of SARS-CoV caused a worldwide epidemic associated with more than 8,000 cases of severe acute respiratory syndrome (SARS) and a case-fatality rate of 10% [7].

Monitoring of novel coronavirus

While the reservoir and mode of transmission of the novel coronavirus virus are under investigation, WHO and ECDC request that any probable or confirmed case that is diagnosed should rapidly (i) be reported to national authorities to enable them to take appropriate public health measures, and (ii) be notified to WHO under the International Health Regulations (2005) and simultaneously through joint reporting system to the Early Warning and Response System (EWRS) for countries in the EU/EEA [3,4,8].

In September 2012, Corman et al described the first diagnostic assays for the novel coronavirus [9]. These involve a two-step screening and confirmation testing algorithm using newly developed specific RT-PCR assays that target the regions upstream of the *E* gene (the *upE* target; recommended for screening) and open reading frame 1b (*ORF1b*; recommended for confirmation) [9]. Additional testing based on sequence analysis of other viral genome targets or whole genome sequence determination from clinical material or culture isolate can also be used to confirm cases [2,6].

A number of pan-coronavirus RT-PCR assays have been described which target the polymerase gene of coronaviruses used for the detection of known and unknown coronaviruses, including coronaviruses currently circulating in humans such as hCoV-229E, hCoV-NL63, hCoV-OC43 and SARS-CoV [10-12]. Some of these pan-coronavirus RT-PCR assays may be also used to detect the novel coronavirus. However, a positive result should be confirmed by screening for the specific targets described for hCoV-EMC, sequencing of the RT-PCR product and/or virus isolation [1,2,6].

Table

Availability of laboratory tests for detection and confirmation of novel coronavirus in European Union/ European Economic Area countries and the member states of the World Health Organization European region, per country, 28 November 2012

Experiment	Number of countries	
	EU/EEA countries (N=30)	WHO European Region (N=46)
Screening tests according to [8]		
No screening or confirmation test	7	17
upE RT-PCR (without positive control)	4	6
upE RT-PCR (with positive control)	19	23
Confirmation test according to [8])		
Confirmation of positive upE RT-PCR by ORF1b RT-PCR	14	17
Confirmation of positive upE RT-PCR by sequencing of ORF1b	13	15
Other confirmation tests		
Confirmation of positive RT-PCR by sequencing of pan-coronavirus RT-PCR product	10	11
Confirmation of positive RT-PCR by whole viral genome sequencing	7	8

EU/EEA: European Union/ European Economic Area; WHO World Health Organization

The rapid communication of technical protocols of validated diagnostic assays and distribution of positive RNA control material is essential to provide public health laboratories with the means to screen and confirm cases of this emerging viral disease and allow for appropriate public health response [4,8,13]. WHO is developing laboratory testing guidance on the detection of the novel coronavirus. To obtain background information for the updated guidance and to identify the needs for support by ECDC and WHO Regional Office for Europe to virology laboratories for case ascertainment across Europe, the capability of national laboratories was jointly investigated by questionnaire surveys.

Survey on novel coronavirus detection capabilities

Two coordinated surveys were administered by email from ECDC and the WHO Regional Office. The ECDC survey was sent to the National Microbiology Focal Points of the 30 EU/EEA countries and in the EU accession country Croatia and to contact points for laboratories in the European Network for Diagnostics of "Imported" Viral Diseases (ENIVD) [14], on 26 November 2012. The Regional Office survey was sent to EuroFlu National Focal Points in 53 countries in the WHO European Region on 26 November 2012, with a deadline set for 29 November. Some EU/EEA institutions belonging to more than one network received and returned both questionnaires. Reminders were sent to the networks after 29 November and an additional 10 replies, received by 3 December, were included in this report.

The ECDC survey questions covered five areas: national laboratories involved in testing for novel coronavirus, availability of laboratory tests and positive controls, laboratory tests under development, sampling strategy and test referral, and need for support. The Regional Office survey questions covered the following areas: confirmation if the WHO-recognised National Influenza Centre (NIC) or other laboratory serve as national reference laboratory for novel coronavirus testing, availability of laboratory tests and positive controls, and need for support.

Data were validated with request for clarification by the survey respondents on 3 December and confirmed data were received by 4 December 2012. Hereafter data from both surveys were merged, duplicate responses removed and validated data analysed jointly by ECDC and Regional Office.

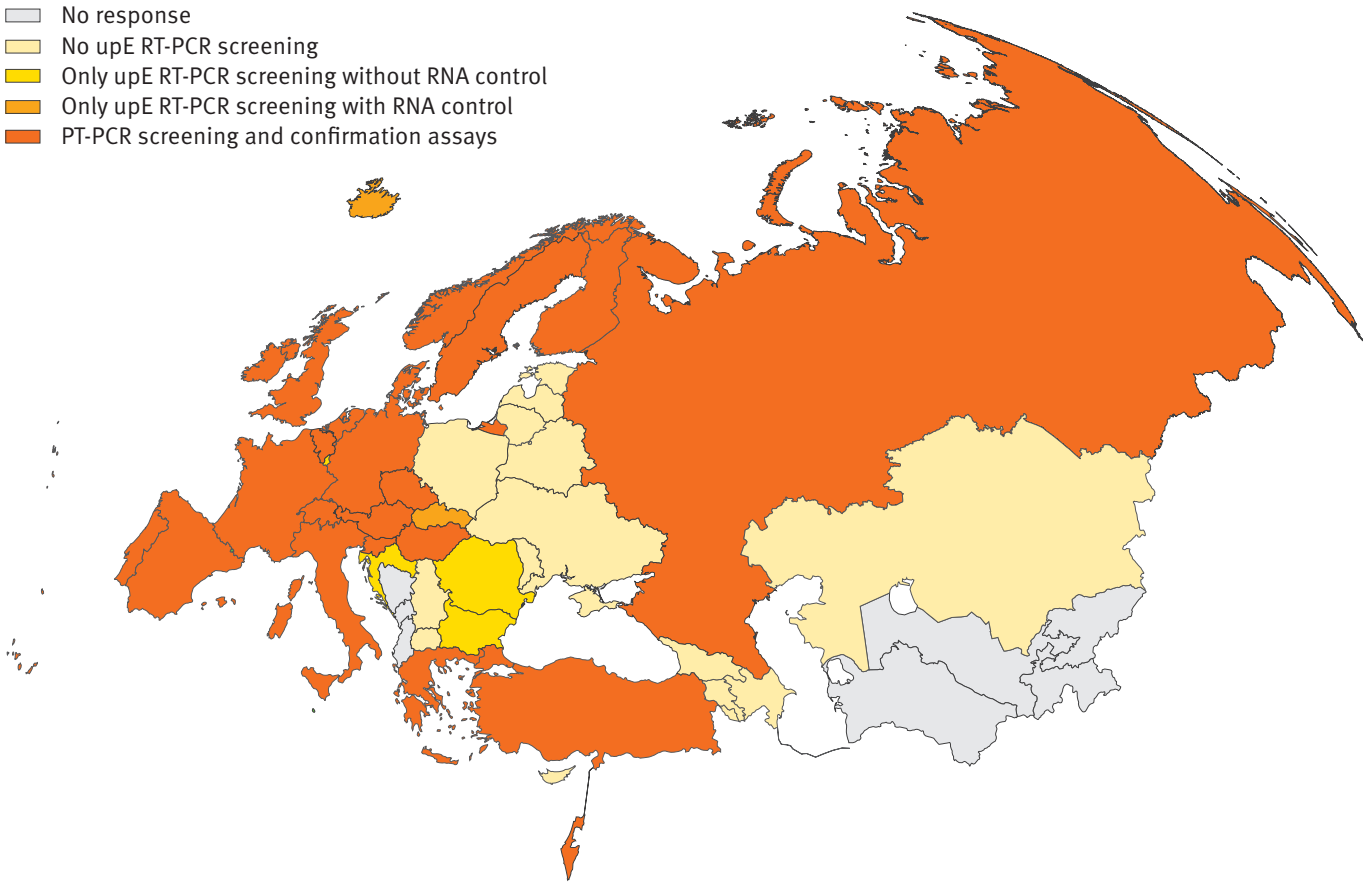
Survey results

The response rates were 93% (28/30 countries) for the EU/EEA countries included in the ECDC survey and 76% (40/53 countries) for the WHO Regional Office survey; taken together, the surveys captured data from 46/53 of WHO European Region Member States and all EU/EEA countries. In total, information was provided by 47 countries responding to one or both questionnaires.

The table indicates which molecular tests were available for novel coronavirus detection and confirmation at national reference or expert laboratory level at the time of the survey. According to recommendations [9,14] screening by internal quality controlled *upE*- RT-PCR assay was available in 23/46 of responding WHO European Region countries and 19/30 EU/EEA

Figure

Countries in the World Health Organization (WHO) European Region with capacity for screening and confirmation of novel coronovairus infection, 28 November 2012



countries. Confirmation of positive screened samples by either *ORF1b* - RT-PCR, other target RT-PCR assays with sequence analysis or whole genome sequence analysis was available in 18/30 EU/EEA countries and in 22/46 responding WHO European Region countries.

The figure indicates the level of screening and confirmation assays available in the 47 responding countries, including 46 WHO European Region Member States, 27 EU Member States and three EEA countries, two of which are also Member States of WHO European Region.

Many countries indicated that their reference laboratories were developing specific molecular detection tests, serological assays or were awaiting positive RNA control material for RT-PCR assays. Therefore, it should be emphasised that the results presented here are an overview of laboratory tests in operation at the time of survey and will require updating as capacities are rapidly increasing in the participating countries.

In 25 countries, the NIC reported to be the national reference laboratory for novel coronavirus and in 17 of these countries it was the only laboratory reporting diagnostic capability. In 10 countries, more than one laboratories were reported to perform novel coronavirus diagnostic tests at national or regional levels. Twenty laboratories that reported diagnostic capability from 12 countries were members of ENIVD.
Twelve of 25 countries with no confirmation capacity at national level reported referral arrangements to ship samples for testing in another country.

Of note, laboratories in six EU/EEA countries indicated that samples had been tested to date for novel coronavirus from approximately 250 patients fulfilling the WHO definition patients under investigation. Of the nine cases reported so far to WHO from Saudi Arabia, Qatar and Jordan, in addition to in-country testing, three have been tested and confirmed by the Erasmus Medical Centre, Rotterdam, the Netherlands, the Health Protection Agency, London, United Kingdom, and the Robert Koch Institute, Berlin, Germany [2,5,6].

Conclusions

The findings of this study indicate an extensive deployment of newly developed novel coronavirus molecular detection assays among public health reference and expert virology laboratories in Europe within only two

months after sequence information on the first reported case was made available. This deployment occurred to a greater extent in EU/EEA countries of the WHO European Region compared with south-east and eastern European countries. Screening tests were available in nearly half of countries of the WHO European Region and cross-border shipment arrangements were in place in many of those lacking domestic testing capacity.

Our results will allow virologists and public health agencies, including ECDC and the WHO Regional Office, to remedy gaps within their laboratory networks. Such measures may include technical laboratory guidance and collaborative arrangements for cross-border referral testing of clinical materials, technical support such as distribution of reference control materials, assistance with development of quality controlled serological assays and, if warranted in the longer term, provision of capacity building courses and external quality assessment schemes.

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References

1. ProMED-mail. Novel coronavirus - Saudi Arabia: human isolate. Archive Number: 20120920.1302733. 20 September 2012. Available from: http://www.promedmail.org/direct.php?id=20120920.1302733
2. Van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, et al .2012. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. mBio. 2012;3(6). pii= e00473-12.
3. World Health Organization (WHO). Global Alert and Response: Background and summary of novel coronavirus infection – as of 30 November 2012. Geneva: WHO; 2012. Available from: http://www.who.int/csr/disease/coronavirus_infections/update_20121130/en/index.html
4. European Centre for Disease Prvention and Control (ECDC). Rapid Risk assessment, update: Severe respiratory disease associated with a novel coronavirus, 26 November 2012. Stockholm: ECDC; 2012. Available from: http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=1007
5. Robert Koch Institute (RKI). Case of novel Corona virus in Germany 23 November 2012. Berlin: RKI; 2012. [Accessed 25 Nov 2012]. Available from: http://www.rki.de/EN/Home/Corona_Virus.html;jsessionid=77C1C0AF8994434233952DC28C20E642.2_cid290
6. Bermingham A, Chand MA, Brown CS, Aarons E, Tong C, Langrish C, et al. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill. 2012;17(40):pii=20290. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20290
7. World Health Organization (WHO).Summary table of SARS cases by country, 1 November 2002 - 7 August 2003. Geneva: WHO; 2003. Available from: http://www.who.int/csr/sars/country/2003_08_15/en/
8. World Health Organization (WHO). Case definition for case finding severe respiratory disease associated with novel coronavirus. Geneva: WHO; 2012. Available from: http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/index.html
9. Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17(39):pii=20285. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20285
10. Vijgen L, Moes E, Keyaerts E, Li S, Van Ranst M. A pancoronavirus RT-PCR assay for detection of all known coronaviruses. Methods Mol Biol. 2008;454: 3-12.
11. Drosten C, Günther S, Preiser W, van der Werf S, Brodt HR, Becker S et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1967-76.
12. Bermingham A, Heinen P, Iturriza-Gómara M, Gray J, Appleton H, Zambon MC. Laboratory diagnosis of SARS. Philos Trans R Soc Lond B Biol Sci. 2004;359(1447):1083-9.
13. Pebody RG, Chand MA, Thomas HL, Green HK, Boddington NL, Carvalho C, et al. The United Kingdom public health response to an imported laboratory confirmed case of a novel coronavirus in September 2012. Euro Surveill. 2012;17(40):pii=20292. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20292
14. European Network for Diagnostics of “Imported” Viral Diseases (ENIVD). Available from: www.enivd.org

Ongoing outbreak of dengue type 1 in the Autonomous Region of Madeira, Portugal: preliminary report

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Following the identification of two autochthonous cases of dengue type 1 on 3 October 2012, an outbreak of dengue fever has been reported in Madeira, Portugal. As of 25 November, 1,891 cases have been detected on the island where the vector *Aedes aegypti* had been established in some areas since 2005. This event represents the first epidemic of dengue fever in Europe since 1928 and concerted control measures have been initiated by local health authorities.

On 3 October 2012, two autochthonous cases of dengue fever were laboratory confirmed in the Autonomous Region of Madeira (RAM), Portugal, following clinical suspicion of dengue, i.e. sudden onset of fever and influenza-like symptoms in patients without travel history to dengue-endemic regions. Laboratory tests performed by the National Institute of Health in Lisbon identified dengue virus serotype 1 (DENV-1) as causing agent. As of 25 November, the Institute of Health and Social Affairs (Instituto de Administração da Saúde e Assuntos Sociais, IASAUDE, RAM) in Madeira reported 1,891 cases of dengue fever. In this preliminary report we highlight the main features of the outbreak and the control measures taken.

