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A new outbreak of brucellosis in Bulgaria detected in July 2015 – preliminary report

R Nenova [1], I Tomova [1], R Saparevska [2], T Kantardjiev [1]
1. National Reference Laboratory High Medical Risk Infections, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
2. Surveillance of Communicable Diseases Department, Regional Health Inspectorate –Kyustendil, Bulgaria

Correspondence: Rumiana Nenova (rnenova62@gmail.com)

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During July 2015 a brucellosis outbreak was detected in Kyustendil district, west Bulgaria. As of 15 August, 31 patients have been diagnosed all with an epidemiological connection to Rila town. Patients have not travelled/worked abroad. Breeding family-owned goats and/or improper use of their milk appear to be the main risk factors for transmission of the infection. This second autochthonous brucellosis outbreak in Bulgaria since 2006, affects the western part of the country.

At the beginning of July 2015, a man in his late 30s presented at the National Reference Laboratory for High Medical Risk Infections (NRL HMRI) in Bulgaria for a consultation and testing in connection with undulant fever up to 38–38.5 °C, that had lasted for ca 30 days. He had had a prior episode of fever starting at the end of April, with an initial empirical antibiotic treatment providing some relief, but soon after the fever had returned, together with myalgia, night sweats and progressive fatigue. The patient resides in Rila town within the Kyustendil district, a district in the west part of the country that has common borders to Serbia and the former Yugoslav Republic of Macedonia (Figure 1). He works as a stock-breeder and declared not consuming milk or milk products of foreign origin. Additionally, he did not report any travel abroad up to one year prior to the onset of symptoms. On the day of the consultation at NRL HMRI, a serum sample from the patient was tested using the Rose Bengal test (BioSystems, Spain) and Brucellacapt (Vircell, Spain), yielding a specific *Brucella* antibody titre of 1:10,240 (diagnostic titre ≥ 1:640). Four days later, during a second visit to the laboratory, a blood culture was initiated on Hemoline Performance Diphasic (Biomerieux, France) at 37 °C with 5% CO_2 and resulted in growth of *Brucella* spp. on day 10. Based on the serological findings and the clinical presentation, the patient was admitted at the University Infectious Disease Hospital in Sofia for treatment of brucellosis.

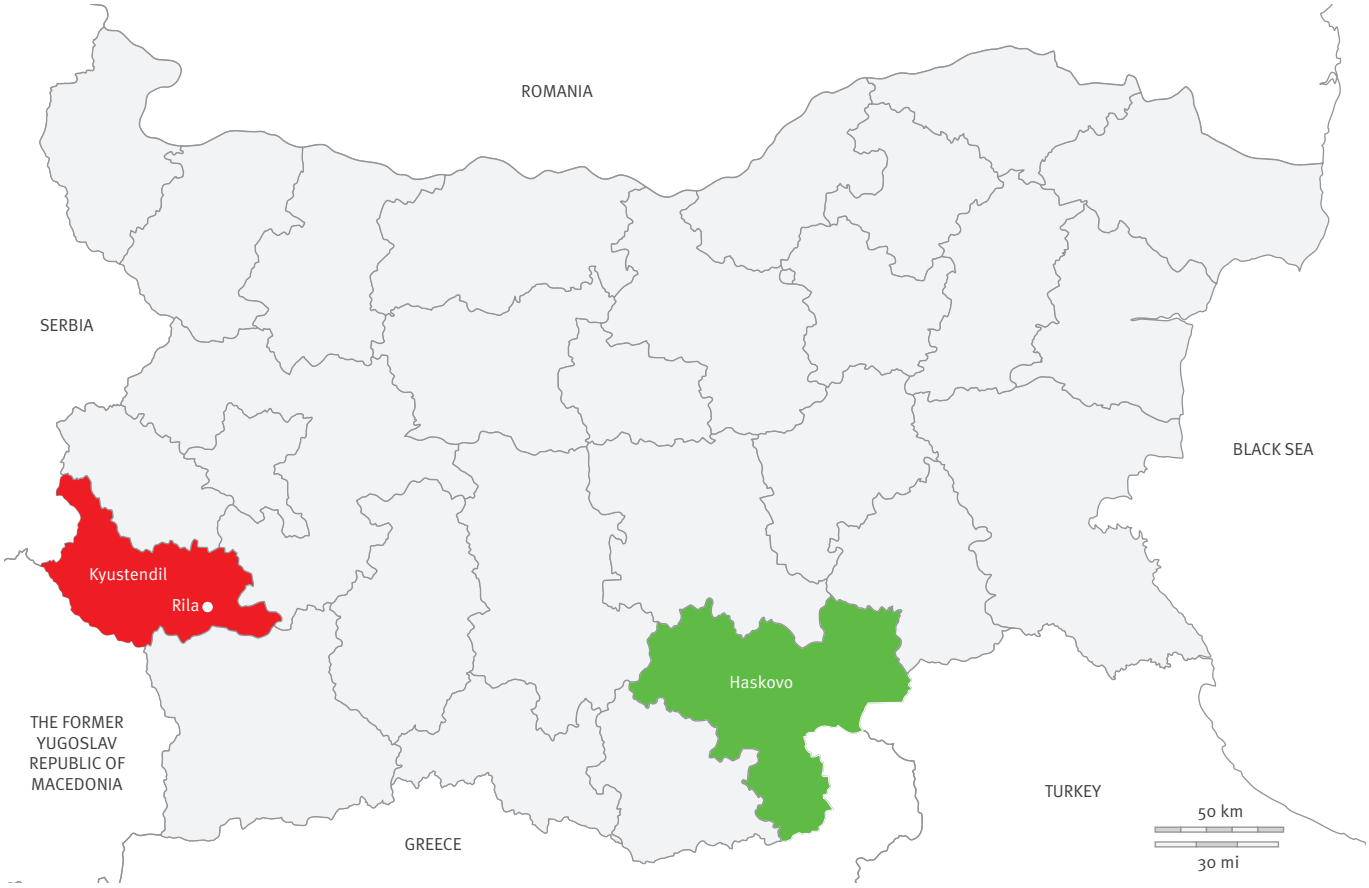
Outbreak in Kyustendil district

Within 24 hours of the diagnosis of the patient, an urgent notification was sent to the Regional Health Inspectorate (RHI), Kyustendil, and the Ministry of Health in accordance with Order 21/2005 for mandatory reportable infectious diseases [1]. Nineteen days after, two other patients, both goat-breeders from the same town as the first patient, were diagnosed with brucellosis. An epidemiological investigation was started immediately in Rila town (2,762 inhabitants). An alert was also sent to the Veterinary Health Service and testing of herds in the area was initiated.

As of 15 August, 31 human infections have been serologically confirmed at the NRL HMRI (Figure 2). Three of the infected persons have professions exposing them to herd animals (2 shepherds and 1 veterinarian), 22 are animal owners/breeders and additionally consumed unpasteurised dairy products from their farms, and six consumed unpasteurised local dairy products only. In accordance with the Public Health Law Act [2], all patients with diagnostic titres are referred to the University Infectious Disease Hospital in Sofia for clinical assessment and treatment. Patients are between 25 and 77 years-old (median: 52.7 years). The most affected age group is that between 60 and 69 years-old (n=8 cases), followed by 30 to 39 year-olds (n=6 cases), two age categories comprising 50 to 59 year-olds and 70 year-olds and over (n=5 cases each), 40 to 49 year-olds (n=4 cases) and 20 to 29 year-olds (n=3 cases). In total, five patients are male and 26 female. Twenty-nine persons are Rila town residents. Two reside outside of the town but visited it or were in its vicinity less than one month prior to illness onset. Of these, one is from another district than Kyustendil, but went to see relatives in Rila town; the other is from a neighbouring city within Kyustendil district, but worked as a veterinarian in an area near the town.

Figure 1

Map showing districts previously (Haskovo) and presently (Kyustendil) affected by autochthonous human brucellosis outbreaks, Bulgaria, 2006–2015



Haskovo district, in green, was the most affected area during a 2006 to 2008 outbreak of brucellosis. Kyustendil district, in red, is where the current 2015 outbreak is still ongoing. All patients in this outbreak have a connection to Rila town, which is also indicated.

Brucellosis in Bulgaria

Brucellosis caused by *B. melitensis* is a widespread zoonosis that can affect every organ and system of the human body [3]. Transmission to humans can occur via direct contact with infected animals and/or consumption of contaminated unpasteurised milk and dairy products. The disease is endemic in many parts of the world, particularly in some areas of the Mediterranean region close to Bulgaria [4]. Despite this, for several decades up to 2006, no autochthonous human brucellosis cases were notified in Bulgaria. In this period, only one outbreak occurred in the country in 2005, but results of a detailed epidemiological investigation revealed that all human cases had been imported [5].

Primary and confirmatory diagnosis of brucellosis in Bulgaria is performed by the NRL HMRI at the National Center of Infectious and Parasitic Diseases, together with the diagnosis of other dangerous infections such as anthrax, glanders, melioidosis, plague, and tularaemia.

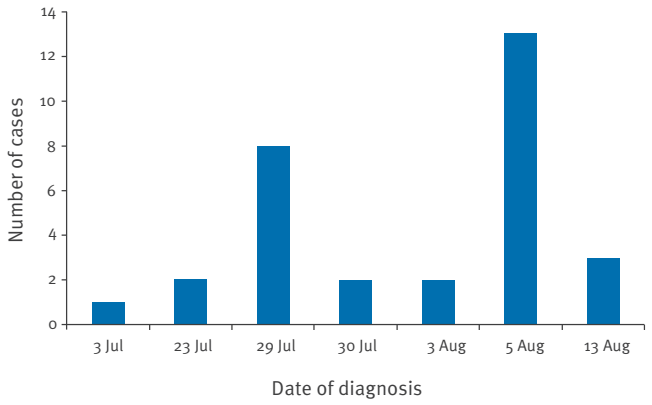
In 2006, seven human cases were microbiologically confirmed in the country, all without any epidemiological data pointing to importation. These patients marked the beginning of an autochthonous outbreak in the south of Bulgaria, with Haskovo district (Figure 1) constituting the most affected area. Epizootiological investigations revealed that the cause of this outbreak was illegal importation of infected animals from a neighbouring endemic country. As a result, by the end of 2008, 71 persons had been infected and more than 600 animals had to be destroyed.

Brucellosis control for herds in Bulgaria is based on the 'test and slaughter' approach. Together with efforts of the Human Public Health authorities, this policy led to a significant reduction in the number of human cases after 2008 [6]. Since 2010, the number of epidemiologically unrelated infections reported annually among Bulgarian residents ranges between zero and two (data not shown).