Background

Dengue is a vector-borne disease caused by a flavivirus with four distinct serotypes (DEN-1-4). The most competent mosquitoes species for disease transmission are *Aedes aegypti* (Linnaeus, 1762) and *Ae. albopictus* (Skuse, 1894) [1]. The last outbreak of dengue in Europe occurred in 1928, in Greece [2] but imported cases of dengue fever in travellers returning from endemic countries are often reported [2-6]. Additionally, sporadic autochthonous cases were recorded recently in areas of France and Croatia where *Ae. albopictus* is present [7,8].

Madeira is the main island of the Autonomous Region of Madeira, a Portuguese territory with 262,302 inhabitants [9]. It is located at around 1,000 km from mainland Portugal and around 500 km from the northern African coast. The other inhabited island of the Region is Porto Santo with 5,483 inhabitants [9]. The climate is temperate Mediterranean, with little temperature variation throughout the year [10]. *Ae. aegypti* was first identified on Madeira in 2005, in Santa Luzia parish of Funchal city [11].

Epidemiological and clinical findings

After the detection of the first two autochthonous dengue fever cases on 3 October 2012, active case finding was initiated and the following case definition has been used since:

A **probable case** should meet both clinical and epidemiological criteria outlined hereafter.

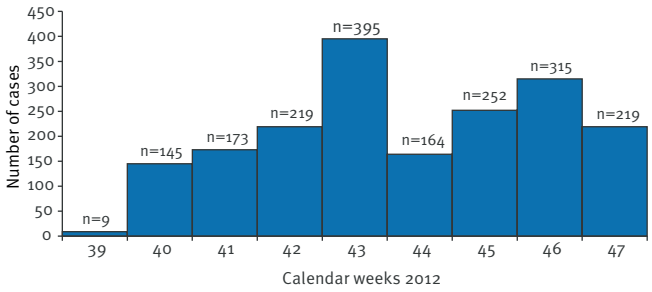
Clinical criteria comprised acute onset of fever and at least two of the following symptoms and signs: headache, retro-orbital pain, myalgia, arthralgia, exanthema, haemorrhagic manifestations or leucopoenia.

Epidemiological criteria are residing in or having visited a dengue-affected area during the 21 days prior to onset of symptoms. Currently, the whole island is considered as 'affected area' which means that the epidemiological criterion is met for all cases occurring on Madeira.

A **confirmed case** is defined as a probable case with at least one of the following laboratory results: (i) presence of dengue-virus-specific IgM antibodies in blood or cerebrospinal fluid (CSF); (ii) significant increase in the concentration of dengue-virus-specific IgG antibodies (seroconversion), or; (iii) detection of dengue

Figure 1

Notified dengue fever cases by week, Madeira, Portugal, 3 October–25 November 2012 (n=1,891)



Source: Instituto de Administração da Saúde e Assuntos Sociais, Autonomous Region of Madeira, Portugal

virus nucleic acid in blood or CSF with RT-PCR or other Nucleic Acid Amplification Test (NAAT).

Pregnant women, patients with chronic diseases and with immunodeficiency were considered at particular risk of getting the disease and of possible complications.

As of 25 November 2012 (calendar week 47), seven weeks after the first autochthonous cases were identified, 1,891 probable cases of dengue fever have been reported, of which 966 were laboratory confirmed

Of the total number of 1,891 cases, 777 (41.1%) were male and 1,114 (58.9%) were female. The median age of the 1,891 cases was 39 years (range 25-64). Up to week 47, the most affected age group was 25 to 64 years-old (Table).

One hundred and eleven patients were hospitalised and no fatalities were recorded. Of the total number of cases (n=1,891), the majority had mild symptoms, with fever, myalgia, headaches and arthralgia.

Current geographic distribution of cumulative dengue cases by parish of residence is presented in Figure 2. The highest proportion of patients is recorded in residents of Santa Luzia parish.

As of 25 November, 29 cases of dengue fever were reported among travellers returning from Madeira. Twelve cases of dengue fever were recorded in travellers to mainland Portugal. In addition, 17 cases were detected in travellers returning to other European countries: United Kingdom (n=6), Germany (n=7), Sweden (n=1), France (n=2) and Finland (n=1).

Laboratory investigation

Sequence analyses of viral genomes (600 nucleotides), performed at the National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal, presented high sequence similarity with DEN-1 viruses circulating in Venezuela and Colombia, suggesting Latin America as the possible origin for the circulating dengue virus [12].

Entomological findings

In 2006, a mosquito surveillance system based on ovitraps, traps that collect eggs by mimicking breeding sites, was established to monitor the presence and the abundance of the vector on the island [13]. By 2009, *Ae. aegypti* was detected outside Funchal city in the neighbouring localities of Câmara dos Lobos and Caniço. In September 2011, cross-sectional entomological surveys were carried out using a total of 273 ovitraps dispersed throughout Madeira (253 ovitraps) and Porto Santo islands (20 ovitraps). Results showed that the mosquito population had expanded to the west (Ponta do Sol and Ribeira Brava counties) and east of Funchal city (Santa Cruz county). The vector was also detected in Porto Moniz, but its presence was not confirmed in subsequent monitoring activities (unpublished data).

After the onset of the outbreak in October 2012, the surveillance network was reinforced and, currently, mosquito activity is monitored by 141 ovitraps and 16 BG-sentinel traps, traps baited with attractants to biting females. These traps are distributed mainly along the south coast of the island and in selected areas such as harbours, the airport, healthcare facilities and potential transmission zones. Up to date the vector has not been found on Porto Santo.

Control measures

In response to the outbreak, several control measures have been undertaken by the health authorities of Madeira. In order to prevent exportation, enhanced vector control measures have been implemented at Madeira's single airport. Currently, all aircrafts departing from the island undergo disinfestation procedures. Mosquito surveillance systems at the airport and at

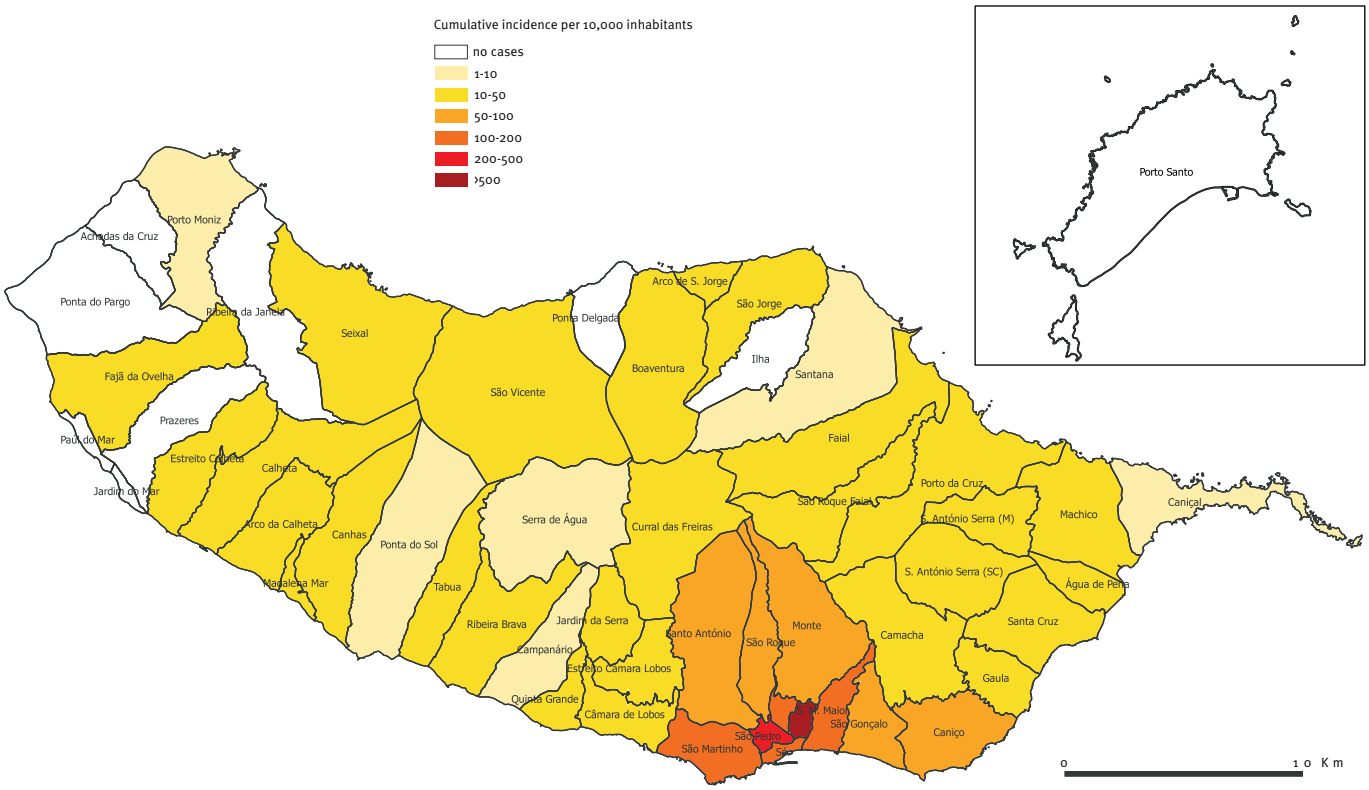
Table

Notified dengue fever cases by age group, Madeira, Portugal, 3 October–25 November 2012 (n=1,891)

Age group (years)	Number of cases	Percentage (%)
0-14	240	12.7
15-24	299	15.8
25-64	1,132	59.9
65+	220	11.6
Total	1,891	100

Figure 2

Cumulative incidence of dengue cases by parish, outbreak on Madeira, Portugal, 3 October–25 November 2012



The map was created in Quantum GIS with support from the European Centre for Disease Prevention and Control.

passenger and cargo harbours have been boosted. Particular care is being given to the passenger ship port, located in the most affected area, Funchal.
Information, mainly focused on the use of personal protective measures and on the elimination of mosquito breeding sites [14], is being disseminated by several mechanisms: (i) individual information to residents through a door-to-door campaign carried-out by environmental technicians of the IASAUDE, RAM; (ii) postings on IASAUDE website and in traditional and social media, and; (iii) community-based activities involving the educational, religious and health sectors. Advice for travellers to Madeira has been issued, containing information about methods to reduce the risk of being bitten by mosquitoes and symptoms of the disease [15].

Guidelines for diagnostic and management of dengue fever were provided to healthcare professionals. Measures to ensure blood safety and the safety of substances of human origin have been implemented and published since the detection of the first cases [16].

Nationwide measures to ensure blood safety in mainland Portugal included deferral of potential blood donors who visited Madeira island for a period of 28 days after leaving the affected area, deferral of potential blood donors with fever or influenza-like symptoms for 28 days after recovery, deferral of confirmed cases of dengue infection for 120 days after recovery and post transfusion haemovigilance.

Local measures applying to the blood services of the affected area included: (i) quarantine of all red cell concentrates resulting from blood collected during the previous 28 days and retrospective testing of relevant archived samples; (ii) deferral of blood donors with fever or influenza-like symptoms up to 28 days after recovery; (iii) deferral of confirmed dengue cases for 120 days after diagnosis; (iv) laboratory screening of all blood donations by RT-PCR for dengue virus; (v) information to blood donors to report any symptoms in the 15 days after donation; (vi) stop of local platelet production and supply of platelets from the Portuguese Institute for Blood and Transplants (Instituto Português de Sangue e Transplantes – IPST). Special attention is paid to the optimal use of blood, appropriate management of the blood supply and haemovigilance.

Discussion

The 2012 dengue outbreak on Madeira is the first of its kind in Europe since 1928. Given its magnitude and

its potential public health impact in a European context, the European Centre for Disease Prevention and Control (ECDC) in Stockholm, Sweden, has, on request of the Portuguese authorities, deployed experts to Madeira to assist the regional and national authorities in investigating and controlling the outbreak and prevent further expansion within Europe.

While *Ae. aegypti* is not considered an established mosquito species in continental Europe, its presence has been sporadically reported in restricted areas in the Netherlands and Russia [17]. The present outbreak however, draws attention to the need to better assess the possibility of dengue epidemics mediated by *Ae. albopictus*, that is present in several European countries. Besides the need to reinforce strategies aimed at averting the introduction of *Ae. aegypti* in Europe [18], it is also important to detect and monitor all imported human cases that might introduce the virus among a very competent mosquito species, *Ae. albopictus*.

At the time of publishing this report, the outbreak on Madeira is still ongoing. However, in week 48, the number of cases reported has decreased by around 54% (from 219 cases to 102 cases) when compared with the previous week. According to the weather forecast for the coming winter weeks, Madeira expects a decrease in temperature. This is assumed to have an impact on the vector, reducing its densities and, consequently, the number of dengue cases might further decline. Nevertheless, even in the absence of new reported human cases the virus may persist on the island either through vertical transmission or maintained by the small number of adult overwintering mosquito females.

Given the challenges posed by the dengue outbreak on Madeira, continued vigilance and a coordinated regional, national and international approach are needed.