Based on regular serological testing performed by the Veterinary Health Service, the last having occurred in October 2014, animal herds in the Kyustendil district were considered brucellosis-free up to the current human outbreak. After its detection, serological testing

Figure 2

Distribution of numbers of brucellosis human cases according to date of diagnosis, ongoing outbreak of brucellosis, Kyustendil district, Bulgaria, 2015 (n=31 cases)



of the animals was resumed, and as of 15 August, 97 of 2,245 tested animals were found positive. For more than five decades before the current outbreak, no autochthonous human cases had occurred in this area. Registers point only to one patient, diagnosed in 2006, whereby according to results of an epidemiological investigation, infection most probably occurred when working at a dairy farm in Italy (data not shown).

Epidemiological investigation in Kyustendil district and control measures

Active epidemiological investigation of the current outbreak in Kyustendil not only unveiled new cases of the disease, but also revealed that raw milk stored at ambient temperature, which in July this year was above 30 °C (http://www.meteo.bg/), was frequently consumed by local residents. Based on preliminary descriptive epidemiological findings, breeding of family goats and/or consumption of unboiled milk and homemade soft cheese appear as potential risk factors for transmission of the infection. Some of the patients have no goats but consumed milk and dairy products supplied from friends with small-scale farms. RHI has spread information (through media and leaflets) about the dangerous nature of the disease, mechanisms of transmission, clinical manifestations and the necessary preventive measures, including the recommendation to boil milk for at least 10 min before consumption. During the active surveillance, door-to-door visits have been made not only to ensure the distribution of this information, but also to seize and destroy all improperly homemade and/or stored dairy products. Until further notice, a ban on the use of domestic milk or milk products, such as soft cheese and butter, as well as uncontrolled slaughtering of animals is imposed. According to data from the Bulgarian Food Safety Agency, dairy products available in markets comply with the European regulations of hygiene and safety.

Epizootiological measures

Animal movement is prohibited. Serological testing of small ruminants and cattle for brucellosis is still ongoing in the area. Animals testing positive are subjected to euthanasia and destruction. Animal owners will receive compensation according to the Law of Veterinary Activity. The affected farms, all of them family owned, have been disinfected with Sanofit 1% (Ukrzoovet). The source of infection for the animals and their movement prior the outbreak discovery is under investigation.

Discussion and conclusion

Although the brucellosis outbreak in Kyustendil is still ongoing, the aim of the current report is to highlight its onset. At time of writing, the field investigation is still under way and information on cases occurring after mid-August 2015 is not yet fully complete/available. On the basis of the past history of Kyustendil district as a brucellosis-free area, and the epizootiological situation in the rest of Bulgaria, the current outbreak is most probably a consequence of unauthorised import of infected animals from endemic country/ies. That infected animals in this outbreak were imported from Haskovo district, which was the last previous area with a brucellosis outbreak in Bulgaria is unlikely, as animals in Haskovo district have been regularly tested for *Brucella* since 2008 and to date none have been found positive. Animals from Kyustendil were not likely exposed to contaminated pastures in the vicinity of endemic countries either, as Rila town and its surrounding pastures lie ca 80 km away from the border with the former Yugoslav Republic of Macedonia and ca 140 km from that with Serbia. Uncontrolled movement of animals to/through the borders is moreover prohibited and, according to data from the National Veterinary Health Service no herds positive for brucellosis have been so far detected along the border. Typing and sequencing of *Brucella* isolates is currently under way and may provide more clues as to the source of the outbreak in the future.

As it is well known, prevention of human brucellosis depends on the control of the disease in animals. Ovine/caprine brucellosis has been eliminated in Bulgaria since 1941 and bovine brucellosis since 1958 [7]. As a result, prior to 2006, there had not been any autochthonous human cases in the country for decades. The current outbreak, as well as the previous one (2006–2008), give grounds to pay particular attention to two important points, namely: (i) the occurrence of positive animals in brucellosis-free regions stress on the need for more stringent measures with regard to uncontrolled trade/migration of animals, especially in regions bordering enzootic countries; (ii) the underestimation of the importance of applying preventive measures in local family-owned farms appears to be the main risk factor for brucellosis among Bulgarian residents. More joint efforts are needed from the responsible public health and veterinary authorities to solve these problems. Brucellosis remains a

serious challenge for many of the countries surrounding Bulgaria [8]. Without rapid implementation and continuous compliance with the appropriate measures we could expect a more frequent re-emergence of this zoonosis in the country.

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Conflict of interest

None declared.

Authors' contributions

RN and IT: laboratory testing and notification of the results, interpretation of the microbiological and epidemiological data, writing and editing the manuscript; RS: epidemiological investigation and sampling; TK: supporting the whole investigation and reviewing the final version.

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Spatial methods for infectious disease outbreak investigations: systematic literature review

CM Smith [1], SC Le Comber [2], H Fry [3], M Bull [4], S Leach [4], AC Hayward [1]

1. UCL Department of Infectious Disease Informatics, Farr Institute of Health Informatics Research, University College London, London, United Kingdom
2. School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom
3. Centre for Advanced Spatial Analysis, University College London, London, United Kingdom
4. Emergency Response Department Science and Technology, Public Health England, Porton Down, United Kingdom

Correspondence: Catherine Smith (catherine.smith.13@ucl.ac.uk)

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Investigations of infectious disease outbreaks are conventionally framed in terms of person, time and place. Although geographic information systems have increased the range of tools available, spatial analyses are used relatively infrequently. We conducted a systematic review of published reports of outbreak investigations worldwide to estimate the prevalence of spatial methods, describe the techniques applied and explore their utility. We identified 80 reports using spatial methods published between 1979 and 2013, ca 0.4% of the total number of published outbreaks. Environmental or waterborne infections were the most commonly investigated, and most reports were from the United Kingdom. A range of techniques were used, including simple dot maps, cluster analyses and modelling approaches. Spatial tools were usefully applied throughout investigations, from initial confirmation of the outbreak to describing and analysing cases and communicating findings. They provided valuable insights that led to public health actions, but there is scope for much wider implementation and development of new methods.

Introduction

Detecting and responding to outbreaks of infectious diseases is a key role of front-line public health organisations [1]. The primary reason for conducting an investigation into an outbreak is prevention of further cases through control measures, while other motivations include addressing public or political concerns, evaluating health programmes and advancing understanding of the disease [2]. Investigations are usually cross-agency exercises and conventionally involve examination of the outbreak in terms of person, time and place.

John Snow famously demonstrated the power of plotting the spatial locations of individuals affected in an outbreak [3]. His map of cholera cases in London in 1854 showed a clear pattern that implicated a water pump as the likely source of the illness. Today, guidelines for investigating outbreaks, including the European Centre for Disease Prevention and Control (ECDC) Outbreak Investigation Toolbox, invariably also recommend consideration of case locations [4-7]. Nevertheless, epidemiological investigations of outbreaks, and research into novel approaches for such investigations, have tended to focus more on analysis of person and time than of place [8]. Development of advanced molecular tools, for example, has allowed transmission of infectious agents among populations to be traced with ever increasing detail. Without also considering the spatial aspects of an outbreak, however, important relationships and therefore aetiological insights may be missed [8].

Geographic information systems (GIS) have increased the availability and range of tools that can be used to analyse outbreaks. A GIS is a database designed to handle geographically-referenced information complemented by software tools for the input, management, analysis and display of data [9]. GIS are used widely in epidemiology and the simplest application in an outbreak investigation is to create maps displaying the relative locations of cases, potential sources and/or risk factors. Maps are an engaging and easy-to-understand means of presenting data and can be used to describe patterns, identify outliers and communicate findings. Cases can be plotted using their point locations or aggregated into administrative areas and displayed as rates. Smoothed incidence maps are an alternative means of visualising point locations as continuous distributions of disease risk, generated by adjusting the density at each point according to the number of cases in adjacent areas [9]. Areas can also be demarcated according to locations of potential sources of infection.

Figure 1

Maps of John Snow's cholera outbreak investigation in London in 1854

A: Dot map of case locations

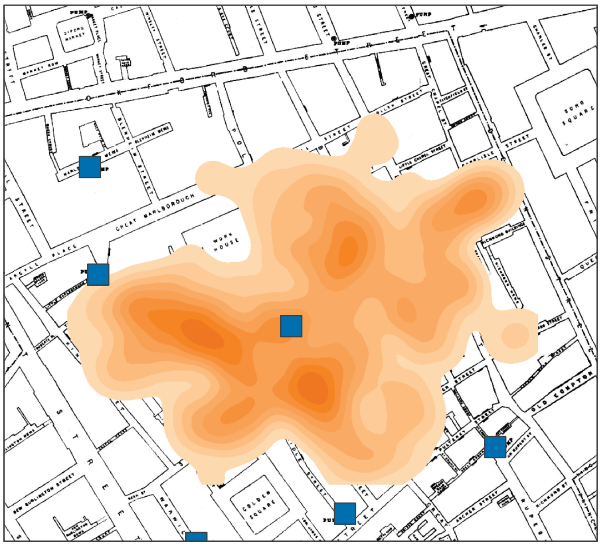
Water pumps

Cholera cases

N

0.25

Kilometers



B: Smoothed intensity of case locations

Case intensity

High

Low

Water pumps

Cholera cases

C: Voronoi diagram demarcating area according to nearest water pump

Voronoi tesselation

Pumps

Cholera cases

Examples of these different approaches to mapping, using Snow's cholera data, are shown in Figure 1.

Spatial relationships not immediately apparent from maps can also be explored using GIS. Measuring distances from cases to potential sources, for example, can be informative if an infection is suspected to derive from an environmental point source. In outbreaks of Legionnaires' disease, this method has been applied to identify cooling towers or other aerosol-producing devices proximal to the cases and therefore generate hypotheses about the likely source [10]. Integration of additional data in the GIS, such as wind direction, can further aid hypothesis generation, for example by identifying areas most likely to be exposed to air emitted from a suspected source during an outbreak of Q fever [11].

Identification and analysis of clusters, areas with higher than expected levels of disease risk, can trigger and be informative during outbreak investigations. Numerous geostatistical methods have been developed to detect clusters, including methods for point and aggregated data [9,12]. 'Global' tests evaluate the entire area for any evidence of clustering, without pinpointing specific clusters, while 'local' (or 'cluster detection') tests identify the positions of specific clusters. Cuzick and Edwards' *k*-nearest neighbour test, for example, is a global method for assessing clustering in case–control point data [13]. It counts the number of nearest neighbours of cases that are also cases, and compares it to the number that would be expected under the null hypothesis that cases and controls were randomly distributed. Kulldorff's spatial scan statistic is a method used to identify local clustering, usually in point data [14]. Observed numbers of cases within windows of various sizes are compared with numbers that would be expected under a random distribution. Circular or elliptical regions of elevated risk of disease are then located. Scan statistics and the *k*-nearest neighbour test have also been adapted to identify spatiotemporal clustering, testing the null hypothesis that cases geographically close to each other occur at random times [15,16].

Spatial relationships in outbreak data can also be analysed through modelling. A range of techniques can be used which, broadly, aim to create informative representations of features, events and processes in geographical space. Environmental risk mapping, for example, uses statistical methods to define relationships between spatially referenced variables and disease risk [9]. Air dispersion models, meanwhile, can be used to identify spatial locations likely to have been exposed to air-borne infections and infer potential release sites [10].

In this study, we explore through a systematic literature review how methods of spatial visualisation and analysis have been employed in infectious disease outbreak investigations. We aimed to use published reports of outbreak investigations (i) to describe the prevalence, utility and outcomes of applying spatial methods and (ii) to make recommendations for improving practice and identify opportunities for further development in this area.

Methods

Search strategy and selection criteria

The aim of our literature search was to identify published reports of infectious disease outbreak investigations that used spatial methods. We defined an outbreak as the occurrence of a series of cases of disease in excess of the number expected in a given time and place. We focused only on outbreaks with local or regional impact and excluded large national or multinational studies of epidemics or pandemics, such as pandemic influenza. Studies describing retrospective analyses of outbreaks that used spatial methods which could theoretically be applied in real-time investigations were included.

We employed a broad search strategy of multiple electronic databases with few restrictions in order to minimise the risk of bias: We searched Embase, Medline and Web of Science for items with terms relating to spatial analysis ('spatial', 'cluster', 'geographic information systems', 'GIS', 'mapping') and outbreaks ('disease outbreak', 'outbreak', 'epidemic'). The search was run on 28 November 2013 and restricted to articles published after 1980 (Embase), 1946 (Medline) and 1900 (Web of Science). No exclusions were made on basis of language or location, and articles were not limited to human disease. Additional relevant articles known to the authors that were not retrieved from the database search were also added to the results.

After deduplication, titles and abstracts were reviewed to identify articles that met our inclusion criteria: Articles had to relate to an infectious disease, they had to describe an investigation of an outbreak (as defined above) and they had to involve application of spatial analysis or mapping. Abstracts that did not include clear information on the inclusion criteria were brought forward for full-text review. Full texts of articles were assessed with the same inclusion criteria.

We then ran a search of the same databases using only the outbreak investigation terms. We simulated the deduplication and screening process that would result from this search by excluding the same proportion of articles at each step as in the original search. This allowed us to obtain a crude estimate of the total number of published reports of infectious disease outbreak investigations and therefore the proportion that used spatial methods.

Data extraction

Each included study was reviewed and information about the spatial methods and outcomes of the studies extracted (Table 1). Descriptive details obtained

Table 1A

Details of included studies, systematic literature review on spatial methods in infectious disease outbreak investigations (n = 80)

Reference	Infectious disease	Location	Publication year	Context	Prospective/ retrospective	Spatial methods used	Stage of investigation[a]	Outcome summary
Acheson [44]	Syphilis	United Kingdom	2011	Sexual transmission	P	Dot map; rate map	4, 7	Some clusters found in high deprivation areas; adverts placed on social networks linked to users' postcodes
Boccia [67]	Salmonellosis	United Kingdom	2004	Food	P	Dot map; spatial case definition; source proximity	3, 4, 5	No significant difference between closest case and control to suspect outlets
Carr [59]	Legionnaires' disease	United Kingdom	2010	Environmental	P	Dot map; case movement map; spatial case definition	3, 4	Identified no hot spots; concluded pseudo-cluster
Hyland [47]	Legionnaires' disease	United Kingdom	2008	Environmental	P	Dot map; case movement map; spatial case definition	3, 4, 5, 6, 7, 8	Sullage tanks identified as source; review of national guidelines
Keramarou [26]	Legionnaires' disease	United Kingdom	2010	Environmental	P	Dot map; case movement map; spatial case definition	3, 4	Two distinct spatiotemporal clusters identified but no definitive source
Kirrage [40]	Legionnaires' disease	United Kingdom	2007	Environmental	P	Dot map; case movement map; spatial case definition; source proximity	3, 4, 5, 7	Identified cluster of cooling towers as likely source; closed and cleaned one of the towers
Neira-Munoz [68]	Cryptosporidiosis	United Kingdom	2007	Water	P	Dot map; thematic map; spatial case definition	3, 4, 6	Hypothesis that low level contamination of drinking water caused outbreak; potential change in water monitoring suggested
Sanson [69]	Foot and mouth disease	United Kingdom	2011	Farm	R	Dot map; spatial case definition; source proximity; case–case distance; air dispersion modelling	5, 6	Distance and direction from index farm significant predictors of infection status; minimum infective dose might be less than previously established
Wallensten [33]	Q fever	United Kingdom	2010	Farm	P	Dot map; spatial case definition; air dispersion modelling	3, 4, 5	Air from each of suspected farms may have exposed town, couldn't rule any out as potential sources
Le Comber [34]	Cholera & malaria	United Kingdom & Egypt	2011	Vector/water	R	Dot map; spatial average; geographic profiling	4, 5	Identified most likely locations of sources of infection
Manfredi Selvaggi [70]	Q fever	Italy	1996	Farm	P	Rate map; thematic map; spatial case finding	3, 4	Infected individuals tended to live closer to sheep migration route
Orsi [71]	Measles	Italy	2010	Community	P	Dot map	4	Identified worst affected areas
Varani [72]	Leishmaniasis	Italy	2013	Vector	P	Dot map	3, 4	Most patients in hilly, rural areas
Norstrom [31]	Acute respiratory disease	Norway	1999	Farm	R	Dot map; smoothed incidence map; spatial case definition; space-time scan statistic; k-nearest neighbour test; Knox test	3, 4, 5	Described progression of outbreak; identified cluster; supports hypothesis of single common source of infection
Nygard [32]	Legionnaires' disease	Norway	2008	Environmental	P	Dot map; case movement map; spatial case definition; source proximity; air dispersion modelling	3, 4, 5, 6, 7	Identified industrial air scrubber as source of outbreak; scrubber closed, new routines for cleaning and national regulations implemented

MMR: measles-mumps-rubella vaccine; NA: not available; P: prospective; R: retrospective; SARS: severe acute respiratory syndrome.

[a] Stages in outbreak investigations defined as: 1. Establishing existence of an outbreak; 2. Confirming diagnosis; 3. Defining and identifying outbreak cases; 4. Describing cases and developing hypotheses; 5. Evaluating hypotheses and drawing conclusions; 6. Comparing with established facts; 7. Executing prevention measures; 8. Communicating findings.

Table 1b

Details of included studies, systematic literature review on spatial methods in infectious disease outbreak investigations (n = 80)

Reference	Infectious disease	Location	Publication year	Context	Prospective/ retrospective	Spatial methods used	Stage of investigation[a]	Outcome summary
Nygard [41]	Giardiasis	Norway	2006	Water	P	Dot map; thematic map; spatial case definition	3, 4, 5, 7	Higher attack rate in zone supplied by water supply A; boil water notice issued; flushed distribution system
Abellan [73]	Legionnaires' disease	Spain	2002	Environmental	R	Dot map; smoothed incidence map; k-function	4 ,5	Cases more aggregated than controls; confirmed environmental origin of outbreak
Garcia-Fulgueiras [57]	Legionnaires' disease	Spain	2003	Environmental	P	Rate map; spatial case definition; source proximity	3, 4, 5, 6	Zone of exposure around hospital associated with illness; replaced cooling tower; Legionella may be able to spread over larger distances from source than previously thought
Jansa [58]	Legionnaires' disease	Spain	2002	Environmental	P	Dot map; spatial case definition	3, 4	Cooling towers identified as source
Hackert [11]	Q fever	The Netherlands	2012	Farm	R	Dot map; smoothed incidence map; spatial case definition; source proximity	3, 4, 5, 6	Incidence increased with proximity to index farm; cases scattered in wedge shape area downwind of farm
Schimmer [74]	Q fever	The Netherlands	2010	Farm	R	Dot map; thematic map; spatial case definition; source proximity; spatial average	3, 4, 5	Gradual diminishing risk from certain farms, identified as probable sources
van der Hoek [50]	Q fever	The Netherlands	2012	Farm	R	Dot map; rate map; thematic map; smoothed incidence map; source proximity	4, 8	Identified 5 hot spots, all around infected dairy goat farms
Gubbels [42]	Campylobacteriosis	Denmark	2012	Water	P	Dot map; thematic map; spatial case definition	3, 4, 7	Cases lived across entire water supply area; concluded contamination of central water supply; implemented boiling order
Nguyen [63]	Legionnaires' disease	France	2006	Environmental	P	Dot map; rate map; case movement map; spatial case definition; spatial case finding; air dispersion modelling	3, 4	Dispersion of plumes from cooling tower correlated with geographical distribution of cases; spread over longer distance than previously thought possible
Kistemann [29]	Salmonellosis	Germany	2000	Hospital	P	Thematic maps; schematic map; spatial case definition	1, 3, 4, 7, 8	Identified functional relationship between cases; measures introduced to prevent future outbreaks
Fitzpatrick [28]	Measles	Ireland	2012	Community	P	Dot map; thematic map	4, 7	Identified emergence of cluster during outbreak in real time; intervention in high rate area - expediated MMR vaccine schedule/ catch-up campaign
Ulugtekin [18]	Measles	Turkey	2007	Community	P	Dot map; thematic maps	4	Identified high incidence areas

MMR: measles-mumps-rubella vaccine; NA: not available; P: prospective; R: retrospective; SARS: severe acute respiratory syndrome.

[a] Stages in outbreak investigations defined as: 1. Establishing existence of an outbreak; 2. Confirming diagnosis; 3. Defining and identifying outbreak cases; 4. Describing cases and developing hypotheses; 5. Evaluating hypotheses and drawing conclusions; 6. Comparing with established facts; 7. Executing prevention measures; 8. Communicating findings.