References

1. European Centre for Disease Prevention and Control (ECDC). Factsheet for health professionals. Dengue fever. Stockholm: ECDC. [Accessed 4 Oct 2012]. Available from: http://ecdc.europa.eu/en/healthtopics/dengue_fever/basic_facts/Pages/Factsheet_health_professionals.aspx
2. Halstead SB, Papaevangelou G. Transmission of dengue 1 and 2 viruses in Greece in 1928. Am J Trop Med Hyg. 1980;29(4):635-7.
3. Jelinek T. Trends in the epidemiology of dengue fever and their relevance for importation to Europe. Euro Surveill. 2009;14(25):pii=19250. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19250
4. Gautret P, Simon F, Hervius Askling H, Bouchaud O, Leparc-Goffart I, Ninove L, et al. Dengue type 3 virus infections in European travellers returning from the Comoros and Zanzibar, February-April 2010. Euro Surveill. 2010;15(15):pii=19541. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19541
5. Nisii C, Carletti F, Castilletti C, Bordi L, Meschi S, Selleri M, et al. A case of dengue type 3 virus infection imported from Africa to Italy, October 2009. Euro Surveill. 2010;15(7):pii=19487. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19487
6. Wichmann O, Gascon J, Schunk M, Puente S, Siikamaki H, Gjorup I, et al. Severe dengue virus infection in travelers: risk factors and laboratory indicators. J Infect Dis. 2007;195(8):1089-96.
7. La Ruche G, Souarès Y, Armengaud A, Peloux-Petiot F, Delaunay P, Desprès P, et al. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro Surveill. 2010;15(39):pii=19676. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19676
8. Gjenero-Margan I, Aleraj B, Krajcar D, Lesnikar V, Klobučar A, Pem-Novosel I, et al. Autochthonous dengue fever in Croatia, August–September 2010. Euro Surveill. 2011;16(9):pii=19805. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19805
9. Instituto Nacional de Estatística, Portugal. Censos 2011. Resultados Preliminares. [2011 Census. Preliminary results]. Lisbon: Instituto Nacional de Estatística. [Accesed 4 Oct 2012]. Portuguese. Available from: http://www.ine.pt/scripts/flex_definitivos/Main.html
10. Departamento de Producción de la Agencia Estatal de Meteorología de España (Área de Climatología y AplicacionesOperativas) y por el Departamento de Meteorologia e Clima, Instituto de Meteorologia de Portugal (Divisão de Observação Meteorológica e Clima). Atlas Climático dos arquipélagos das Canárias, da Madeira e dos Açores (1971-2000). [Climate Atlas of the Archipelagos of the Canary Islands, Madeira and the Azores (1971-2000)]. Lisbon: Instituto de Meteorologia de Portugal. 2011. Portuguese.
11. Almeida AP, Gonçalves YM, Novo MT, Sousa CA, Melim M, Gracio AJ. Vector monitoring of Aedes aegypti in the Autonomous Region of Madeira, Portugal. Euro Surveill. 2007;12(46):pii=3311. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3311
12. National Institute of Health Dr. Ricardo Jorge. Febre de dengue na Madeira, Diagnóstico laboratorial. [Dengue fever in Madeira. Laboratory diagnosis]. Lisbon: National Institute of Health Dr. Ricardo Jorge. 9 Oct 2012. Available from: http://www.insa.pt/sites/INSA/Portugues/ComInf/Noticias/Paginas/DengueMadeiraDiagLab.aspx
13. Gonçalves Y, Silva J, Biscoito M. On the presence of Aedes (Stegomyia) aegypti Linnaeus, 1762 (Insecta, Diptera, Culicidae) in the island of Madeira (Portugal). Bol Mus Mun Funchal. 2008;58 (322):53-9
14. Serviço de Saúde da RAM. [Dengue fever: Personal protective measures]. Funchal: Serviço de Saúde da RAM. [Accessed 4 Oct 2012]. Portuguese. Available from: http://iasaude.sras.gov-madeira.pt/mosquitos/
15. Directorate-General of Health, Portugal. Comunicado do Diretor-Geral da Saúde com recomendações para os cidadãos que viajem para a Ilha da Madeira, na sequência dos casos de dengue reportados naquela região. [Notice of the Director General of Health with recommendations for citizens traveling to the island of Madeira, in the context of dengue fever cases reported in the region]. Lisbon: Directorate-General of Health. 9 Oct 2012. Portuguese. Available from: http://www.dgs.pt/
16. Directorate-General of Health. Casos de dengue na Regiao Autonoma da Madeira. Abordagem para casos de dengue. [Dengue cases in the Autonomous Region of Madeira. Approaching dengue cases]. Lisbon: Directorate-General of Health. 3 Oct 2012. Portuguese. Available from: http://www.dgs.pt/upload/membro.id/ficheiros/io17887.pdf
17. European Centre for Disease Prevention and Control [ECDC]. VBORNET Maps – Mosquitoes. Stockholm: ECDC. [Accessed 4 Oct 2012]. Available from: http://ecdc.europa.eu/en/activities/diseaseprogrammes/emerging_and_vector_borne_diseases/Pages/VBORNET_maps.aspx
18. European Centre for Disease Prevention and Control (ECDC). Guidelines for the surveillance of invasive mosquitoes in Europe. Technical report. Stockholm: ECDC. Aug 2012. Available from: http://ecdc.europa.eu/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf

Use of a geographic information system to map cases of measles in real-time during an outbreak in Dublin, Ireland, 2011

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In 2011, there was a large measles outbreak in Dublin. Nationally 285 cases were notified to the end of December 2011, and 250 (88%) were located in the Dublin region. After the first case was notified in week 6, numbers gradually increased, with 25 notified in June and a peak of 53 cases in August. Following public health intervention including a measles-mumps-rubella (MMR) vaccination campaign, no cases were reported in the Dublin region in December 2011. Most cases (82%) were children aged between 6 months and 14 years, and 46 cases (18%) were under 12 months-old. This is the first outbreak in Dublin to utilise a geographic information system for plotting measles cases on a digital map in real time. This approach, in combination with the analysis of case notifications, assisted the department of public health in demonstrating the extent of the outbreak. The digital mapping documented the evolution of two distinct clusters of 87 (35%) cases. These measles cases were infected with genotype D4-Manchester recently associated with large outbreaks across Europe. The two clusters occurred in socio-economically disadvantaged areas and were attributable to inadequate measles vaccination coverage due in part to the interruption of a school-based MMR2 vaccination programme.

Introduction

Measles is a highly infectious disease that can result in serious morbidity and mortality. It has been a notifiable disease in Ireland since January 1988. An effective and safe vaccine against measles is available, and two doses of a measles-containing vaccine are recommended for individual protection. In addition, 95% uptake of each dose across the general population is required to prevent outbreaks. Measles vaccination was introduced to Ireland in 1985, initially as a monovalent vaccine and since 1988 as a component of the measles-mumps-rubella (MMR) vaccine. The national vaccination policy from 2008 recommends children receive the first dose of MMR vaccine (MMR1) at the age of 12 months from their family doctor. The second dose of MMR vaccine (MMR2) is given at the age of 4–5 years generally through the school-based vaccination programme [1].

Although the number of measles cases in Ireland has decreased substantially since the introduction of the MMR vaccine [2], there have still been a number of significant measles outbreaks in Ireland [3]. In 2010, a total of 426 measles cases were notified nationally. These cases clustered among certain population groups, including members of the Irish Traveller and Roma communities and children whose parents object to vaccination on philosophical or religious grounds [4].

The World Health Organization (WHO) recognises eight clades (A-H) of measles viruses which comprise 23 established genotypes [5]. The WHO Measles and Rubella Laboratory Network (LabNet) requires virological surveillance of wild-type measles viruses and genetic characterisation of a 450 nt fragment of the nucleoprotein gene to monitor virus transmission, discriminate between imported and endemic cases, and document interruption of transmission [6,7]. Molecular epidemiological analysis of wild-type measles viruses in Ireland over the previous decade have shown exclusively clade D viruses to be circulating. Genotype D8 strains present in 2002/03 were supplanted by genotype D7 in 2003, and from 2004 to 2012, numerous distinct clusters of genotype D4 predominated [8]. The D4-Enfield strain (MVs/Enfield.GBR/14.07[D4]), documented first in Irish Travellers in the United Kingdom (UK), was identified in Irish cases in 2007/08 in a school outbreak in Dublin associated with travel to Germany [8]. A variant of D4-Enfield, termed D4-Hamburg, was described in the Irish Traveller and Roma communities in Ireland in the latter part of 2009 and early 2010 (e.g. MVs/Kerry.IRL/40.09[D4]) with subsequent spread to other citizens who refused vaccination [4,9]. This

genotype was also linked to cases in Northern Ireland (MVs/Belfast.GBR/50.09[D4]) [10].

The Dublin region is divided into eight Community Care Areas (CCA1- CCA8). Each CCA is an administrative division of the health service with responsibility for immunisation activities in their area. In Ireland, data on the MMR1 uptake rate at 24 months of age are available at the national and regional level on a quarterly basis [11]. The national and Dublin region uptake rate was 77% in 1999 (the first year data became routinely available for national reporting), however, marked improvements in MMR1 uptake have occurred since then. For the third quarter of 2011, the national uptake rate was 93% and the Dublin region uptake rate was 91%. Although these figures represent an improvement in vaccination coverage, they are still below the WHO recommended target of 95% required to provide herd immunity.

The measles outbreak that occurred in Dublin in 2011 involved 250 cases. The majority (82%) were children aged between 6 months and 14 years. The aim of this study was to digitally plot cases of measles as they occurred in real time during the outbreak using a geographic information system (GIS) that also mapped the varying levels of socio-economic deprivation across Dublin. This facilitated the identification of clusters of measles in areas of deprivation and assisted the direction of Public Health interventions.

Methods

The measles data used in this report were extracted from the Computerised Infectious Disease Reporting system (CIDR) on 14 March 2012. Crude incidence rates of measles cases were calculated nationally and for CCA1-CCA8 using population data from the 2011 Census [12].

When a case of measles was notified, the home address details from the CIDR were cross-referenced with the GIS within the application Health Atlas Ireland (HAI). This provided XY co-ordinates for each notified case, and these co-ordinates were then digitally mapped. Throughout this measles outbreak, a continuously updated map of the Dublin region was available showing the exact location of all cases. The outbreak map was also colour-coded to show the socio-economic deprivation level by electoral division using the Haase and Pratschke index [13].

HAI is a web application portal supporting health service planning and monitoring in Ireland. The system integrates open-source GIS, database and statistical components. The GIS arm integrates the national post office GeoDirectory that allows address location and address cleansing functionality nationally.

Notified measles cases were assigned case classifications using the Case Definitions for Notifiable Diseases [14]. The measles case definition is composed of clinical, laboratory and epidemiological criteria:

Clinical: any person with fever and maculopapular rash and at least one of the following: cough, coryza or conjunctivitis;
Laboratory: At least one of the following: Isolation of measles virus from a clinical specimen, detection of measles virus nucleic acid in a clinical specimen, measles virus-specific antibody response characteristic for acute infection in serum or oral fluid, or detection of measles virus antigen by direct fluorescent antibody test in a clinical specimen using measles-specific monoclonal antibodies;
Epidemiological: any person with an epidemiological link by human-to-human transmission.

Cases were classified as follows:

Possible case: any person meeting the clinical criteria;
Probable case: any person meeting the clinical criteria and with an epidemiological link;
Confirmed case: any person not recently vaccinated and meeting the clinical and the laboratory criteria.

Measles virus genotyping was performed according to WHO guidelines by bidirectional sequencing of the 3' hypervariable region of the nucleoprotein gene (NP-HVR) on an ABI3500 genetic analyzer [15]. Genbank accession numbers JQ866618, JQ866619 and JX315600 were assigned for the measles genotypes described in this study following submission to the Measles Nucleotide Surveillance (MeaNS) database (http://www.who-measles.org), a joint project between the Health Protection Agency (London, UK) and the WHO.

A maximum likelihood phylogenetic tree of measles genotype D4 sequences from the outbreak was built based on partial (456 bp) nucleoprotein gene sequences. The tree includes reference measles genotype D4 sequences obtained from GenBank and measles virus sequences detected in Ireland since 2004. The phylogenetic tree was built using PAUP*) version 4.0 Beta 10 [16] and annotated in FigTree version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

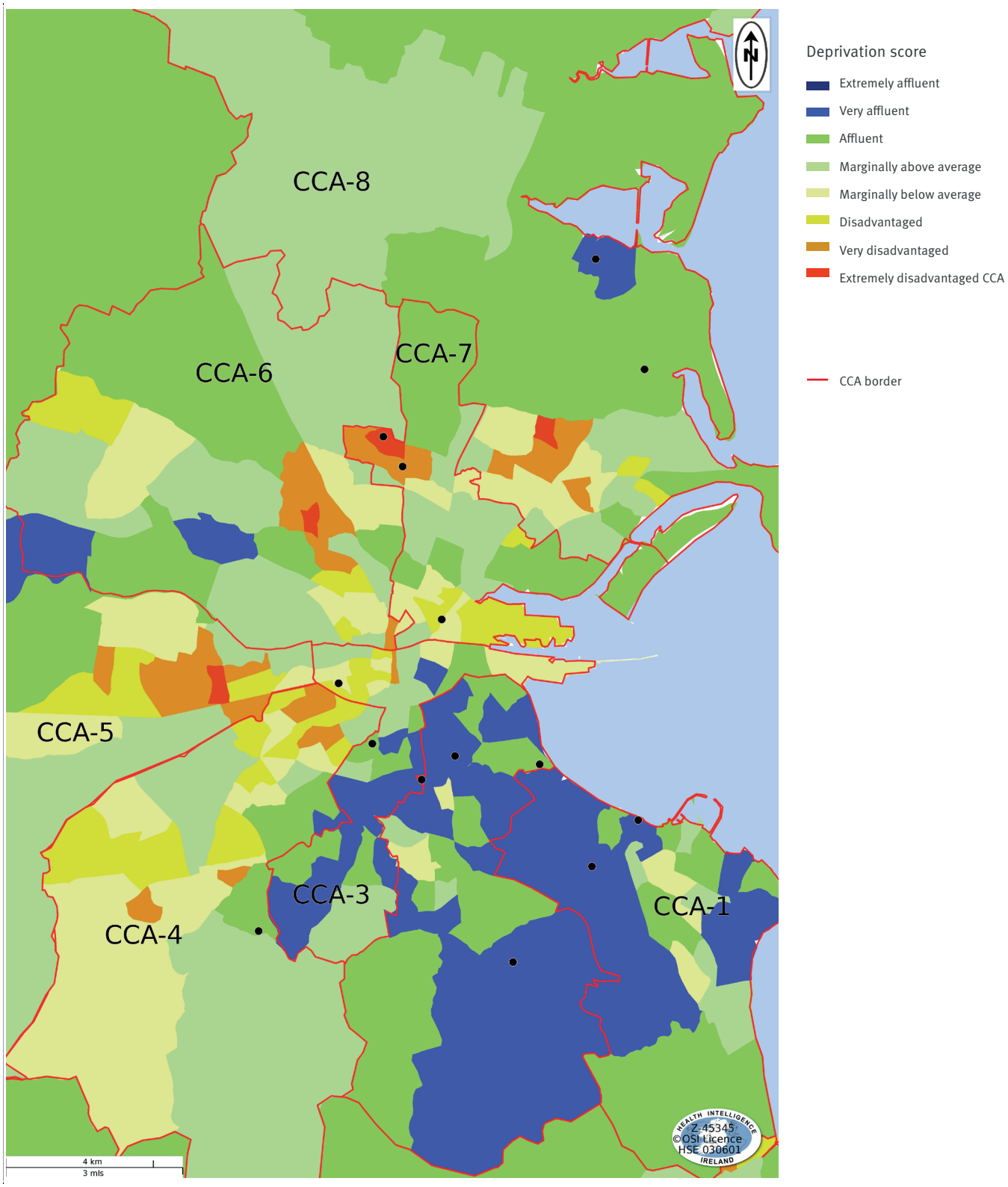
Outbreak description

In 2011, the first case of measles was notified in week 6 in a 22 month-old child from North Dublin (CCA-8) who did not attend daycare facilities. This case was laboratory-confirmed. From weeks 6 to 15, an average of one to two cases per week were notified, however, no epidemiological links between these cases could be identified. These 13 cases were widely distributed across the Dublin region with no obvious geographical clustering (Figure 1).