Table 1c

Details of included studies, systematic literature review on spatial methods in infectious disease outbreak investigations (n = 80)

Reference	Infectious disease	Location	Publication year	Context	Prospective/ retrospective	Spatial methods used	Stage of investigation[a]	Outcome summary
Lai [75]	Influenza	Hong Kong	2010	Community	R	Dot map; smoothed incidence map; standard deviation ellipse; Moran's I; Getis-Ord Gi* statistic	4, 5	Identified hot spots and directional trend
Lai [76]	SARS	Hong Kong	2004	Community	R	Dot map; rate map; smoothed incidence map; standard deviation ellipse; origin–destination plots; Moran's I; nearest neighbour analysis	4, 5	Clear clustering identified; directional bias and radius of spread of superspreading events demonstrated
Sze-To [77]	Varicella	Hong Kong	2011	Hospital	R	Schematic map; air dispersion modelling	4, 5	Model matches epidemiological distribution of cases
Wong [39]	Influenza	Hong Kong	2010	Hospital	R	Schematic map; spatial case definition; source proximity; air dispersion modelling	3, 4, 5, 6	Proximity to air purifier associated with infection; suggests possible role for aerosol transmission
Yu [78]	SARS	Hong Kong	2005	Hospital	R	Schematic map; spatial case definition; air dispersion modelling	3, 4, 5	Attack rates higher in bays closer to index patient; suggests airborne transmission played important role
Bali [27]	Hepatitis E	India	2008	Water	P	Dot map; spatial case finding; spatial case definition	3, 4	Cases mapped to water supply distribution area
Nisha [43]	Dengue fever	India	2005	Vector	P	Dot map; spatial case finding; scan statistic	3, 4, 7	Identified cluster; fogging and larval reduction; drawing up standard protocol for GIS in outbreaks
Saha [79]	Shigellosis	India	2009	Water	P	Rate map; spatial case definition	3, 4	Incidence higher downstream of damaged pipeline
Sarkar [46]	Diarrhoea	India	2007	Water	P	Dot map; thematic map; spatial case definition; source proximity; spatial case finding; spatial scan statistic	3, 4, 5, 7, 8	Showed dispersed nature of outbreak; no significant clustering; funds released to improve drainage network
Sowmyanarayanan [56]	Hepatitis A	India	2008	Water	P	Dot map; spatial scan statistic	4, 5	Cluster not significant; outbreak generalised across area
Fang [80]	Influenza	China	2013	Community	R	Dot map; thematic map; environmental risk prediction model	4	Identified high incidence areas; predicted areas with high risk to inform future control efforts
Liang [81]	SARS	China	2007	Community	R	Rate map	4	Rate increased with distance from city centre, supported spatial quarantining of city for future outbreaks
Ali [82]	Dengue fever	Bangladesh	2003	Vector	R	Dot map; thematic map; smoothed incidence map; source proximity; spatial case finding; kriging	3, 4, 5	Clusters identified, generally closer to major hospitals; spatial association between clusters and vector populations
Tenzin [83]	Rabies	Bhutan	2010	Community	R	Dot map; spatial average; standard deviation ellipse	4	Visualised spread of outbreak; seemed to follow road network that had many free-roaming dogs

MMR: measles-mumps-rubella vaccine; NA: not available; P: prospective; R: retrospective; SARS: severe acute respiratory syndrome.

[a] Stages in outbreak investigations defined as: 1. Establishing existence of an outbreak; 2. Confirming diagnosis; 3. Defining and identifying outbreak cases; 4. Describing cases and developing hypotheses; 5. Evaluating hypotheses and drawing conclusions; 6. Comparing with established facts; 7. Executing prevention measures; 8. Communicating findings.

Table 1d

Details of included studies, systematic literature review on spatial methods in infectious disease outbreak investigations (n = 80)

Reference	Infectious disease	Location	Publication year	Context	Prospective/ retrospective	Spatial methods used	Stage of investigation[a]	Outcome summary
Nishiguchi [84]	Influenza	Japan	2009	Farm	R	Dot map; scan statistic	3, 4, 5	Identified cluster and factors associated with farms inside cluster
Siddiqui [85]	Cholera	Pakistan	2006	Water	R	Dot map; spatial case definition; k-nearest neighbour test	3, 4, 5	Clustering in one of the outbreaks investigated; water reservoir identified as likely source
Miranda [86]	Ebola	Philippines	2002	Breeding facility	R	Schematic map	4	Documented progression of outbreak
Le [87]	Porcine high fever disease	Vietnam	2012	Farm	R	Dot map; smoothed incidence map; spatial and space–time scan statistic; k-nearest neighbour test; Knox test; space–time k function	4, 5	Little evidence for clustering; thought not to be important in this outbreak
Addiss [88]	Legionnaires' disease	United States	1989	Environmental	P	Dot maps; spatial case definition; source proximity	3, 4, 5	Rate decreased with distance from one cooling tower; implicated as probable source of outbreak
Blondin [89]	Blastomycosis	United States	2007	Environmental	R	Dot map; thematic map; source proximity	4, 5	No common source identified; infection likely to have been acquired close to homes
Brown [60]	Legionnaires' disease	United States	1999	Environmental	P	Dot map; thematic map; spatial case definition; source proximity	3, 4, 5	Transmission mostly in close proximity to cooling towers
Chung [90]	West Nile fever	United States	2013	Vector	R	Dot map; rate map; Getis-Ord Gi* statistic	4, 5	As outbreak progressed it became clustered and hot spot was identified
McKee [36]	Shigellosis	United States	2000	Water	P	Dot map; k-nearest neighbour test	4, 7	Space–time clustering found; identified communal wading pools as probable source; targeted information campaigns and education
Mongoh [91]	Anthrax	United States	2008	Farm	R	Dot map; thematic map	4	Displayed spatial distribution of premises with cases n study
Pfister [92]	Blastomycosis	United States	2011	Environmental	P	Dot map; spatial case definition; spatial average	3, 4	Centre of outbreak identified, north of river; yard waste disposal identified as likely source
Roy [25]	Blastomycosis	United States	2013	Environmental	P	Dot map; spatial case definition; scan statistic	1, 3, 4	Confirmed the presence of the outbreak
Bowie [93]	Toxoplasmosis	Canada	1997	Water	P	Dot map; thematic map	4, 5	Outbreak-related cases in area served by water distribution system
Epp [94]	Anthrax	Canada	2010	Farm	R	Thematic map; smoothed incidence map; velocity vector map; spatial case definition; space time scan statistic; k-nearest neighbour test; k-function; Oden's Ipop	4, 5	Three separate movements of spread identified; clusters located

MMR: measles-mumps-rubella vaccine; NA: not available; P: prospective; R: retrospective; SARS: severe acute respiratory syndrome.

[a] Stages in outbreak investigations defined as: 1. Establishing existence of an outbreak; 2. Confirming diagnosis; 3. Defining and identifying outbreak cases; 4. Describing cases and developing hypotheses; 5. Evaluating hypotheses and drawing conclusions; 6. Comparing with established facts; 7. Executing prevention measures; 8. Communicating findings.

Table 1e

Details of included studies, systematic literature review on spatial methods in infectious disease outbreak investigations (n = 80)

Reference	Infectious disease	Location	Publication year	Context	Prospective/ retrospective	Spatial methods used	Stage of investigation[a]	Outcome summary
Parkinson [22]	Anthrax	Canada	2003	Farm	R	Dot map; thematic maps	4	Described physical characteristics of outbreak and documented spatial descriptive patterns
Pasma [95]	Influenza	Canada	2008	Farm	R	Thematic map; spatial average; standard deviation ellipse; k-nearest neighbour test; spatial scan statistic; Knox test; nearest neighbour analysis; Mantel's test	4, 5, 6	Identified and located clusters; outbreak established in densely populated areas, moved to less densely populated areas; suggests focus for surveillance
Morrison [96]	Dengue fever	Puerto Rico	1998	Vector	R	Dot map; Knox test; k-function analysis; Barton–David test	3, 4	Significant case clustering within households over short periods of time; but in general, cases had pattern similar to population as a whole; control measures need to be applied to entire municipality
Chadee [97]	Meningococcal meningitis	Trinidad	2006	Community	P	Dot map; case–case distance	1, 4	Revealed two clusters
Chadee [55]	Dengue fever	West Indies	2005	Vector	R	Dot map; k-nearest neighbour test	4	Cases occurred in clusters when mosquito densities were high enough
Affolabi [24]	Tuberculosis	Benin	2009	Community	R	Dot map; case movement map	1, 4	Identified potential cluster
Bartels [98]	Cholera	Ethiopia	2010	Water	P	Dot map	4	Cases mapped along river; thought to be most likely source
Luquero [49]	Cholera	Guinea-Bissau	2011	Water	P	Dot map; rate map; smooth incidence maps; spatial case finding; scan statistic; k-function	3, 4, 5, 7, 8	Two clusters identified; improved sanitation systems and hygiene collection in affected area
Rivas [23]	Influenza	Nigeria	2010	Farm	R	Dot map; thematic maps; spatial case definition; case–case distance; risk factor proximity	3, 4, 5	Supports hypothesis that major highway network promoted epidemic spread
Roquet [20]	Cholera	Senegal	1998	Water	R	Dot map; rate map	4	Identified high incidence areas
Bessong [99]	Diarrhoea	South Africa	2009	Water	P	Dot map; spatial case finding	3, 4	Identified hot spots of the outbreak; two water extraction points implicated
Fevre [35]	Trypanosomiasis	Uganda	2001	Vector	R	Dot map; spatial case definition; source proximity; spatial scan statistic	3, 4, 5	Significant cluster detected; distance from market significant risk factor
Sasaki [30]	Cholera	Zambia	2008	Water	R	Dot map; rate map; Voronoi diagram; nearest neighbour analysis; Moran's I	4, 5	Significant clustering found in areas with lower coverage of latrines and effective drainage systems
Fernandez [45]	Cholera	Zimbabwe	2011	Water	R	Dot map; thematic map; rate map; empirical Bayes smoothing	4, 5, 7	Spatial pattern linked to historical social construction of city characterised by distinct regions of socioeconomic status

MMR: measles-mumps-rubella vaccine; NA: not available; P: prospective; R: retrospective; SARS: severe acute respiratory syndrome.

[a] Stages in outbreak investigations defined as: 1. Establishing existence of an outbreak; 2. Confirming diagnosis; 3. Defining and identifying outbreak cases; 4. Describing cases and developing hypotheses; 5. Evaluating hypotheses and drawing conclusions; 6. Comparing with established facts; 7. Executing prevention measures; 8. Communicating findings.

Table 1f

Details of included studies, systematic literature review on spatial methods in infectious disease outbreak investigations (n = 80)

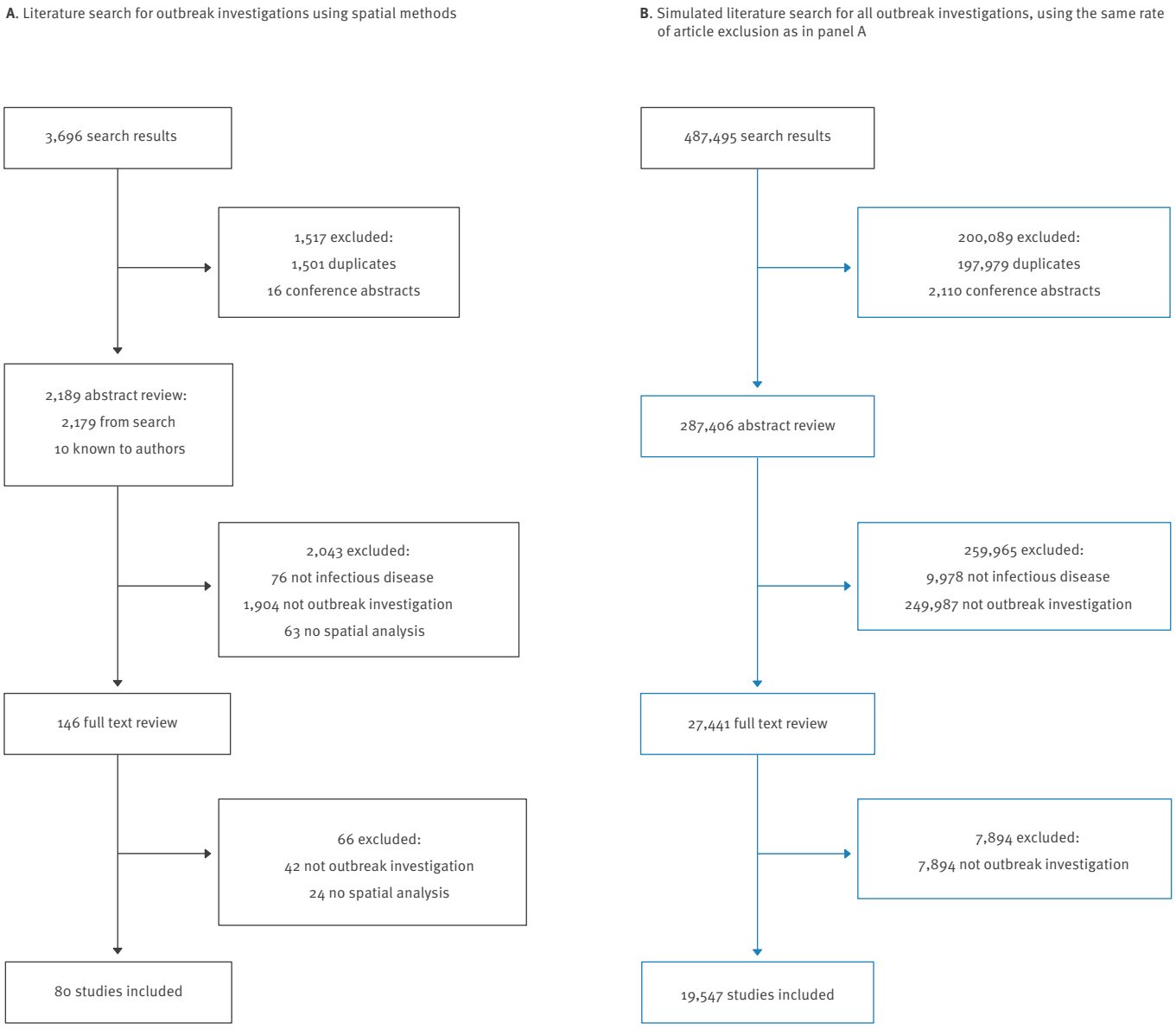
Reference	Infectious disease	Location	Publication year	Context	Prospective/ retrospective	Spatial methods used	Stage of investigation[a]	Outcome summary
Angulo [100]	Variola minor	Brazil	1979	Community	R	Dot map; smoothed incidence map	4	Demonstrated importance of schools in epidemic spread
Barcellos [21]	Leptospirosis	Brazil	2000	Water	R	Dot map; thematic maps; risk factor proximity	4	Concentration of cases observed; identified characteristics of areas
Barreto [19]	Schistosomiasis	Brazil	1993	Vector	P	Dot map; thematic maps	4	Children with frequent water contact around open bodies of water, no sewage disposal, absence of water supply associated with infection
de Moura [101]	Toxoplasmosis	Brazil	2006	Water	P	Dot map; rate map	4, 6, 7	Cases more likely to be served by water reservoir A than B; closed reservoir.
Passos [102]	Rabies	Brazil	1998	Community	R	Dot map	4	Cases corresponded to parts of city with most slums and lower income populations
Rotela [48]	Dengue fever	Argentina	2007	Vector	R	Dot map; smoothed incidence map; Knox test; environmental risk prediction model	4, 5	Identified clusters and developed predictive risk model
Rivas [103]	Foot and mouth disease	Uruguay	2003	Farm	R	Dot map; thematic map; source proximity	4, 5	Generated hypothesis that early epidemic virus disseminated took advantage of road network, then spread outwards
Firestone [104]	Influenza	Australia	2011	Farm	R	Dot map; smoothed incidence map; spatial social network analysis; space-time scan statistic; kriging	4, 5, 6	Local spread through contact network to distance of 15 km; identified 5 significant clusters
Waldron [105]	Cryptosporidiosis	Australia	2011	Water	R	Dot map	4	Identified hot spots and movement of cluster over time
White [38]	Legionnaires' disease	New Zealand	2013	Environmental	P	Dot map; thematic map; scan statistic; Moran's I	4, 5, 6, 8	Identified clusters; case distribution consistent with plume effect from probable source
Turcios-Ruiz [106]	Necrotising enterocolitis	NA	2008	Hospital	P	Schematic map; spatial case definition; Grimson test	3, 4, 5	Clustering identified; suggested possible association with caregivers working in affected area

MMR: measles-mumps-rubella vaccine; NA: not available; P: prospective; R: retrospective; SARS: severe acute respiratory syndrome.

[a] Stages in outbreak investigations defined as: 1. Establishing existence of an outbreak; 2. Confirming diagnosis; 3. Defining and identifying outbreak cases; 4. Describing cases and developing hypotheses; 5. Evaluating hypotheses and drawing conclusions; 6. Comparing with established facts; 7. Executing prevention measures; 8. Communicating findings.

Figure 2

Study selection, systematic literature review on spatial methods in infectious disease outbreak investigations (n = 3,696)



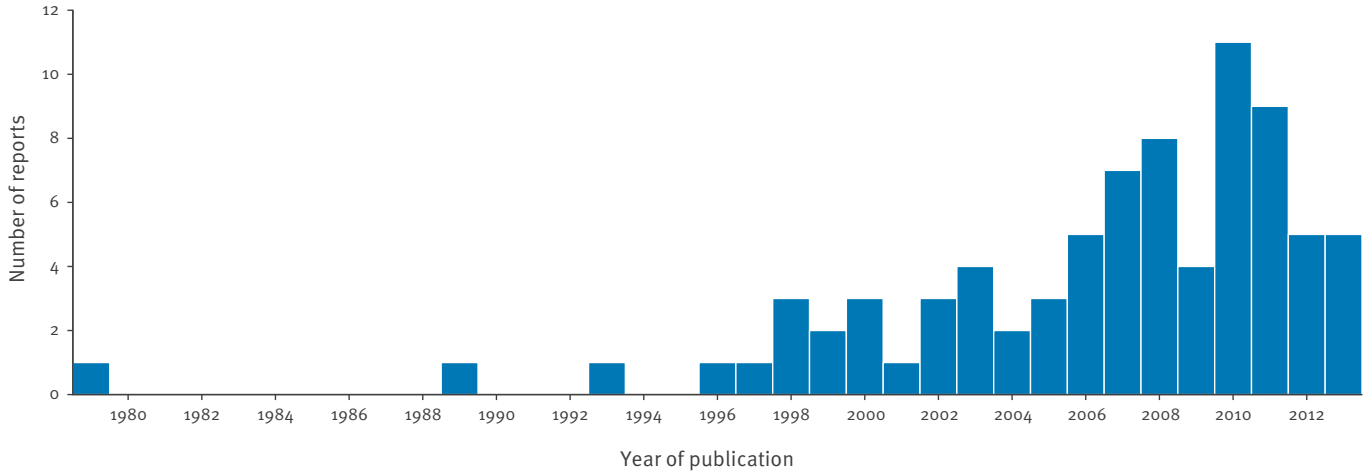
Blue boxes are estimated numbers. Key details of all 80 included articles are described in Table 1.

were the location of the outbreak, date of publication, type of infection, context or suspected source, and whether the study was prospective or retrospective. Methodological details were the type of spatial methods used and the tools employed. Outcomes were results of the investigations that related specifically to the use of spatial methods and any comments on their advantages or limitations. We summarised reports according to the date of publication, type of infection, location and context of the outbreak. Spatial methods used were categorised into four broad classes: visualisation, cluster analysis, modelling and other spatial analyses.

To demonstrate the utility of spatial methods during outbreak investigations, and therefore how they could be used in the future, we identified the stage(s) of the investigation to which they were applied. Outbreak investigations can be delineated into steps in various ways, and for the purpose of this review we used the following steps, adapted from the ECDC's Field Epidemiology Manual [17]: 1. Establishing existence of an outbreak, 2. confirming diagnosis, 3. defining and identifying outbreak cases, 4. describing cases and developing hypotheses, 5. evaluating hypotheses and drawing conclusions, 6. comparing with established facts, 7. executing prevention measures, 8. communicating findings.

Figure 3

Reports of outbreak investigations using spatial methods (n = 80)



Results

Article screening and estimation of proportion with spatial methods

After excluding duplicates, we identified a total of 2,189 articles for abstract screening. Of these, 146 were selected for full text review and 80 of them were included in the analysis. Reasons for article exclusion are summarised in Figure 2A. Conducting the search without any terms specific to spatial analysis identified 487,495 articles. Assuming the same rate of article exclusion at each step in the review process, we estimated the total number of published articles relating to outbreak investigations of infectious diseases at ca 20,000 (Figure 2B). The overall proportion of published outbreak investigation reports that explicitly described spatial methods was therefore around 0.4%.