During week 16, two outbreaks were identified. The first occurred in a tertiary educational institution in North Dublin, where two students developed clinical measles. One was laboratory-confirmed and the other was epidemiologically linked to the confirmed case. No international travel was associated with these cases. Neither case had received any MMR vaccinations. The

Figure 1

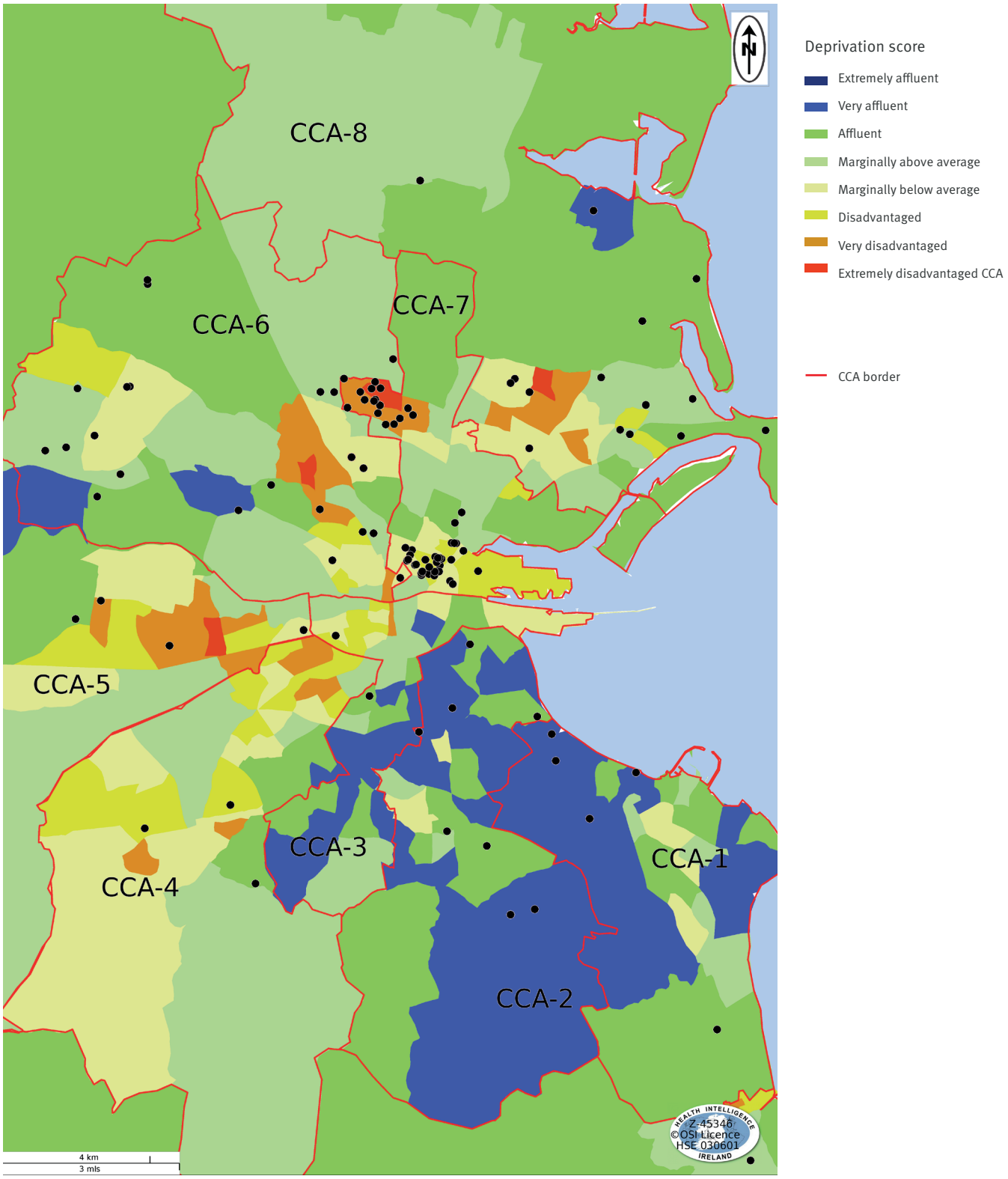
Measles cases in Dublin, Ireland, weeks 1–16, 2011 (n=18)



The black spots indicate areas in Dublin where cases occurred. The map is colour-coded to show the different levels of socio-economic deprivation across Dublin.

Figure 2

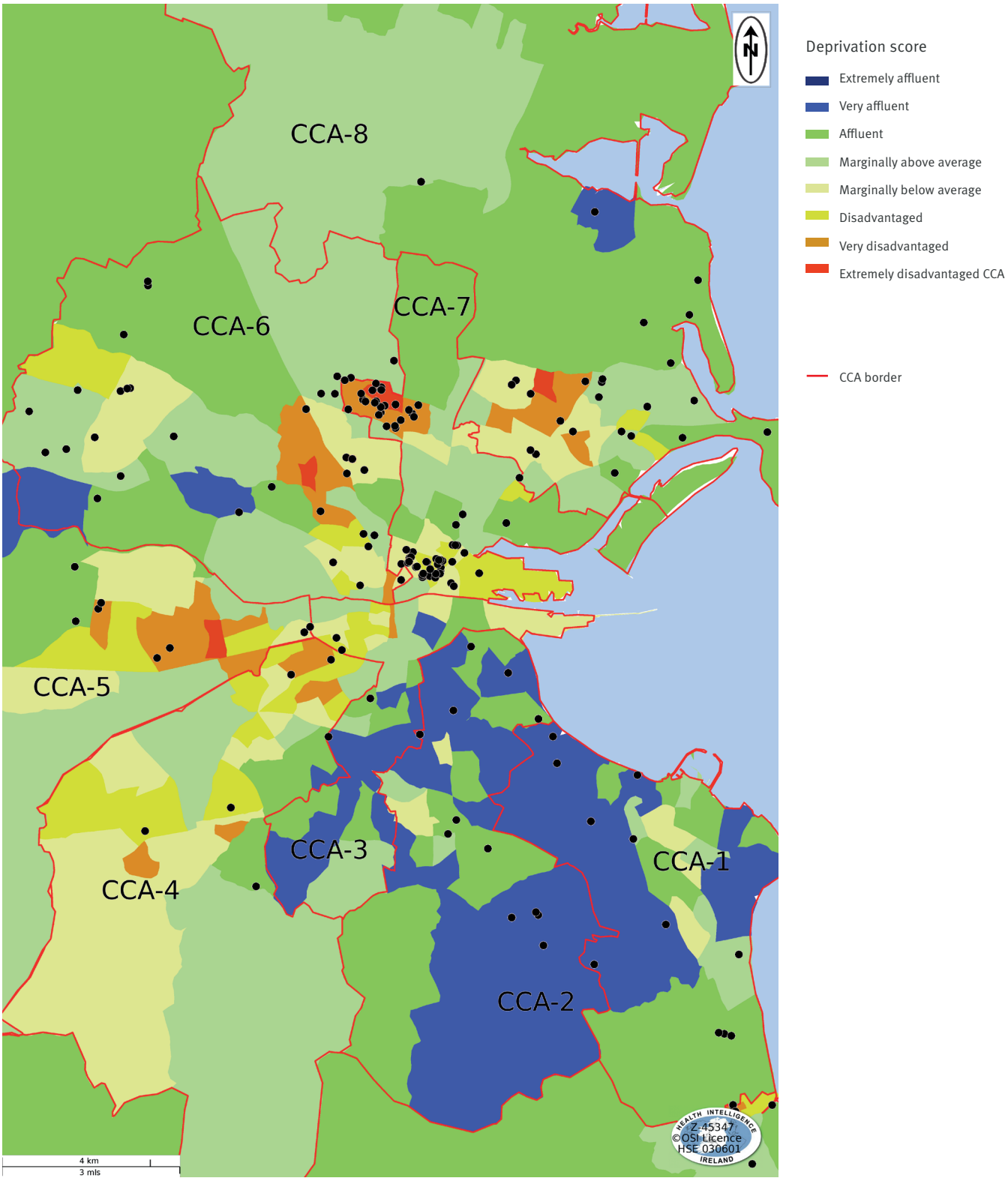
Measles cases in Dublin, Ireland, weeks 1– 36, 2011 (n=161)



The black spots indicate areas in Dublin where cases occurred. The map is colour-coded to show the different levels of socio-economic deprivation across Dublin.

Figure 3

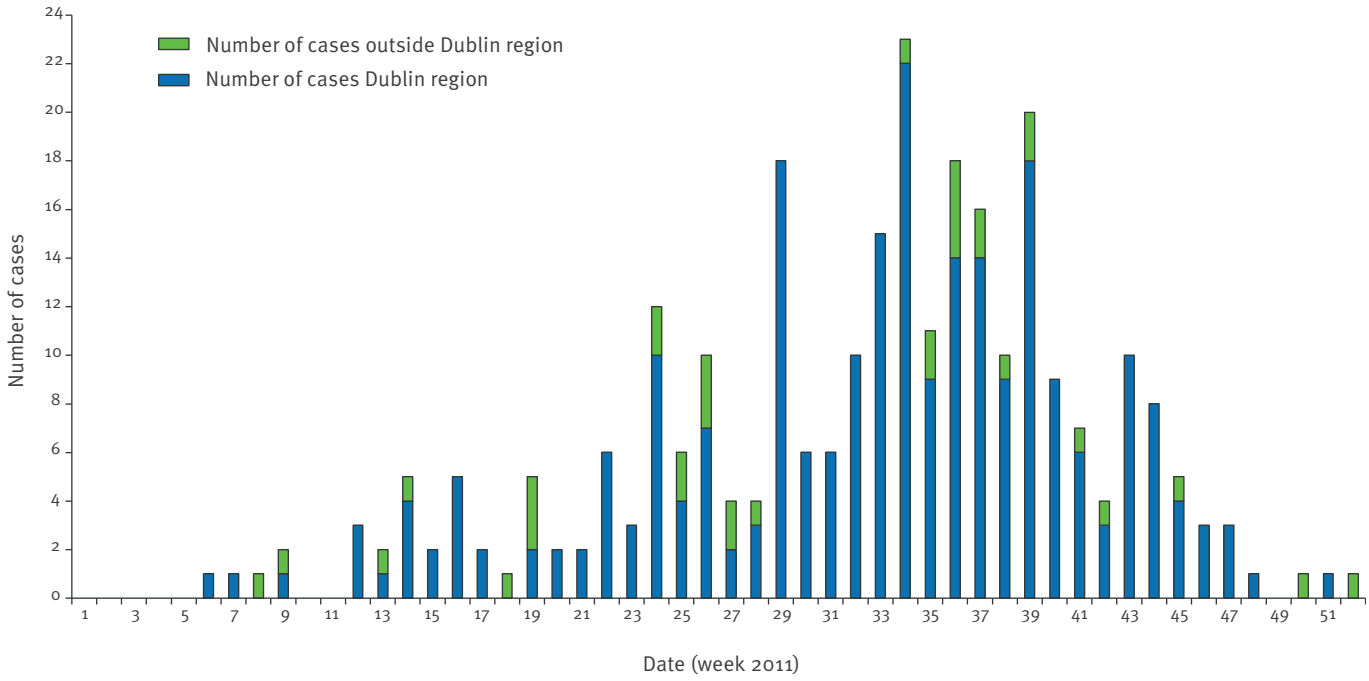
Measles cases in Dublin, Ireland, weeks 1–52, 2011 (n=250)



The black spots indicate areas in Dublin where cases occurred. The map is colour-coded to show the different levels of socio-economic deprivation across Dublin.

Figure 4

Measles notifications by week, Dublin region and outside Dublin region (rest of Ireland), weeks 1–52, 2011 (n=285)



second outbreak in week 16 occurred in a primary school in South Dublin (CCA-2) with three children confirmed to have measles. None of these children had received MMR vaccinations.

Between weeks 17 and 36, an additional 16 outbreaks in Dublin were notified. Seven of these outbreaks were found in Community Care Area-7 (CCA-7) which is the most densely populated CCA in Dublin with 3,632 persons per km^2. The largest outbreak in 2011 began during week 35 in a residential summer camp for disadvantaged children in the inner city. The summer camp is located in North County Dublin and provides one week of daytime activities and overnight accommodation for children. A total of 25 confirmed cases of measles were notified and epidemiologically linked to this camp. Overall by week 36, the initial clustering of cases in two distinct and socially disadvantaged areas of CCA-7 was evident on digital mapping (Figure 2).

From week 37 to 52, public health medical staff investigated five measles outbreaks in Dublin. Two of these outbreaks occurred in family homes, two in childcare facilities and one in a hospital. The number of measles notifications dropped dramatically from week 45 onwards. Further clustering of cases in two geographically distinct regions of CCA-7 occurred during weeks 37 to 52 (Figure 3).