Characteristics of studies included

Publication of outbreak investigations with spatial methods has increased markedly since 2000, with over half (n = 42) of the studies published since 2008 (Figure 3). Most articles (n = 66; 83%) concerned infections in human populations, of which the most frequently investigated infections were Legionnaires' disease (n = 12), cholera (n = 7) and influenza (n = 7) (Table 2). Correspondingly, the most common transmission contexts for human infections were water/sanitation (n = 20), followed by environmental (n = 14) and community (n = 10) (Table 3).

Healthcare-associated infections were reported in five of the articles while food-borne and sexually transmitted infections were reported once apiece. Veterinary infections were almost exclusively linked to farms or other breeding facilities (n = 12) and influenza was the most frequently investigated infection affecting animals (n = 4). Prospective outbreak investigations comprised around half (n = 39) of the articles included, with the remainder describing retrospective analyses of outbreak data.

Figure 4 displays the outbreaks by country, with the most reports in the United Kingdom (UK) (n = 10) or the United States (US) (n = 8), and by continent, with a third of reports in Europe (n = 27) and fewer in Africa (n = 10).

Spatial methods

Spatial methods used are listed and classified according to type in Table 4.

All articles presented or referred to at least one method of visualising case distributions to describe outbreaks in space. Plotting cases as dots on a map is the simplest form of visualisation and was used in the majority (n = 68; 85%) of studies. Dot maps were either presented using case locations only, or were enhanced with further information such as their vaccination status [18], migratory status [19] or date of disease onset [20]. Thematic maps provide context to case locations by displaying the spatial distributions of other variables. Such maps were used in 25 studies and variables plotted included socioeconomic status [21], soil type [22] and road density [23]. Maps of disease rates were used in 14 studies, with data usually aggregated according to administrative boundaries. Smoothed incidence maps were used in 13 studies. Other methods for visualising outbreaks that were used in fewer studies included standard deviation ellipses and velocity vector maps. Both use the locations of cases to describe the direction of spread of outbreaks.

Cluster analyses were used in 24 studies (30%), and spatial scan statistics were the most frequently used (n = 13 studies). *k*-nearest neighbour tests, *k*-function analyses and the Knox test were also used frequently (n = 7, 5 and 5 studies, respectively). Modelling approaches were used in 13 studies, including seven which used air dispersion models to identify areas that

Table 2

Infectious diseases investigated by category (n = 80 reports)

Infection category	n	Infection	n[a]	References
Respiratory	23	Legionnaires' disease	12	[26,32,38,40,47,57-60,63,73,88]
		Influenza	7	[23,39,75,80,84,95,104]
		SARS	3	[76,78,81]
		Acute respiratory disease	1	[31]
Intestinal	18	Cholera	7	[20,30,34,45,49,85,98]
		Cryptosporidiosis	2	[68,105]
		Diarrhoea	2	[46,99]
		Salmonellosis	2	[29,67]
		Shigellosis	2	[36,79]
		Campylobacteriosis	1	[42]
		Giardiasis	1	[41]
		Necrotising enterocolitis	1	[106]
Viral haemorrhagic fever	8	Dengue fever	5	[43,48,55,82,96]
		Ebola	1	[86]
		Porcine high fever disease	1	[87]
		West Nile fever	1	[90]
Viral skin infections	7	Measles	3	[18,28,71]
		Foot and mouth disease	2	[69,103]
		Varicella	1	[77]
		Variola minor	1	[100]
Protozoal	5	Toxoplasmosis	2	[93,101]
		Leishmaniasis	1	[72]
		Malaria	1	[34]
		Trypanosomiasis	1	[35]
Rickettsioses	5	Q fever	5	[11,33,50,70,74]
Bacterial zoonotic	4	Anthrax	3	[22,91,94]
		Leptospirosis	1	[21]
Mycoses	3	Blastomycosis	3	[25,89,92]
Viral CNS infections	2	Rabies	2	[83,102]
Viral hepatitis	2	Hepatitis A	1	[56]
		Hepatitis E	1	[27]
Helminthiases	1	Schistosomiasis	1	[19]
Other bacterial	1	Meningococcal meningitis	1	[97]
Sexually transmitted	1	Syphilis	1	[44]
Tuberculosis	1	Tuberculosis	1	[24]

CNS: central nervous system; SARS: severe acute respiratory syndrome.

[a] The total is 81 because one study reported two investigations.

may have been exposed to air from suspected contaminated environmental sources.

A range of other spatial methods based on geographic attributes of cases were also identified. These included methods for defining (n = 31 studies) and identifying (n = 8 studies) cases, summarising the average locations of cases (n = 5 studies) and assessing proximity to potential sources (n = 16 studies).

Analytic methods were used less frequently in prospective than retrospective articles: Cluster methods were used in 16 (39%) retrospective compared with eight (21%) prospective studies, and modelling in 10 (24%) and three (8%) retrospective and prospective analyses, respectively.

The most frequently cited GIS software was ArcGIS/ArcView, used in 30 studies, with MapInfo the other commonly used programme (n = 7). Various other packages including R, ClusterSeer, GeoDa and SaTScan were used for specific analyses.

Application of spatial methods in outbreak investigations

Applications of spatial methods to different stages during outbreak investigations are described below (see also Table 1).

1. Establishing existence of an outbreak

Few studies (n = 4) used spatial methods to assist with establishing the existence of an outbreak. Methods that were used aimed to identify unusual patterns of cases, either visually or through formal statistical tests of clustering.

For example, Affolabi and colleagues described complementary use of molecular and geographic methods to identify an outbreak of tuberculosis in Benin [24]. Among a series of 194 *M. tuberculosis* isolates, 17 belonged to the Beijing genotype and exhibited an identical 12-loci subtype. Mapping of patients' residences, workplaces and movements revealed a corresponding spatial cluster, confirming that the cases were likely to be linked. In another study, Roy and colleagues plotted the locations of cases of blastomycosis in Wisconsin after noting an increase in the number of reports [25]. They visually identified clustering within five neighbourhoods and used the spatiotemporal scan statistic to confirm that this was statistically significant.

2. Confirming diagnosis

Although knowledge of the endemicity of diseases in the geographic regions in which outbreaks arise is useful in developing plausible preliminary diagnostic hypotheses, spatial methods alone are not able to confirm a diagnosis and were therefore not used for this purpose in any of the studies.

3. Defining and identifying outbreak cases

Geographic boundaries in which outbreak cases were defined were stated explicitly in over a third (n = 31) of the studies. For instance, Keramarou and colleagues' investigation of an outbreak of Legionnaires' disease included only cases that lived or worked in the outbreak area, defined as a 12 km corridor on either side of a major road [26].

Table 3

Contexts of outbreak investigations of human and animal diseases (n = 80 reports)

Context	Human[a]		Animal	
	n	References	n	References
Water/sanitation	20	[20,21,27,30,34,36,41,42,45,46, 49,56,68,79,85, 93,98,99,101,105]	0	
Environmental	14	[25,26,32,38,40,47,57-60,63,73,88,92]	1	[89]
Community	10	[18,24,28,71,75,76,80,81,97,100]	2	[83,102]
Vector-borne	10	[19,34,35,43,48,55,72,82,90,96]	0	
Farm/breeding facility	5	[11,33,50,70,74]	12	[22,23,31,69,84,86,87, 91,94,95,103,104]
Healthcare-associated	5	[29,39,77,78,106]	0	
Food	1	[67]	0	
Sexually transmitted	1	[44]	0	
Total [b]	**66**		**15**	

[a] Includes outbreaks affecting humans that had animal origin.

[b] The total is 81 because one article reported two investigations.

Spatial methods were also used to assist with active case finding in eight studies. Bali and colleagues describe a search for cases of hepatitis E prompted by identification of three cases in a small town in northern India [27]. A house-to-house survey in this region identified 3,170 cases of jaundice with an attack rate of 5.2%.

4. Describing outbreak cases and developing hypotheses

Use of dot mapping to support an outbreak in real time is described by Fitzpatrick and colleagues, who investigated a rise in measles cases in Dublin, Ireland [28]. Continuously updating their maps throughout the outbreak allowed them to identify clustering of cases as soon as it developed and ultimately assisted with targeting of control interventions.

Simple maps were also used to develop hypotheses about the origins of outbreaks. For example, Kistemann and colleagues plotted cases by date of onset in an investigation of a nosocomial *Salmonella* outbreak [29]. Their schematic map revealed the central kitchen as the only functional relationship linking the cases, which they therefore hypothesised to be the source of the infection.

Sasaki and colleagues created a Voronoi diagram to demarcate their study area using locations of water taps [30]. Plotting incidence rates in the different areas defined by these water tap boundaries helped to visualise clear spatial clustering of cholera cases associated with poor water and sanitation facilities. Smoothed incidence maps were used in an investigation by Norström and colleagues into acute respiratory disease in Norwegian cattle herds. They used smoothing based on kernel density estimation to describe the progression of the outbreak, which was shown to spread locally before jumping to new areas [31].

A common method to develop hypotheses about sources of infections was to construct concentric circles of varying radii around potential sources and compare the attack rates in each. Nygard and colleagues used this technique in an investigation of Legionnaires' disease in Norway [32]. They calculated attack rates in five rings of increasing distance around eight potential sources and observed a trend of decreasing rate ratios with increasing distance from an air scrubber. Other metrics used to describe cases included calculating their average location and proximity to risk factors.

Possible air-borne spread of Q fever from farms near Cheltenham, UK was investigated by Wallensten and colleagues using the Numerical Atmospheric-dispersion Modelling Environment (NAME) model [33]. Plotting the modelled distribution showed that air from each of the suspected farms may have exposed the town. Geographic profiling is another modelling technique used to generate hypotheses about the locations of sources of infections. Le Comber and colleagues used this method to identify most likely locations of mosquito breeding sites using residential locations of a series of cases of malaria in Cairo, Egypt [34].

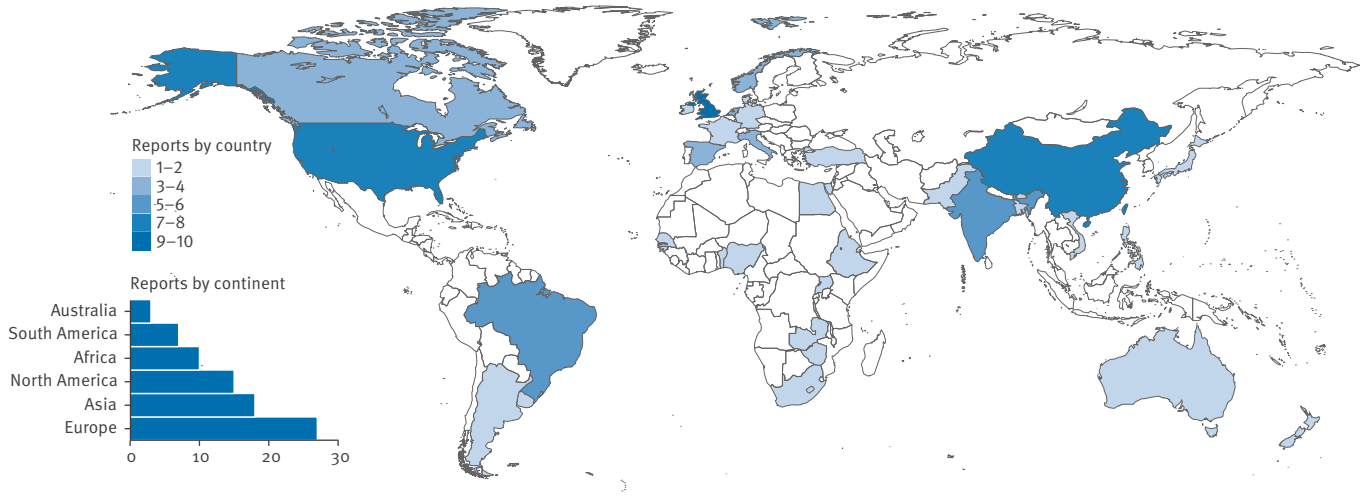
5. Evaluating hypotheses and drawing conclusions

More than half of the studies (n = 42) used statistical tests, such as cluster and regression analyses, to conduct formal evaluations of hypotheses arising from observations of case distributions. Fevre and colleagues, for example, assessed clustering of cases of trypanosomiasis under the hypothesis that a cattle market was the source of the outbreak [35]. A significant cluster encompassing the location of the market was detected using the spatiotemporal scan statistic, supporting this theory.

In an investigation on a military installation in North Carolina, McKee and colleagues used the *k*-nearest

Figure 4

Locations of outbreak investigations using spatial methods by country and continent (n = 80)



neighbour method to identify significant spatiotemporal clustering of shigellosis [36]. They used dot maps to locate the area with intense transmission and targeted it with educational interventions to bring the outbreak under control.

Combinations of multiple tests for clustering were used in some studies, such as Norström and colleagues' investigation of acute respiratory disease in Norwegian cattle herds [31]. They combined the Knox test [37], a global test for space–time clustering, with the *k*-nearest neighbour test and space–time scan statistic. These tests allowed them, respectively, to define the smallest distance and time frame in which the events had been clustered, to determine whether cases tended to be close to other cases and to locate the most significant clusters. All methods indicated presence of space–time clustering, adding weight to the conclusion that a common contagious source was responsible for the outbreak.

The hypothesis that risk of infection decreased with increasing distance from a suspected source was tested in several studies through regression analysis. Hackert and colleagues, for example, used linear regression of log-transformed attack rates to assess a cluster of human cases of Q fever in the Netherlands [11]. Incidence increased by a statistically significant exposure–response gradient with proximity to a dairy goat farm, which they concluded was likely to be the primary and sole source.

6. Comparing results with established facts

Results from spatial analyses in some cases provided updates to knowledge about the dynamics of the infectious agents concerned, such as their minimum infective dose and mode of transmission. For example, in an outbreak of Legionnaires' disease in Christchurch, New Zealand, cases were identified at a distance of 12 km from the implicated cooling tower [38]. White and colleagues therefore proposed updates to World Health Organization guidelines which at the time placed the area at risk from such sources at 3.2 km.

Wong and colleagues used a computational fluid dynamics model to study the spread of an influenza outbreak in a hospital setting [39]. Concentrations of hypothetical virus-laden particles from modelled air distributions correlated closely with locations of infected patients. This suggested a possible role for aerosol transmission of influenza, which is predominantly associated with transmission by droplets and direct contact.

7. Executing prevention measures

Spatially targeted interventions to control the outbreak or prevent future cases were described in many studies. Measures that aimed to control outbreaks included cleaning implicated cooling towers [40], issuing water boiling orders to areas served by contaminated supplies [41,42], vaccination catch-up campaigns [28], removal of breeding sites for mosquito larvae [43] and targeted information campaigns [36]. For example, Acheson and colleagues placed postcode-targeted information on social networks during an outbreak of heterosexually acquired syphilis in Teesside, UK [44].

Attempts to prevent future outbreaks included improvement of infrastructure [45,46], change of policy [29,32,47] and generation of risk maps [48]. Luquero and colleagues, for instance, used results of their analysis to recommend specific regions in which to focus preparedness activities to avoid future cholera outbreaks in Guinea-Bissau [49].

8. Communicating findings

All studies in this review had, by definition, used their spatial analyses in communication of findings through reports in peer-reviewed publications. Several studies also highlighted the usefulness of maps in reports or

Table 4

Spatial methods used in outbreak investigations (n = 80)

Method category	n (prospective, retrospective)	Method	n	References
Visualisation	80 (39, 41)	Dot map	68	[11,18-28,30-36,38,40-50,55,56,58-60, 63,67-69,71-76,80,82-85,87-93,96-105]
		Thematic map	25	[18,19,21-23,28,29,38,41,42,45,46,50, 60,68,70,74,80,82,89,91,93-95,103]
		Rate map	14	[20,30,44,45,49,50,57,63,70,76,79,81, 90,101]
		Smoothed incidence map	13	[11,31,48-50,73,75,76,82,87,94,100,104]
		Case movement map	7	[24,26,32,40,47,59,63]
		Schematic map	6	[29,39,77,78,86,106]
		Standard deviation ellipse	4	[75,76,83,95]
		Origin–destination plot	1	[76]
		Velocity vector map	1	[94]
		Voronoi diagram	1	[30]
Spatial exploration	47 (28, 19)	Spatial case definition	32	[11,23,25-27,29,31-33,35,39-42,46,47, 57-60,63,67-69,74,78,79,85,88,92,94,106]
		Source proximity	16	[11,32,35,39,40,46,50,57,60,67,69,74, 82,88,89,103]
		Spatial case finding	8	[27,43,46,49,63,70,82,99]
		Spatial average	5	[34,74,83,92,95]
		Case–case distance	3	[23,69,97]
		Risk factor proximity	2	[21,23]
		Spatial social network analysis	1	[104]
Cluster	24 (8, 16)	Kulldorff's spatial/ spatiotemporal scan statistic	13	[25,31,35,38,43,46,49,56,84,87,94,95,104]
		Cuzick–Edwards *k*-nearest neighbour test/Jacquez's *k*-nearest neighbours for space time interaction	7	[31,36,55,85,87,94,95]
		Knox test	5	[31,48,87,95,96]
		k-function/space–time *k*-function	5	[49,73,87,94,96]
		Moran's I	4	[30,38,75,76]
		Nearest neighbour analysis	3	[30,76,95]
		Getis Ord G*i*(*d*) statistic	2	[75,90]
		Barton–David's test	1	[96]
		Grimson test	1	[106]
		Oden's Ipop	1	[94]
		Mantel's test	1	[95]
Spatial modelling	13 (3, 10)	Air dispersion modelling	7	[32,33,39,63,69,77,78]
		Environmental risk prediction model	2	[48,80]
		Kriging	2	[82,104]
		Empirical Bayes smoothing	1	[45]
		Geographic profiling	1	[34]

presentations to communicate results to health officials [29,47], policymakers [49,50] and the public [38]. Sarkar and colleagues, for example, presented dot maps of cases of acute diarrhoeal disease in a village in southern India to the local community and health authorities [46]. Their maps visualised the proximity of cases to a contaminated water supply, and the presentation resulted in release of funds to improve sanitation in the area.

Discussion

In this review, we have identified 80 published articles of infectious disease outbreak investigations that used spatial methods, less than half a per cent of our estimated total of 20,000 outbreak reports. Although the simple dot map was the most commonly used method, a wide range of techniques were applied, including more sophisticated data visualisations and analytic tools. Across the range of studies, there were examples of spatial tools being usefully applied throughout the course of an outbreak investigation; from initial confirmation of the outbreak to describing and analysing

Table 5

Application of spatial methods to steps in outbreak investigation

1. Establish the existence of an outbreak	• Visualise case distribution (e.g. dot map) • Identify and confirm clustering (e.g. spatial scan statistic)
2. Confirm diagnosis	• Spatial methods alone cannot confirm diagnoses. Consider spatial epidemiology of infection to develop preliminary diagnostic hypotheses.
3. Define and identify outbreak cases	• Set geographic limits in which cases are considered part of the outbreak (e.g. postcode area hospital ward) • Select controls in case–control study based on same geographic limits • Use maps to assist with active case finding
4. Describe cases and develop hypotheses	• Visualise distribution of cases in relation to known risk factors or potential sources (e.g. rate map, thematic maps) • Describe progression of outbreak (e.g. dot maps at different stages, standard deviation ellipse) • Identify centre of outbreak (e.g. spatial mean) • Identify high-risk areas (e.g. attack rates in zones at different distances from potential sources) • Assess likelihood of potential sources (e.g. geographic profiling)
5. Evaluate hypotheses and draw conclusions	• Test for overall clustering (e.g. k-nearest neighbour test) • Locate significant clusters (e.g. spatial scan statistic) • Identify significant trends in attack rates with distance from potential sources (e.g. linear regression of log-transformed attack rates)
6. Compare with established facts	• Calculate maximum dispersal distance from probable source to cases • Model concentrations of infected particles to understand transmission dynamics (e.g. computational fluid dynamics, NAME atmospheric modelling)
7. Execute prevention measures	• Spatial targeting of interventions to control outbreak (e.g. order to boil water in area served by contaminated reservoir) • Spatial targeting of health promotion campaigns (e.g. using postcodes on social networks) • Identify geographic areas at risk of future outbreaks (e.g. risk mapping)
8. Communicate findings	• Use maps to communicate results to health officials/policymakers, to the public and in scientific journals

cases and communicating findings. Spatial techniques often provided valuable insights that supplemented traditional epidemiological analyses of person and time and led to public health actions.

Outbreak investigations of infectious diseases occurring in any context were included in this study. Thus, we extended the scope of two previous reviews that focused, respectively, on use of spatial methods in outbreaks of Legionnaires' disease [10] and on spatiotemporal methods to investigate transmission of infections in hospital settings [51]. In doing so, we have highlighted imbalances in application of spatial methods in different types of investigations. For example, it was notable that only one study reported an outbreak of food-borne illness. Annual summary statistics from 2013 report a total of 5,196 food- and waterborne outbreaks in the European Union (EU) [52] and 831 reports of food-borne outbreaks in 2012 in the US [53]. Although only a small proportion of these are likely to have been published in academic journals, this still indicates a substantial shortfall in use of spatial methods in this context.