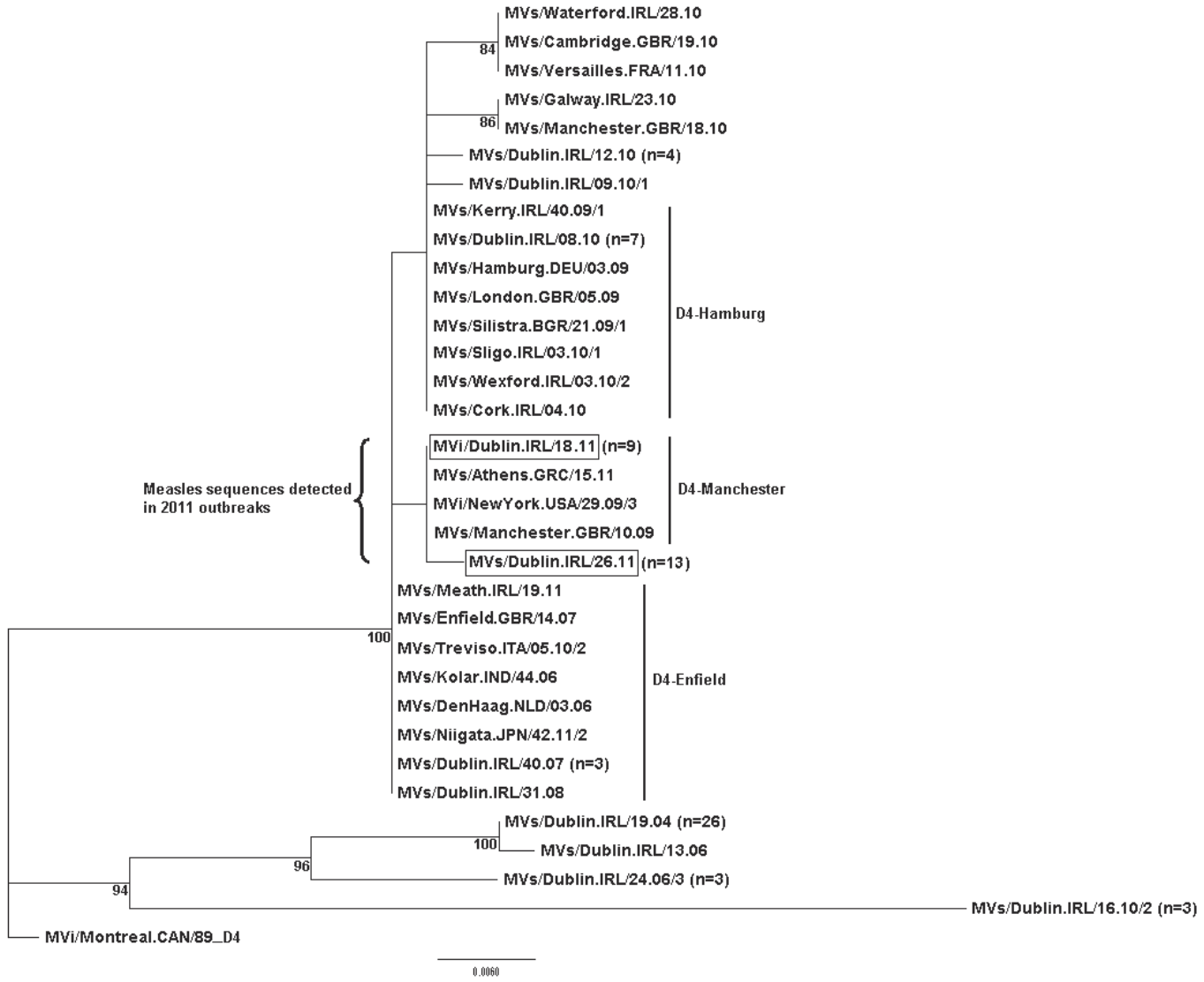
By the end of week 52, 285 cases were notified nationally, of which 250 (88%) were located in the Dublin region (Figure 4). By week 52, the national crude incidence rate (CIR) for measles cases was 5.9/100,000. In the Dublin region, the CIR for 2011 was 15.0/100,000, however, in CCA-7 it was 63.9/100,000. Of the 250 cases (54% male) notified in the Dublin region, 127 (51%) were laboratory-confirmed, 58 (23%) were probable cases and 65 (26%) were possible cases.

Measles virus genetic analysis was performed on 23 cases from Dublin in 2011 by sequencing the NP-HVR, and phylogenetic analysis confirmed 22 were genotype D4 (Figure 5). A single genotype D8 case was also detected in week 35 without history of travel, suggesting this genotype was also circulating in Ireland. That strain, MVs/Dublin.IRL/35.11/2[D8], was 100% identical over the 456 bp typing region to genotype D8 cluster 1 viruses described in India since 2007 (MVi/Villupuram.IND/07.07) and more recently in the UK (MVs/Chester.GBR/3.09) and Italy (MVs/Padova.ITA/07.11/1). Two distinct lineages of measles genotype D4 were found to be circulating in Dublin in 2011 between weeks 18 to 41, one represented in Figure 5 by MVi/Dublin.IRL/18.11 (n=9), 100% identical to D4-Manchester (MVs/Manchester.GBR/10.09), and the other exemplified by MVs/Dublin.IRL/26.11 (n=13), identified between weeks 26 to 41, 2011. The latter measles virus was found to be associated with the outbreak in CCA-7 and corresponds to a distinct variant of D4 with a single synonymous mutation compared to MVs/Manchester.GBR/10.09 (Figure 5).

At present there is no published data on MMR2 uptake at 5 years of age in Ireland. Each CCA in the Dublin region has an immunisation department that files MMR2 returns. In 2011, CCA-7 reported an MMR2 uptake at 5 years of 75%. The remaining CCAs in the Dublin region reported an MMR2 uptake at 5 years of

Figure 5

Phylogenetic analysis of measles genotype D4 outbreaks, Dublin, Ireland, 2011 (n=22)



The measles virus NP-HVR sequences associated with the outbreaks in Dublin are highlighted.

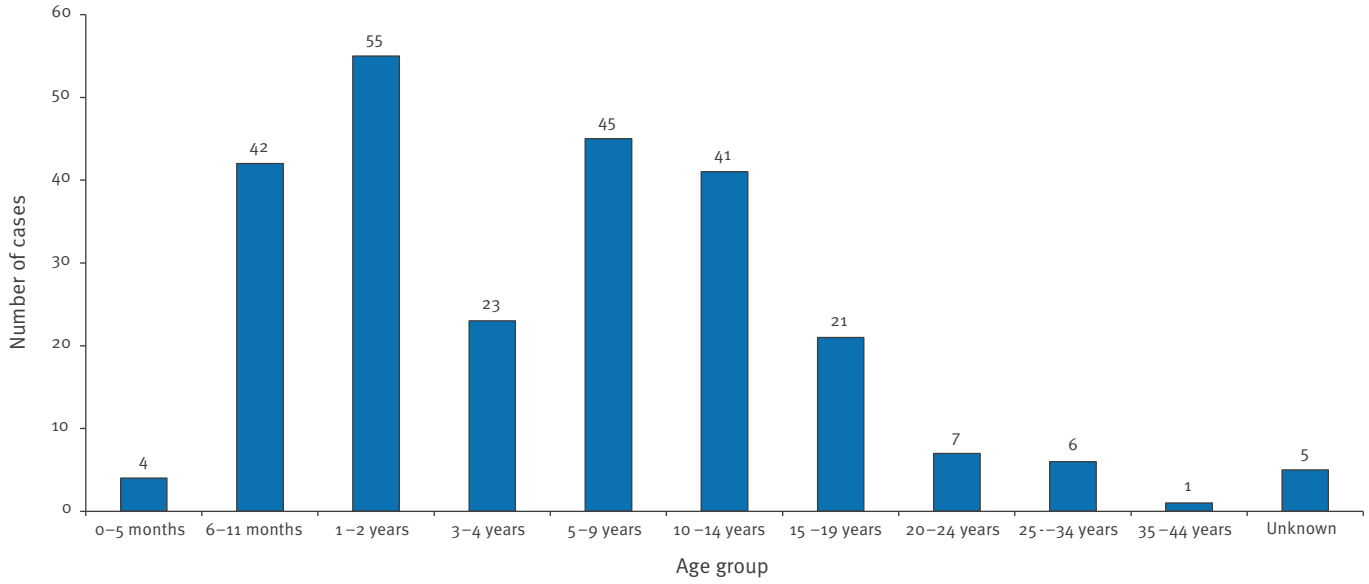
greater than 90% in 2011. These data were not independently verified. All age groups were affected during the current outbreak (Figure 6). The majority of cases (82%) were in children aged between 6 months and 14 years, with 46 (18%) cases occurring in those under 12 months of age.

Of the 250 notified cases, 145 (58%) had received no MMR vaccination and for 38 (15%) cases vaccination status was documented as either "unknown" or "not specified". Fifty-seven (23%) cases were reported to have had one dose of MMR vaccine, while 10 (4%) cases reported to have received two doses (documented evidence available only for two of them). Twenty-four measles cases (10%) were hospitalised in the Dublin region and no deaths were recorded. The reasons for admission included seizures (n=1), pneumonia (n=2), pyrexia (n=1), dehydration due to nausea and vomiting (n=1), tonsillitis (n=1), and the remaining were not specified (n=18). In CCA-7, 10 cases were admitted to hospital (42% of all hospitalised cases).

The GIS component of HAI achieved an initial 78% XY co-ordinate match for the 250 measles case addresses supplied to it by the CIDR. The remaining 22% of addresses had to be matched by manual review of the data entered in the address field of the CIDR. Of those initially unmatchable addresses, 85% (n=47) contained substantial spelling mistakes and the remaining 15% (n=8) did not contain the minimum information for the software to match the address to the reference geodirectory.

Figure 6

Measles cases by age, Dublin, Ireland, weeks 1–52, 2011 (n=250)



Control measures

An outbreak control team was convened with medical, nursing and administrative representatives from public health, community medicine, HPSC and the National Immunisation Office (NIO). The outbreak control measures undertaken included:

General guidance

Information on control measures to contain the measles outbreak was distributed to all relevant institutions such as hospitals and general practice surgeries. This guidance stated that all children should receive MMR vaccination at 12 months and 4–5 years of age. If a child had not received two MMR vaccines by five years of age, it was recommended that they should be vaccinated opportunistically as soon as possible. All relevant guidance was posted on the websites of the NIO and the HPSC [17].

Contact tracing

Public health medical staff followed up measles cases as a priority by advising on appropriate immunisation, arranging swabs and liaising with schools, childcare facilities and parents.

Media campaign

Radio and print media were utilised to inform the public of the measles outbreak and the importance of having children vaccinated with MMR.

Healthcare staff

Healthcare professionals were reminded of the importance of infection control measures to prevent measles transmission within clinics and hospitals by rapidly isolating cases when they presented.

North Inner City intervention (including CCA-7)

Due to the number and age profile of measles cases it was recommended that children aged 6–11 months in CCA-7 receive MMR vaccine during the outbreak period if they had contact with a probable or confirmed case of measles within the previous 72 hours. These children were then advised to complete their measles vaccination schedule in accordance with the current Irish national guidelines. It was also recommended that MMR2 be expedited for children older than one year who had already received MMR1 if they had been in contact with a probable or confirmed case of measles in the previous 72 hours.

From 7 to 16 September 2011, emergency vaccination teams attended primary schools, focussing on those located in CCA-7. The vaccination teams consisted of medical, nursing and administrative staff from the Community Medical Service and the Department of Public Health. Twenty-eight of 51 schools in the affected area were prioritised for vaccination, and MMR vaccine was offered to those children who were not up to date with their vaccination schedule. A total of 1,772 MMR vaccine doses were administered.

Discussion

The measles outbreak in 2011 demonstrates that Ireland has to make substantial progress to meet the WHO goal of eliminating measles in Europe by 2015 [18]. The outbreak in Dublin highlighted that measles cases clustered in two distinct areas of CCA-7 in North Inner City Dublin. The likely reason for this was MMR vaccination uptake rates below 95%. In CCA-7, MMR1 and MMR2 are given to children in the surgery of the

family doctor, whereas in all other CCAs in the Dublin region, MMR2 is offered to children by vaccination teams that attend schools and provide the vaccine on site with parental consent. CCA-7 discontinued providing a school-based MMR2 vaccination programme in 2000 primarily due to resource issues. Each September, the local immunisation office for CCA-7 receives the name, age and address of every child commencing primary school education in CCA-7. This local immunisation office then sends a letter to the parents of these children indicating they should bring their child to the family doctor to receive the MMR2 vaccine free of cost. As MMR2 uptake rates are lower for CCA-7 than surrounding CCAs, this suggests that a school-based MMR2 vaccination programme is more successful at improving MMR2 uptake levels and therefore should be reinstituted in CCA-7 to minimise the transmission of measles to susceptible children and adolescents [19,20].

The national quarterly MMR1 uptake rates at 24 months [4] indicate that since 1999, all national quarterly reports have recorded MMR1 uptake rates of below 95% at 24 months. This suggests that Ireland has built up a large reservoir of unvaccinated individuals capable of sustaining future outbreaks of measles. Similar situations have been anticipated and occurred in larger European countries such as Germany, Italy and France, where MMR vaccine coverage has remained below 95% for a substantial number of years [21].

The two large geographical clusters in the city developed in areas designated disadvantaged and extremely disadvantaged [13] in CCA-7. CCA-7 had an MMR2 uptake rate of 75% for 2011, as reported by the local immunisation office, even though MMR vaccination was available at no cost from the local family doctor. An important question facing public health and local government in CCA-7 is how to convince parents to bring their unvaccinated children to the family doctor in a deprived area awaiting the re-introduction of school-based MMR2 vaccination teams. One programme currently available is the voluntary Community Mothers Programme [22]. This involves experienced mothers visiting and offering support to first-time mothers in deprived areas of Dublin. The programme has achieved substantial improvements in vaccination uptake rates for those children whose mothers are enrolled in the programme [22-24]. An important component of this programme is that visiting experienced mothers are from the same area as the first-time mother. However, in order for the Community Mothers Programme to have a meaningful effect on vaccination uptake rates in CCA-7 it would have to be extended to cover a greater number of families over a more prolonged period of time. The location of measles outbreaks in areas of deprivation is also an issue for other European countries [25] and shows that vaccination education and delivery designed for the general population is unlikely to be successful in deprived inner city areas. Tailored evidence-based interventions, such as the Community Mothers Programme, in these locations are required [26]. The combination of low vaccination rates, deprivation, high population density and a lack of specific preventative measures for disadvantaged areas together led to the two clusters in CCA-7.

The finding that 18% of cases were younger than 12 months indicates that the issue of low vaccination uptake has had a disproportionate effect on the infant population in Dublin. This change in the age profile of measles infections in Ireland may in part be explained by the reduced incidence of natural measles infection in women of child-bearing age and therefore a decrease in the level of maternal protective anti-measles IgG. It is unlikely that a mother who has received MMR will transfer adequate protective antibody to prevent measles infection in children under 12 months of age. As a result infants younger than 12 months may be increasingly dependent on further improvements in vaccine coverage, which needs to reach 95% to afford the necessary herd immunity.

Since 2007, six different measles virus genotypes (B3, D4, D5, D8, D9, H1) have been described in Europe [27]. The 2011 outbreak in Dublin comprised two distinct genotype D4 strains, one of which was 100% identical over the typing region to D4-Manchester which is widely circulating in Europe [28] and to a newly described D4 variant with a single synonymous nucleotide substitution compared to MVs/Manchester.GBR/10.09. Measles virus genotype D4 represents the predominant autochthonous strain identified in recent outbreaks in Dublin and the rest of Ireland before 2012 and also in Austria, Belgium, Bulgaria, France, Germany, Greece, Italy, the Netherlands, Romania, Spain and the United Kingdom [8,9,29]. We have identified a single measles genotype D8, cluster 1 virus circulating contemporaneously with the genotype D4 viruses during the outbreak in 2011. Genotype D8 is endemic in Bangladesh, Nepal and India [30] and has also been described in outbreaks in Italy and France [31,32]. Measles genotype G3 is endemic in southeast Asia, and transmission of this genotype appeared to have been interrupted with the last cases described in 2006, however, since late 2010 cases of G3 were described in the UK, Spain, France, Germany, Switzerland and also in a Canadian national with a recent history of travel to Europe [33-35]. The results of the genotyping data in this study suggest a limited number of clade D viruses (genotype D4 and D8) are presently circulating in Ireland with no evidence for circulation of the novel G3 described elsewhere in Europe. This is characteristic of endemic transmission of measles virus and contrasts with geographic regions approaching measles elimination, where an endemic virus is absent and the co-circulation of multiple imported genotypes is generally seen [36]. The molecular characterisation of circulating measles viruses is of increasing importance in confirming the absence of endemic infection in Ireland as Europe aims for the elimination of measles by 2015.

The use of the geographical information system for the real-time digital mapping of cases identified an issue with the recording of addresses on the computerised infectious disease reporting system. Health Atlas Ireland's geodirectory contains an exhaustive list of addresses within Ireland obtained from the national postal directory. The finding that 22% of addresses from the CIDR were not recorded accurately suggests improvements with regard to inputting the data should be sought. Accurate address information is extremely important for mapping large numbers of cases within short periods of time. This highlights that any georeferencing system is only as good as the quality of the data that it receives. The GIS utilised during this outbreak identified geographic clusters at the elementary level through the plotting of disease occurrences at residential addresses of patients. Our system did not account for varying background population density. An area of high population density will have a greater number of cases than an area of lower population density even when the crude incidence rates for both areas are the same. It is preferable that when GIS is employed during an outbreak that the system incorporates kernel functionality [37] to filter the noise caused by spatially varying population density in order to identify spatial clusters [38]. However, a separate calculation using census data [12] confirmed that the CIR for CCA-7 was greater than that of the surrounding regions. Another limitation of the GIS technique in infectious disease epidemiology and outbreak investigation is that mapping of diseases will describe the 'where' but not the 'why there' of the outbreak [39]. Nevertheless map patterns can provide potential clues as to what is causing the disease which can then be followed up by further investigation [40]. Modern GIS software that incorporates multiple map layers with advanced geostatistical analysis is expensive. This cost is further increased by the necessity of having to employ personnel or train existing personnel on how to use the complex software. Public health departments under financial constraints must carefully weigh the advantages and disadvantages of GIS before allocating budgets.

The use and level of complexity of geographical information systems being used internationally varies greatly and is dependent on the resources available. In Canada, a GIS has been developed that can simulate the spread of a communicable disease, such as measles, within a populated area. The system is built on a complex algorithm that describes both the communicable disease stages and the life paths of people responsible for the transmission of disease within an urban area [41]. This system is composed of multiple layers including information on population density, the transportation network and different types of land use. Turkey relatively recently used a GIS to retrospectively assess a measles outbreak in Istanbul. The process involved geo-referencing the addresses of measles cases and attaching various epidemiological information such as vaccination status by using symbols [42]. When GIS is utilised in developing countries it must have minimal costs attached. The Ministry of Health in Nicaragua in conjunction with Columbia University in New York have combined Google Earth imaging with a GIS subsidised through the Global Fund to produce a low-cost innovative method to track infectious diseases in resource-poor settings [43]. This consequently allows public health to focus attention on areas where cases cluster.

International literature has confirmed that compulsory vaccination at school entry achieves higher uptake rates when compared to other methods [44,45]. Therefore a more prudent approach to prevent measles outbreaks occurring in Dublin would be to introduce compulsory MMR vaccination for those wishing to attend school education. Ireland's situation reflects the larger epidemic of measles occurring on the continent of Europe at present [46] and this particular outbreak has demonstrated how novel technologies such as GIS can assist with the management of outbreaks. However, as public health budgets diminish, this measles outbreak has also shown that the resources to provide school-based MMR vaccination programmes should be protected and expanded in order to help prevent future outbreaks of measles in Ireland.

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References

1. Corcoran B. Driving change in immunisation. The role of the national immunisation office 2005-2011. Health Service Executive: National Immunisation Office, 2012. Available from : http://www.immunisation.ie/en/EXTRADOWNLOADS/PDFFile_17039_en.pdf
2. Health Protection Surveillance Centre (HPSC). Annual reports on measles in Ireland. Dublin: HPSC. Available from: http://www.hpsc.ie/hpsc/A-Z/VaccinePreventable/Measles/Publications/AnnualReportsonMeaslesinIreland/
3. McBrien J, Murphy J, Gill D, Cronin M, O'Donovan C, Cafferkey MT. Measles outbreak in Dublin, 2000. Pediatr Infect Dis J. 2003;22(7):580-4. Epub 2003/07/18.
4. Gee S, Cotter S, O'Flanagan D, on behalf of the national incident management team. Spotlight on measles 2010: Measles outbreak in Ireland 2009-2010. Euro Surveill. 2010;15(9):pii=19500. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19500
5. Rota PA, Brown K, Mankertz A, Santibanez S, Shulga S, Muller CP, et al. Global distribution of measles genotypes and measles molecular epidemiology. J Infect Dis. 2011;204 Suppl 1:S514-23.

6. Rota PA, Brown KE, Hubschen JM, Muller CP, Icenogle J, Chen MH, et al. Improving global virologic surveillance for measles and rubella. J Infect Dis. 2011;204 Suppl 1:S506-13.
7. Rota PA, Featherstone DA, Bellini WJ. Molecular epidemiology of measles virus. Curr Top Microbiol Immunol. 2009;330:129-50.
8. Carr MJ, Conway A, Waters A, Moran J, Hassan J, Hall WW, et al. Molecular epidemiology of circulating measles virus in Ireland 2002-2007. J Med Virol. 2009;81(1):125-9.
9. Mankertz A, Mihneva Z, Gold H, Baumgarte S, Baillot A, Helble R, et al. Spread of measles virus D4-Hamburg, Europe, 2008-2011. Emerging infectious diseases. 2011;17(8):1396-401. Epub 2011/08/02.
10. Smithson R, Irvine N, Hutton C, Doherty L, Watt A. Spotlight on measles 2010: Ongoing measles outbreak in Northern Ireland following an imported case, September-October 2010. Euro Surveill. 2010;15(43):pii=19698. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19698
11. Health Protection Surveillance Centre (HPSC). Immunisation uptake statistics quarterly reports. Dublin: HPSC. Available from: http://www.hpsc.ie/hpsc/A-Z/VaccinePreventable/Vaccination/ImmunisationUptakeStatistics/QuarterlyReports/
12. Central Statistics Office (CSO). This is Ireland - Highlights from census 2011. Dublin: CSO; 2012. Report No. Available from: http://www.cso.ie/en/media/csoie/census/documents/census2011pdr/Census%202011%20Highlights%20Part%201%20web%2072dpi.pdf
13. Haase T, Pratschke J. The new measures of deprivation in the Republic of Ireland. Dublin: Pobal; 2008; Available from: http://trutzhaase.eu/publications/the-new-measures-of-deprivation-in-the-republic-of-ireland/
14. 14.Health Protection Surveillance Centre (HPSC). Case definitions for notifiable diseases. Dublin: HPSC; Mar 2012. Available from: http://www.hpsc.ie/hpsc/NotifiableDiseases/CaseDefinitions/
15. World Health Organization. Global measles and rubella laboratory network--update. Wkly Epidemiol Rec. 2005;80(44):384-8.
16. Swofford DL. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sunderland, Massachusetts: Sinauer Associates; 2003.
17. Health Protection Surveillance Centre (HPSC). Measles guidance. Dublin: HPSC. Available from: http://www.hpsc.ie/hpsc/A-Z/VaccinePreventable/Measles/Guidance/
18. Regional Committee for Europe. Renewed commitment to elimination of measles and rubella and prevention of congenital rubella syndrome by 2015 and sustained support for polio-free status in the Copenhagen: World Health Organisation regional Office for Europe; Sep 2010. Available from: http://www.euro.who.int/__data/assets/pdf_file/0008/119546/RC60_edoc15.pdf
19. Lindley MC, Boyer-Chu L, Fishbein DB, Kolasa M, Middleman AB, Wilson T, et al. The role of schools in strengthening delivery of new adolescent vaccinations. Pediatrics. 2008;121 Suppl 1:S46-54.
20. Joyce-Cooney J, Meagher G, Quinn C, Cavanagh I. Achieving 95% uptake in an immunisation programme--not an impossibility. Ir Med J. 2003;96(9):268-70.
21. Lévy-Bruhl D, Pebody RG, Veldhuijzen I, Valenciano M, Osborne K. ESEN: a comparison of vaccination programmes – Part three: measles mumps and rubella. Euro Surveill. 1998;3(12):pii=112. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=112
22. Molloy B. Community mothers programme. Annual report 2010. Dublin: Health Service Executive; 2010; Available from: http://www.lenus.ie/hse/bitstream/10147/136802/1/CommMothers2010.pdf
23. Johnson Z, Howell F, Molloy B. Community mothers' programme: randomised controlled trial of non-professional intervention in parenting. BMJ. 1993;306(6890):1449-52.
24. Thornton L, Barry J, Kelly A. A policy paper on public health care issues for low income families with children. Dublin: Combat Poverty Agency; Nov 2000. Available from: http://lenus.ie/hse/bitstream/10147/46616/1/2372.pdf
25. Wright JA, Polack C. Understanding variation in measles-mumps-rubella immunization coverage--a population-based study. Eur J Public Health. 2006;16(2):137-42.
26. Baker D, Garrow A, Shiels C. Inequalities in immunisation and breast feeding in an ethnically diverse urban area: cross-sectional study in Manchester, UK. J Epidemiol Community Health. 2011;65(4):346-52.
27. Mankertz A, Mulders MN, Shulga S, Kremer JR, Brown KE, Santibanez S, et al. Molecular genotyping and epidemiology of measles virus transmission in the World Health Organization European Region, 2007-2009. J Infect Dis. 2011;204 Suppl 1:S335-42.
28. Pfaff G, Lohr D, Santibanez S, Mankertz A, van Treeck U, Schönberger K, et al. Spotlight on measles 2010: Measles outbreak among travellers returning from a mass gathering, Germany, September to October 2010. Euro Surveill. 2010;15(50):pii=19750. Available: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19750
29. European Centre for Disease Prevention and Control (ECDC). European monthly measles monitoring (EMMO). Stockholm: ECDC; Jun 2011; Available from: http://ecdc.europa.eu/en/publications/Publications/2011_June_measles_montly.pdf
30. Duraisamy R, Rota PA, Palani G, Elango V, Sambasivam M, Lowe L, et al. Molecular characterization of wild-type measles viruses in Tamil Nadu, India, during 2005-2006: relationship of genotype D8 strains from Tamil Nadu to global strains. J Med Virol. 2012;84(2):348-57. Epub 2011/12/16.
31. Filia A, Tavilla A, Bella A, Magurano F, Ansaldi F, Chironna M, et al. Measles in Italy, July 2009 to September 2010. Euro Surveill. 2011;16(29):pii=19925. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19925.
32. Waku-Kouomou D, Freymuth F, du Chatelet IP, Wild TF, Horvat B. Co-circulation of multiple measles virus genotypes during an epidemic in France in 2008. J Med Virol. 2010;82(6):1033-43.
33. Brown KE, Mulders MN, Freymuth F, Santibanez S, Mosquera MM, Cordey S, et al. Appearance of a novel measles G3 strain in multiple European countries within a two month period, 2010. Euro Surveill. 2011;16(17):pii=19852. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19852
34. Cilla G, Montes M, Artieda J, Piñeiro L, Arriola L, Pérez-Trallero E. Measles genotypes D4 and G3 reintroduced by multiple foci after 15 years without measles virus circulation, Gipuzkoa, the Basque Country, Spain, March to June 2011. Euro Surveill. 2011;16(43):pii=19997. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19997.
35. López Hernández B, Laguna Sorinas J, Marín Rodríguez I, Gallardo García V, Pérez Morilla E, Mayoral Cortés JM. Spotlight on measles 2010: An ongoing outbreak of measles in an unvaccinated population in Granada, Spain, October to November 2010. Euro Surveill. 2010;15(50):pii=19746. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19746.
36. Rota PA, Rota JS, Redd SB, Papania MJ, Bellini WJ. Genetic analysis of measles viruses isolated in the united states between 1989 and 2001: absence of an endemic genotype since 1994. J Infect Dis. 2004;189 Suppl 1:S160-4.
37. Bailey T, Gatrell A. Interactive spatial data analysis. London: Longman; 1995.
38. Goovaerts P, Jacquez GM. Accounting for regional background and population size in the detection of spatial clusters and outliers using geostatistical filtering and spatial neutral models: the case of lung cancer in Long Island, New York. Int J Health Geogr. 2004;3(1):14.
39. Howe G. National Atlas of Disease Mortality in the United Kingdom. London: Nelson; 1963.
40. McKee Jr KT, Shields TM, Jenkins PR, Zenilman JM, Glass GE. Application of a geographic information system to the tracking and control of an outbreak of shigellosis. Clin Infect Dis. 2000;31(3):728-33.
41. Perez L, Dragicevic S. An agent-based approach for modeling dynamics of contagious disease spread. Int J Health Geogr. 2009;8:50.
42. Ulugtekin N, Alkoy S, Seker DZ. Use of a geographic information system in an epidemiological study of measles in Istanbul. J Int Med Res. 2007;35(1):150-4.
43. Chang AY, Parrales ME, Jimenez J, Sobieszczyk ME, Hammer SM, Copenhaver DJ, et al. Combining Google Earth and GIS mapping technologies in a dengue surveillance system for developing countries. Int J Health Geogr. 2009;8:49.
44. Fogarty KJ, Massoudi MS, Gallo W, Averhoff FM, Yusuf H, Fishbein D. Vaccine coverage levels after implementation of a middle school vaccination requirement, Florida, 1997-2000. Public Health Rep. 2004;119(2):163-9.
45. Centers for Disease Control and Prevention (CDC). Effectiveness of a middle school vaccination law--California, 1999-2001. MMWR Morb Mortal Wkly Rep. 2001;50(31):660-3.
46. Steffens I, Martin R, Lopalco PL. Spotlight on measles 2010: Measles elimination in Europe – a new commitment to meet the goal by 2015. Euro Surveill. 2010;15(50):pii=19749. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19749

The application of geographic information systems and spatial data during Legionnaires' disease outbreak responses

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A literature review was conducted to highlight the application and potential benefit of using geographic information systems (GIS) during Legionnaires' disease outbreak investigations. Relatively few published sources were identified, however, certain types of data were found to be important in facilitating the use of GIS, namely: patient data, locations of potential sources (e.g. cooling towers), demographic data relating to the local population and meteorological data. These data were then analysed to gain a better understanding of the spatial relationships between cases and their environment, the cases' proximity to potential outbreak sources, and the modelled dispersion of contaminated aerosols. The use of GIS in an outbreak is not a replacement for traditional outbreak investigation techniques, but it can be a valuable supplement to a response.

Background

Legionnaires' disease (LD) is an atypical type of pneumonia caused by bacteria of the genus *Legionella* [1-3]. The disease mainly affects people over 50 years of age, and generally men more than women [4,5]. Smokers, people with certain occupations, and people with underlying medical conditions may be at a higher risk of infection [1]. The early symptoms of Legionnaires' disease can include an influenza-like illness with muscle aches, tiredness, headaches, dry cough and fever [1,2]. The fatality rate of Legionnaires' disease can vary from 1% to 17% of cases in the general population and may be higher in the risk groups [5-9]. The right skewed incubation period distribution has a median of six days but can range between two and 19 days [10].

Susceptible persons typically become infected when they inhale *Legionella* bacteria in aerosolised form. There is no evidence of person-to-person transmission [11]. *Legionella* organisms are found widely in the environment. They multiply under favourable conditions created by man-made water systems, such as hot and cold water systems, whirlpools, water in air conditioning cooling systems, and cooling towers, from where they can be aerosolised.

The majority of LD cases are reported as single (sporadic) cases which can occur throughout the year, with most cases occurring in late summer and early autumn [3,4,12]. However, clusters and outbreaks also occur [6-9]. During an LD outbreak descriptive epidemiological and (clinical and environmental) microbiological investigations are often sufficient to identify the outbreak source when it becomes clear that all cases have visited a common location. However, there are instances where there is no obvious, common link between cases. It is in these situations that geographic information systems (GIS) can provide supplementary insight.

A GIS can be described as the integration of software and hardware for the digital capture, management, analysis and visualisation of geographically referenced data. The majority of health data are inherently spatial and have a location, be it an address or a broader administrative unit. GIS enable interpretation of this information spatially, looking for patterns, trends and relationships that might exist between disease (or other occurrences), demography, environment, space and time. GIS therefore have wide-ranging applications in public health, including outbreak response.

If a common source is responsible for an increase in LD cases it is reasonable to assume that those infected with LD have been in relatively close spatial proximity to the same source at some point over the likely incubation period range. By using GIS to analyse the spatial distribution of cases and how they have interacted with their environment, including their proximity to potential sources such as cooling towers, it is possible to identify areas in geographical space that are perhaps common between cases and perhaps suggestive of where the source of an outbreak might be located. In this way GIS can help identify an outbreak source, target additional investigation or corroborate findings

from other types of investigation. The aim of this paper was to review the available peer-reviewed scientific literature to highlight the application and potential benefit of using GIS within LD outbreak investigation. This paper will not review the use of GIS for LD outbreak detection or other analysis of surveillance data.

Literature search strategy

The literature was searched at the end of November 2011. The data was sourced using the Scopus (www.scopus.com) and PubMed (www.ukpmc.ac.uk/) citation databases of peer-reviewed literature using the following search terms:

"((cluster analysis OR space OR spatial OR gis OR geographical) AND (legionnaires disease outbreak OR legionellosis))"

The returned titles and abstracts were reviewed by the first author and full texts were obtained for those publications that appeared relevant to the scope of the review. The selected full texts were then further reviewed by all the authors and selected for inclusion, if they provided details on the application of GIS or some type of spatial analysis within an LD outbreak investigation. Articles were excluded if they did not give practical details of the use of GIS or spatial analysis in the context of a *Legionella* outbreak. Additional published materials that were cited in the articles returned by the initial search, and met the selection criteria, were also sourced for inclusion. Unpublished examples of GIS-based analyses employed in LD outbreak response were not considered because they had not been subjected to peer review.

Results

Literature search

Of the 137 articles retrieved in the literature search, four met the inclusion criteria and were included in this review. A further four articles, cited in these articles were also included, together with an additional article that met the inclusion criteria and was known to the authors, but did not appear in the literature search.

Data collection

It is evident that the body of literature covering the application of GIS within LD outbreak response is fairly small; however from the examples available it is clear that the application of GIS relies on the availability of detailed patient case data and, depending on the type of analysis, other data such as information on potential source locations or demographic and meteorological data.

Patient case data

Typically, the incubation period of Legionnaires' disease is between two and 19 days [10]. It is therefore highly desirable to collect data for each patient case (and possibly controls) covering that period of time before the onset of symptoms. In terms of spatial data, home location is commonly recorded as a minimum requirement to identify a case's location in geographic space; however it is likely that patients will not be stationary at their home location but travel throughout their environment, for work or recreation, over the time period in question. Table 1 summarises the patient case data collected for a range of outbreaks reported in the reviewed literature. The majority of those studies [6,7,13-18] collected other data in addition to home location to gain a fuller understanding of the spaces occupied by cases (and possibly controls). Collecting case data to this level of detail is a challenge in itself, both in terms of resource availability for the outbreak control team and the physical ability of cases (who may be seriously ill) to recall and provide such detailed information. As such, such detailed data are often absent from an outbreak investigation, and the application of GIS-based analyses is therefore not possible or seriously restricted.

Potential source locations

Where the source of an LD outbreak is not clear from the initial descriptive epidemiological investigation, it is often the case that cooling towers or other aerosol-emitting facilities are found to be the responsible sources [3]. By collecting details of the locations of these potential sources, GIS can be used to assess the relative likelihood that a source could be responsible for an outbreak based on the spatial movements of patient cases in relation to each potential source location. In a number of countries it is a statutory requirement to register a cooling tower with a particular administrative body, either at a local, regional or national level; however in other countries it is not [20]. It is likely that a desktop assessment using mapping tools, and some field reconnaissance may be required to quality-check such registers and to also identify other potential outbreak sources.

Demographic data

Data about the population in an area are often utilised to calculate attack rates, providing relative measures of disease occurrence or effects [6,7,13,15,16].

Meteorological data

A number of studies have also made use of meteorological data for the purpose of atmospheric dispersion modelling [6,7] in an attempt to identify whether a modelled release from a suspected facility (such as a cooling tower) is consistent with the spatial pattern of infection. Climatic variables within atmospheric dispersion models can include wind speed, wind direction, temperature, humidity and atmospheric stability measures.

Data analysis

The real value of a GIS for an LD outbreak investigation is to take spatially implicit 'textual' information, such as addresses and descriptions of travel movements, and make them spatially explicit geometric features (coordinates) with linked attributes. This information

Table 1

Summary of case data collected in Legionnaires' disease outbreaks for analyses based on geographic information systems

Outbreak	Author(s)	Cases	Case data collected for GIS-based analyses
Pas-de-Calais, France Nov 2003–Jan 2004	[6] Nguyen et al. (2006)	86 cases	As part of a matched case–control study, data on each location visited and time spent at each location (as well as method of transport) was mapped.
Fredrikstad and Sarpsborg, Norway May 2005	[7] Nygard et al. (2008)	56 cases	The location of cases' home addresses was mapped as well as their movements for the 14 days prior to the onset of symptoms.
Murcia, Spain Jul 2001	[13] Garcia-Fulgueiras et al. (2003)	>800 suspected cases reported, 449 confirmed.	As part of a case–control study (consisting of 85 cases and 170 controls), home locations, work locations and travel movements (as well as method of transport) were mapped for the 14 days before onset of symptoms.
Hereford, England Oct–Nov 2003	[14] Kirrage et al. (2007)	28 cases	The location of cases' home addresses was mapped as well as their movements for the 14 days prior to onset of symptoms.
Barcelona, Spain Oct–Nov 2000	[15] Jansa et al. (2002)	54 cases	The location of cases' home addresses was mapped as well as the locations they had visited during the two days prior to their admission to hospital.
Delaware, United States Jul–Sep 1994	[16] Brown et al. (1999)	29 cases	As part of a case–control study participants were asked to identify areas on a gridded map that they had visited, as well as the number of visits made and the length of time spent in each area. Data were collected for the 14 days prior to the onset of symptoms.
South Wales Sep 2010	[17] Keramarou & Evans (2010)	22 cases	The location of cases' home addresses as well as any other locations visited were mapped for the 14 days prior to the onset of symptoms.
Alcoi, Spain July–Sep 2009	[18] Coscolla et al. (2010)	11 cases	The location of cases' home addresses were mapped as well as their movements.
Alcoi, Spain Sep 1999–Dec 2000	[19] Martinez-Beneito et al. (2006)	36 cases in the 1st outbreak, 11 cases in the 2nd outbreak and 97 cases in the 3rd outbreak	As part of a case–control study home addresses were mapped.

can be plotted onto a map and used within analytical operations. Textual information, such as addresses, can often be sufficient to suggest the source of an outbreak if, for example, all cases report having been to the same location, such as a spa pool. However, in those instances where the source remains unclear, the information provided in the case questionnaire can be mapped. Visualising that data on a map could reveal a pattern of infection that may be suggestive of a source or focus the investigation on a particular area. In addition, a number of analytical techniques have been described in the literature that utilise patient case data, as well as other sources of spatial information, to analyse the spatial relationships that exist between cases and their environment.

Potential source proximity analysis

A common strategy employed is to identify potential sources of an outbreak, such as cooling towers, and then to analyse the spatial relationships between each case and each of these potential sources. Kirrage et al. [14] employed this technique within their investigation of the 2003 outbreak in Hereford, United Kingdom. Having identified the locations of cooling towers within the area of the outbreak, each cooling tower location was 'buffered' by 250 m, 500 m, and 1,000 m. A composite score quantified the risk of exposure and therefore the likely source of contamination amongst seven sites of interest in and around the city centre. When reviewing the composite scores, two sites were identified as being associated with significantly more cases. Additional epidemiological and microbiological investigation then enabled the rapid identification of a single cooling tower as the source of the outbreak.

Garcia-Fulgueiras et al. [13] adopted a similar approach as part of their investigation into the world's largest LD outbreak to date with more than 800 suspected cases (449 confirmed) in Murcia, Spain. As part of the case–control study a variety of data were mapped, including home and work addresses, travel movements and method of transport. Also, thirty zones were defined around potential sources of contaminated aerosols (such as cooling towers). The authors analysed movements through each of these zones and revealed a strong association, in all eight multivariate analyses described in the paper, between passing through the zone surrounding a hospital cooling tower and being ill with LD.

In the same way that simple counts or scores can be attached to a buffer or zone, attack rates can be

calculated to provide a relative measure of disease occurrence within a population. Nygard et al. [7] used attack rate analysis to help identify a commercial air scrubber as being the responsible source for the 2005 outbreak in Fredrikstad and Sarpsborg, Norway. The assumption behind this technique is that the risk of infection will decrease with distance from a facility that is responsible for an outbreak. This part of their investigation revealed that people living within 1 km of a particular industrial air scrubber were most at risk, and that was the only source for which the risk decreased with increasing distance.

Dispersion modelling

Nygard et al. [7] used the AirQUIS Gaussian puff model INPUFF [21] to simulate the dispersion of aerosols emitted from a number of potential sources of infection incorporating data on wind direction, velocity, temperature and atmospheric stability. They had to assume values for particle size of the aerosols, pipe diameter, output velocity and emission rate. Whilst acknowledging some of the limitations of the modelled outputs, the plumes were used to establish the proportion of patients who would have been exposed to the various potential sources by either living or visiting a location within the modelled dispersal region during the incubation period. The results showed the best fit, with the same source highlighted by the attack rate analysis.

Similarly, Nguyen et al. [6] also employed aerosol dispersion modelling in an attempt to simulate the dispersion of aerosols from a suspected cooling tower. An atmospheric dispersion modelling system (ADMS) [22] was used to simulate emissions from the suspected facility during each wave of the outbreak. The outputted maps of aerosol dispersions revealed a good fit between the modelled plumes and the geographical distribution of cases.

As is acknowledged by both Nguyen et al. [6] and Nygard et al. [7], there are a number of difficulties in modelling the airborne dispersal of contaminated aerosols. In essence a plume model attempts to track the concentration or dose of a contaminant through space and time following its release into the atmosphere. A simple Gaussian model makes the simplifying assumption that wind is of a fixed speed and direction for the duration of the release and whilst having an inspiration in turbulent fluid theory, the form of the Gaussian plume is dependent on empirical estimates of downwind dispersion. The effects of buildings and vehicles changing the flow are not included in such a model. Essentially dispersion models serve two major functions: firstly, to estimate the exposed population following a potential release, and secondly, to infer potential release sites from the pattern of observed infections. For the former, insufficient evidence has been compiled to suggest an infectious dose of *Legionella* in humans (or the probability of infection following inhalation of a dose), or how long the bacteria can survive in the atmosphere once aerosolised. As such, converting the contours from a plume model into exposure and potential infections is difficult without additional strong assumptions being made. The latter use for dispersion modelling is challenging in the majority of outbreaks as the uncertainty regarding the time of infection means that the location of the infection is unclear. Furthermore the total at-risk population in time and space may be unclear. One is often left with simply stating whether the pattern of infection is consistent with a modelled release, rather than making any stronger statements.

Case-based analysis

If no suspect sources are identified, the focus of analysis will have to be on the spatial interactions of each patient case with their general environment. In other words, there is a need to analyse interactions between the places where people live, the places people have visited and the routes they have taken.

Coscolla et al.'s [18] study into the 2009 LD outbreak in Alcoi, Spain involved the collection of detailed patient case data including home location, any other locations visited and routes of transport. Within a GIS each location and route was then buffered by a 500 m radius, with those buffers representing areas in which contact with contaminated aerosols may have taken place and where infection might have occurred. Areas where different cases' buffers intersected were considered to represent locations more likely to contain the source of infection, with the initial hypothesis being that the outbreak originated from a common, static point source (e.g. a cooling tower). However, the authors noted obvious spatial variation in the data with two different neighbourhoods of the city being linked with particular waves of infection over the course of the outbreak. A secondary hypothesis was proposed: that the source of contamination was mobile. An asphalt paving machine was identified as being the responsible infection source. It was used in both neighbourhoods at times consistent with the pattern of infection attached to each wave of the outbreak.

As part of the investigation by Jansa et al. [15] into the 2000 outbreak in Barcelona, Spain, incidence rates by census tracts (geographic boundaries created for the aggregation and reporting of census data) containing approximately 400 people, revealed significant spatial variation. Within the affected area, the incidence rates revealed that the northern part of the district was more heavily affected (6.4/1,000) than the southern area (2.23/1,000). The identified area was subsequently shown to be in closest proximity to the cooling towers identified as responsible for the outbreak by further environmental and microbiological investigation.

Similarly, attack rate analysis was utilised by Nguyen et al. [6] as part of a wide range of analytical methods investigating the 2003–04 outbreak in Pas-de-Calais, France. Their analysis revealed the attack rate was highest in the Harne commune in which the suspected cooling tower was located.

Martinez-Beneito et al. [19] applied a spatial statistical methodology to investigate three consecutive outbreaks in the industrial city of Alcoi, Spain between September 1999 and December 2000. 36 cases were identified in the first outbreak, 11 in the second outbreak, and 97 in the third outbreak. The authors identified a group of controls who were staying in hospital in the same period as cases in the first outbreak and who were of the same sex and roughly the same age. Residential postcodes were obtained and a spatial point process model was constructed with the aim of identifying whether the geographical distribution of the cases could be considered to be random. Ripley's K function [23], a descriptive statistic for identifying deviation from spatial homogeneity, was estimated for cases and controls and a difference between these statistics was then calculated and tested for statistical significance. Results of significance tests suggested higher aggregation of cases than of controls in all outbreaks. Risk surface maps were also estimated for each outbreak. These were given based on the difference between the observed probability of being a case at a particular location and the expected probability of being a case within the city. Thus areas of high risk were highlighted on which attempts to find a source for each outbreak should focus.

Brown et al.'s [16] study looked into a method for calculating dose of exposure. The outbreak was strongly linked to a hospital in Wilmington, Delaware, United States. Attack rate analysis revealed that the highest relative risk existed among hospital staff and those living within a census tract adjacent to the hospital. In total 29 cases met the study's case definition criteria for LD, and 21 of these were included in the case–control study, with three controls being matched to each case. A standardised questionnaire to interview cases and their controls was used that focussed on the area near the hospital where the attack rate was highest. Interviewees were provided with a gridded map of the area. They were asked to mark possible locations for their exposure in the two weeks before onset of illness. Further information was recorded about the number of visits made and the length of time spent in each grid cell. Separate regression models were used to determine the change in frequency and duration of potential exposure in each grid cell and the change in risk associated with a change in distance from the hospital. Risk of illness was found to decrease with increasing distance from the hospital, but to increase for each additional hour spent in grid cells within 0.125 miles of the hospital. The median dose of modelled potential exposure was higher for cases than controls.

Discussion

The use of GIS in LD outbreak investigation is not a replacement for traditional descriptive epidemiological and microbiological investigative techniques, but it should be viewed as a valuable addition to the public health professional's toolbox. However, it is important to keep in mind that each outbreak is a unique event, and as such not all analytical techniques reviewed in this article will be appropriate in all circumstances. The body of peer reviewed literature covering the application of GIS for LD outbreak investigation is currently relatively small, so the extent to which GIS is used more generally across public health organisations for this purpose is unclear.

Four types of spatial data have been identified in this review as being potentially useful to an outbreak response: case data (i.e. locations visited in incubation period including their home); potential sources in the locality (i.e. a registry of cooling tower locations and field investigation of other sources); information about the broader demography of the population (i.e. how many people live in the administrative regions identified or a control group to compare to cases) and finally meteorological data (i.e. wind speed and direction if dispersion modelling is being performed). To facilitate a response, mechanisms for collecting and storing such data should be in place before an outbreak occurs. These mechanisms should be considered an important aspect of LD outbreak preparedness and have the potential of speeding up and improving substantially the use of these techniques.

Two broad families of statistical analysis were identified from the literature: one using case data to infer zones for further/higher priority field analysis; the other focussing on known potential sources and checking whether the pattern of infection of cases is consistent with a release emanating from there. A third type of analysis that overlaps with these two approaches, dispersion modelling, can be useful if the release occurred over a short time period, but the results of such analysis are likely to be compromised by the uncertainty in infection time of each case and the infectious dose. If resources allow, a carefully designed case–control study that includes appropriate controls might better support source hypothesis testing than using dispersion modelling.

The nature of the outbreak, as well as data availability, will influence the selection of a GIS-based investigative approach. The analytical options, based on data availability, are summarised in Table 2. The techniques that test presumptive sources against the observed distribution of cases can identify a single source, or a number of sources, that are more likely to have been responsible for the outbreak than others. As such these types of analyses can help focus additional investigation, particularly if there are a large number of potential outbreak sources initially being considered. Even in the absence of detailed case data, home locations alone have been successfully utilised, in conjunction with demographic data and potential source location data, to map rates of disease occurrence at varying distances from potential sources. It should be stressed that these techniques are reliant on good quality information about potential source locations, and if the actual source of the outbreak is absent from your

Table 2

Summary of analytical techniques used in Legionnaires' disease outbreak investigations, given data availability

Data type	Examples of possible analyses	Considerations
Patient case data (patient home locations only)	Home locations can be plotted for outbreak visualisation. Density analyses (such as kernel density analysis) may be used to highlight areas in space with a high density of cases. Area(s) of higher density may suggest that the outbreak source is within relatively close spatial proximity. If case–control study data are available, comparative analyses between cases and controls can be performed. Basic cluster analyses can be utilised to identify whether spatial clustering is greater in cases than controls (e.g. Martinez-Beneito et al. [19]). Clustering of cases may suggest that an outbreak source is located within relatively close spatial proximity or may identify a region for further (field) investigation.	Simply plotting patient case home locations can provide a spatial context to an outbreak. Using only home location can bias any analyses, as home location is not necessarily the location of infection.
Patient case data (patient home locations + travel histories)	Case travel histories can be plotted for outbreak visualisation (e.g. Coscolla et al. [18]). Density analyses (such as kernel density analysis) may be used to highlight areas in space with a high density of spatial interactions between cases. Area(s) of higher density may suggest that the outbreak source is within relatively close spatial proximity.	Case data that includes travel histories give a more complete record of the spaces occupied by each case (where infection may have taken place). Clear overlaps may be identified but bias may be introduced (each case's travel history must be carefully weighted so that their contributions are equal and reported movements are fairly accounted). Note that without comparator information travel routes may simply highlight popular commuter routes
Patient case data + potential source location data	Zones or buffers can be established around each of the potential source locations. Overlay analysis can then be used to identify which cases live, work or have travelled within each zone. You would expect the responsible source to display a high number of cases living, working or travelling within its zone, compared to other sources (e.g. Kirrage et al. [14]). If case–control study data are available then comparative analyses between cases and controls can be performed (e.g. Garcia-Fulgueiras et al. [13]).	Centrally archived lists of sources may be obsolete (new unregistered sources or decommissioned sources might exist in the locality). Without well designed case–control/cohort study or demographic data, inference on patient data is likely to be biased (i.e. some areas may be visited rarely by certain groups).
Patient case data + demographic data	Demographic data allows for attack rate analysis, providing a relative measure of disease occurrence within a population. Attack rate analysis can be undertaken using populations attached to small-area administrative units and can potentially highlight areas with higher levels of disease occurrence. Such areas should be within close spatial proximity to an outbreak source (e.g. Nguyen et al. [6] and Jansa et al. [15]). Cluster analysis can be used to identify abnormal grouping of cases in space and time, with new techniques being developed that can measure the degree of association between cases. If case–control study data are available then comparative analyses between cases and controls can be performed (e.g. Brown et al. [16]).	Demographic data are normally based on home locations, however, daytime population figures may be significantly different due to the movements of working populations. Knowledge that cases are clustered in space and time may not reduce an area of interest for potential sources, but may potentially confirm other investigations. Case control data may actually be more appropriate/detailed than general population data that reflect only residence.
Patient case data + potential source location data + demographic data	Radial attack rate analysis buffers each potential source at multiple distances and calculates the attack rate within each buffer. For the responsible facility you would expect to observe a pattern where the attack rate decreases with an increase in distance from the facility (e.g. Nygard et al. [7]).	Demographic data are normally based on home locations, however, daytime population figures may be significantly different due to the movements of working populations. Case control data may actually be more appropriate/detailed than general population data.
Patient case data + potential source location data + demographic data + meteorological data	Dispersion modelling allows you to identify whether a modelled plume from a potential source is consistent with the observed pattern of infection (e.g. Nguyen et al. [6] and Nygard et al. [7]).	Dispersion models can provide intuitive outputs if, for example, the release is over clear short time window, there is only one possible source, or people have not moved. A lack of information on dose response and general uncertainty over infection time for cases means that, in many situations, dispersion models will not inform the outbreak control team's hypotheses.

dataset, it will not be considered within your analysis and subsequently will not be identified.

Alternative approaches examine case data in isolation, looking to identify areas in geographic space that display a higher concentration of spatial interaction amongst cases. Some techniques, such as kernel density, attempt to smooth case point data out across space. In this context, it should be noted that without a comparator population from a control group such analysis is difficult to interpret and is simply an alternative visualisation of the point data. However, even whilst lacking quantitative power, it might highlight areas of particular interest. Others aggregate the observed case numbers to small administrative units for which attack rates can be calculated. Upon identifying areas in space that are seemingly common between cases, investigation can be targeted to look for potential outbreak sources within that vicinity. Care should be taken during the interpretation of outputs that the regions identified are not unduly biased by commuting or other similar behaviours in the underlying population. These techniques can be applied to very detailed case data covering the entire travel histories of patient cases over the course of the likely incubation period. However, as above, they can also be applied in situations where only residential address information is available to provide additional insight into an outbreak.

Outbreaks of Legionnaires disease may be seen as a proxy or analogous to other disease outbreaks, such as Q fever. However, caution should be taken when applying these techniques by judging whether the methodology is appropriate to the specific disease. This is especially true given the fact that the applicability and usefulness of the techniques depend very much on characteristics such as the incubation period of the disease, release and/or transmission characteristics, susceptibility, symptomatology, detection and diagnostics.

A GIS can clearly supplement an outbreak response by quickly visualising both case and potential outbreak source information, as well as providing spatial analytical capabilities to interrogate that data. In order to utilise GIS for these purposes it is important to have clear data collection protocols in place ahead of time, and an awareness of the technical and legal issues around storing and managing such information (particularly patient-identifiable data). The usefulness of GIS to outbreak investigations are largely dependent on the availability of good quality case data, and any enhancements to the way such information is collected would ultimately enhance the application of the spatial analytics used to assist in outbreak responses.

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References

1. Fields BS, Benson RF, Besser RE. Legionella and Legionnaires' disease: 25 years of investigation. Clin Microbiol Rev. 2002;15(3):506-26.
2. Stout JE, Yu VL.Legionellosis. New Engl J Med. 1997;337(10):682-7.
3. World Health Organization (WHO). Legionella and the prevention of legionellosis. Geneva: WHO; 2007. ISBN 92 4 156297. Available from: http://www.who.int/water_sanitation_health/emerging/legionella.pdf
4. European Centre for Disease Prevention and Control (ECDC). Annual epidemiological report on communicable diseases in Europe 2009. Revised Edition. Stockholm: ECDC; 2010 Jun. Available from: http://ecdc.europa.eu/en/publications/Publications/0910_SUR_Annual_Epidemiological_Report_on_Communicable_Diseases_in_Europe.pdf
5. Benin AL, Benson RF, Besser RE. Trends in legionnaires disease, 1980-1998: declining mortality and new patterns of diagnosis. Clin Infect Dis. 2002; 35(9):1039-46.
6. Nguyen TMN, Ilef D, Jarraud S, Rouil L, Campese C, Che D, et al. A community-wide outbreak of Legionnaires' disease linked to industrial cooling towers: How far can contaminated aerosols spread? J Infect Dis. 2006;193(1):102-11.
7. Nygard K, Werner-Johansen O, Ronsen S, Simonsen O, Kanestrom A, Ask E, et al. An outbreak of Legionnaires' disease caused by long-distance spread from an industrial air scrubber in Sarpsborg, Norway. Clin Infect Dis. 2008;46(1):61-9.
8. Den Boer JW, Yzerman PFE, Schellekens J, Lettinga KD, Boshuizen HC, Van Steenbergen JE, et al. A large outbreak of Legionnaires' disease at a flower show, the Netherlands, 1999. Emerg Infect Dis. 2002; 8(1):37-43.
9. Greig JE, Carnie JA, Tallis GF, Ryan NJ, Tan AG, Gordon IR, et al. An outbreak of Legionnaires' disease at the Melbourne Aquarium, April 2000: investigation and case-control studies. Med J Aust. 2004;180(11);566-72.
10. Egan JR, Hall IM, Lemon DJ, Leach S. Modeling Legionnaires' disease outbreaks: estimating the timing of an aerosolized release using symptom-onset dates. Epidemiology. 2011;22(2):188–98.
11. Ricketts KD, Charlett A, Gelb D, Lane C, Lee JV, Joseph CA. Weather patterns and Legionnaires' disease: a meteorological study. Epidemiol Infect. 2009;137(7):1003-12.
12. Health Protection Agency (HPA). Legionnaires' disease. London: HPA. [Accessed 25 Nov 2011]. Available from: http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/LegionnairesDisease/
13. Garcia-Fulgueiras A, Navarro C, Fenoll D, Garcia J, Gonzalez-Diego P, Jimenez-Bunuales T, et al. Legionnaires' disease outbreak in Murcia, Spain. Emerg Infect Dis. 2003; 9(8):915-21.
14. Kirrage D, Reynolds G, Smith GE, Olowokure B. Investigation of an outbreak of Legionnaires' disease: Hereford, UK 2003. Respir Med. 2007;101(8):1639-44.
15. Jansa JM, Cayla JA, Ferrer D, Gracia J, Pelaz C, Salvador M, et al. An outbreak of Legionnaires' disease in an inner city district: Importance of the first 24 hours in the investigation. Int J Tuberc Lung Dis. 2002;6(9):831-8.
16. Brown CM, Nuorti PJ, Breiman RF, Hathcock AL, Fields BS, Lipman HB, et al. A community outbreak of Legionnaires' disease linked to hospital cooling towers: An epidemiological method to calculate dose of exposure. Int J Epidemiol. 1999;28(2):353-9.
17. Keramarou M, Evans MR, for the South Wales Legionnaires' Disease Outbreak Control Team. A community outbreak of Legionnaires' disease in South Wales, August-September 2010. Euro Surveill. 2010;15(42): pii=19691. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19691
18. Coscolla M, Fenollar I, Escribano I, Gonzalez-Candelas F. Legionellosis Outbreak Associated with Asphalt Paving Machine, Spain, 2009. Emerg Infect Dis. 2010; 16(9):1381-7.

19. Martinez-Beneito MA, Abellan JJ, Lopez-Quilez A, Vanaclocha H, Zurriaga O, Jorques G, et al. Source detection in an outbreak of Legionnaires' disease. In: Case studies in spatial point process models. Baddeley A, Gregori P, Mateu J, Stoica R, Stoyan D (eds). Springer: 2005; p. 169-182.
20. Ricketts KD, Joseph C, Lee J, Wewalka G, European Working Group for Legionella Infections. Survey on legislation regarding wet cooling systems in European countries. Euro Surveill. 2008;13(38):pii=18982. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18982
21. Petersen WB, Lavdas LG. INPUFF 2.0 – A multiple source Gaussian puff dispersion algorithm. U.S. Research Triangle Park, NC: Environmental Protection Agency; 1986. Report No.: EPA-600/8-86-024.
22. Cambridge Environmental Research Consultants (CERC). ADMS industrial air pollution model. Cambridge: CERC. [Accessed 25 Nov 2011]. Available from: http://www.cerc.co.uk/environmental-software/model-documentation.html
23. Ripley BD. Spatial statistics. Wiley Series in probability and mathematical statistics. New York: John Wiley & Sons; 1981.