Our review also allowed assessment of the extent to which spatial methods have been used in Europe. Although there was a large number of reports from Europe compared with other parts of the world, those reports derived from only 10 counties. These were predominantly in western Europe, with one report from Turkey the only investigation in eastern areas. Sharing expertise through the European Centre of Disease Prevention and Control could help to reduce this gap and strengthen outbreak investigation capacity across the continent. Expanding the use of these tools is also important in other parts of the world. Only 10 reports described outbreaks in Africa, the same number as in the UK alone, which clearly does not correlate with the distribution of the global burden of infectious diseases.

There are several limitations of spatial methods, and barriers to their use, which may account for the unequal and under-use of these tools as identified here. Firstly, reliable spatial analyses can only be conducted with accurate location data. This can be a particular challenge in developing countries in which good quality maps of residential areas are often not available [54]. Several investigations of outbreaks in such settings conducted field surveys and used Global Positioning Systems (GPS) to accurately record patient residence or risk factor locations [30,35,43,46,55,56]. However, this is a time- and cost-intensive approach and will not always be feasible. In settings in which good quality maps of residential data are available, quality of case location data is still not assured: Errors can arise from incomplete or mistranscribed addresses, out of date GIS databases or incomplete information on potential source locations. During outbreaks of Legionnaires' disease, for example, some investigators had to conduct visual searches or make public enquiries to ascertain the locations of aerosol-producing devices because there was no central registry [26,32,40,57-60].

Simplification of case locations to static points, usually residential locations, also impacts the utility of location data. In reality, individuals can become exposed to infectious agents at any place where they spend time and, similarly, traditional census population denominators that record night-time populations are not necessarily reflective of population distributions during the day [61,62]. Although a number of studies made attempts to record case movements [24,26,32,40,47,59,63], none accounted for diurnal fluctuations in populations. Ideally, this spatial uncertainty should be accounted for in data collection, analysis and visualisation stages to improve reliability of estimates of spatial risk, and new analytic methods may be required to achieve this.

Secondly, even if reliable location data are available, presentation of information on maps can be open to misinterpretation. Dot maps, for instance, were used widely but do not take into account the geographic distribution of the underlying population and can therefore mask important trends. Similarly, patterns in aggregated data are sensitive to changes in the boundaries into which they are grouped, a phenomenon known as the modifiable aerial unit problem [9]. Presentation of data on maps fails to highlight these limitations, and relatively few prospective investigations used statistical methods to formally confirm observations identified from visual displays of data.

Thirdly, researchers may be deterred from using spatial analytic methods because they involve selection of parameter values, often with an element of subjectivity. Methods that display or identify clustering require specification of the degree to which distant points may be considered part of the same neighbourhood. For the spatial scan statistic, the user must define the maximum spatial extent of clusters in terms of the percentage of the population that can be included, in *k*-nearest neighbour analysis, the number of neighbours included must be specified, and equivalent parameters must be selected for other spatial cluster and modelling analyses [9]. Altering these parameters can have a profound influence on the results, and a trial and error approach is often required to arrive at an appropriate value. This can raise issues of multiple hypothesis testing, although some methods, including the spatial scan statistic and Tango's maximised excess events test [64], are able to adjust for this while evaluating clustering at multiple scales. Results of spatial analyses can also suffer from lack of specificity. For example, in several studies of Legionnaires' disease, spatial methods identified areas most likely to be the source of the infection, but could not discriminate between potential sources that were close together [40,57,58].

Another barrier to the effective use of spatial methods that is often cited is the expense of specialised GIS software and the need for trained personnel to operate it. Although some GIS programmes are available free of charge, the most commonly used was a commercial package, ArcGIS. However, it is also noteworthy that spatial scan statistics were the most frequently adopted analytic methods. Scan statistics can be implemented with relatively little training through SaTScan, a programme free to download from the Internet. This suggests a possible model for wider adoption of other more advanced techniques.

The results of our study point to a number of recommendations for improved practice and opportunities for further development of spatial methods. Given the potential utility of existing tools demonstrated here, under-use of these methods has doubtless resulted in missed opportunities for more effective real-time outbreak investigations. Public health officials must be supported to address this issue, and a useful first step would be development of protocols describing the application of appropriate analyses. Table 5, which relates spatial methods to specific stages in outbreak investigations, provides a framework for this. Provision of training, for example through short courses, and interdisciplinary working with specialists in geographic analysis would also be beneficial to improve the skills base of the workforce.

The majority of studies identified in this review that used analytic methods described retrospective analysis of data collected during outbreaks. These reports demonstrated the potential utility of analytic methods, but will be of greater public health benefit when used in real time. Assembly of GIS databases in advance is essential to allow spatial analyses during prospective outbreak investigations. Improving data accessibility will save time during investigations, improve accuracy of analyses and prevent duplication of effort. Reports of analyses using spatial methods would also benefit from some degree of standardisation. For example, reporting of the sources and level of precision of spatial data would enable more accurate interpretation of the results by researchers not familiar with the study site. This could be achieved, for example, through extension of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement with items specific to spatial data [65].

Finally, there is scope for development of new tools for analysis and visualisation of spatial data. A move towards web-based applications with user-friendly interfaces would be a natural progression, provided that these platforms included adequate training materials and data governance infrastructure. This would make spatial analyses more accessible to non-experts and could facilitate wider use of interactive displays of data and animations. The quantity and detail of geolocated data available to researchers is also increasing. GPS-enabled mobile devices and applications for self-reported or crowd-sourced information (for example *sickweather* [66], based on reports on social networks) have the potential to provide near real-time data including information on individuals' movements. Development of new analytic techniques will be needed to ensure that these data are effectively exploited and

potential benefits are met. In the context of outbreak investigations, possible applications include contact tracing and improved estimation of exposure to environmental risk factors.

The primary limitation of this study was the challenge of designing the database search strategy. Although we employed a broad search which identified a large number of abstracts for screening, the number of studies identified here will inevitably be an underestimate of the outbreak investigations that used spatial methods. Our search will not have captured studies that used spatial methods but did not refer to them explicitly in the title, abstract, subject headings or MeSH terms. Restricting the search to articles published in academic journals also excluded reports in the grey literature. Inclusion of such reports would increase the number of investigations using spatial methods, but would be unlikely to reveal novel approaches or tools not identified here. Articles published since the database search was run at the end of 2013 are also not included in this study. Recent years have seen an increase in reports using spatial methods, probably due to increased availability of GIS software. This trend is likely to have continued, and recent publications will focus on current public health issues, for example the recent Ebola outbreak in West Africa.

There was also a possible publication bias in this study: spatial analyses may have been more likely to be presented in published reports if they were found to be useful. Concerns of breaching patient confidentiality could have further limited the number of studies that published maps. Nevertheless, the proportion of studies using spatial methods was very small, and even if our estimate is an order of magnitude too low, it would still represent less than 5% of the estimated total number of investigations published.

Conclusion

Investigations of outbreaks of infectious diseases require synthesis of information and expertise from a range of fields. Spatial analyses can make many valuable contributions, with simple maps alone providing fundamental insights about the distribution of cases. However, advancements in GIS technology and increasing availability of good quality spatial data provide an opportunity for development and implementation of more sophisticated tools. Adoption of these new techniques, and wider use of existing methods, has the potential to support more effective investigations and therefore limit the public health impacts of infectious disease outbreaks.

Conflict of interest

None declared.

Authors' contributions

CS and ACH designed the study. CS did the literature search, analyses and wrote the first draft. ACH, SLC, HF, MB and SL revised and edited the final report

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Phylogenetic and epidemiological analysis of measles outbreaks in Denmark, 2013 to 2014

LD Rasmussen [1], J Fonager [1], LK Knudsen [2], PHS Andersen [2], J Rønn [1], MW Poulsen [1], KT Franck [1], TK Fischer [1,3]
1. Microbiological Diagnostics and Virology, Statens Serum Institut, Copenhagen, Denmark
2. Department of Infectious Disease Epidemiology, Statens Serum Institut, Copenhagen, Denmark
3. Centre for Global Health, University of Southern Denmark, Odense, Denmark

Correspondence: Lasse Dam Rasmussen (LARA@ssi.dk)

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Despite the introduction of safe, effective vaccines decades ago and joint global public health efforts to eliminate measles, this vaccine-preventable disease continues to pose threats to children's health worldwide. During 2013 and 2014, measles virus was introduced into Denmark through several independent importations. This resulted in a number of secondary cases (n = 7), with two clusters in 2013 and one in 2014. In total, there were 44 cases of measles. Most cases (n = 41) were laboratory confirmed by detection of measles virus genome by real-time reverse transcription (RT)-PCR and IgM antibodies. The viruses from confirmed cases were genotyped by sequencing. Only one genotype circulated each year, i.e. D8 and B3, respectively. Sequencing of measles virus from different clinical specimens from the same patients revealed that sequence variants of measles viruses might co-exist and co-transmit during an outbreak. The majority of the cases were unvaccinated (n = 27) or recipients of one dose of measles-mumps-rubella (MMR) vaccine (n = 7). In addition, two fully vaccinated adult cases were reported in 2014. We demonstrate the transmission of measles virus in a population in which the two-dose MMR vaccination coverage rate was 80% and how even vaccinated individuals may be at risk of contracting measles once transmission has been established.

Introduction

Measles is caused by a negative-sense single-stranded RNA virus belonging to the *Morbillivirus* genus. The wild-type measles virus consists of 24 genotypes that are grouped into eight clades [1]. Measles is one of the leading causes of childhood mortality worldwide: about 145,700 people died from measles in 2013, most of them children under the age of five years [2].

The first measles-mumps-rubella (MMR) vaccination programme was implemented in the 1960s in the United States [3]. The World Health Organization (WHO) has set the goal of eliminating measles in Europe by 2015 and globally by 2020 [4]. In 2013, the WHO Regional Office for Europe produced a supplement for accelerated action during 2013 to 2015 [5]. In this supplement, WHO identified a number of acceleration strategies to achieve this goal by the end of 2015: (i) immunisation system strengthening; (ii) establishing and improving case-based surveillance; (iii) improving laboratory and epidemiological data integration; and (iv) improving outbreak response. These strategies have been adapted and are some of the key elements in the Danish national disease elimination efforts.

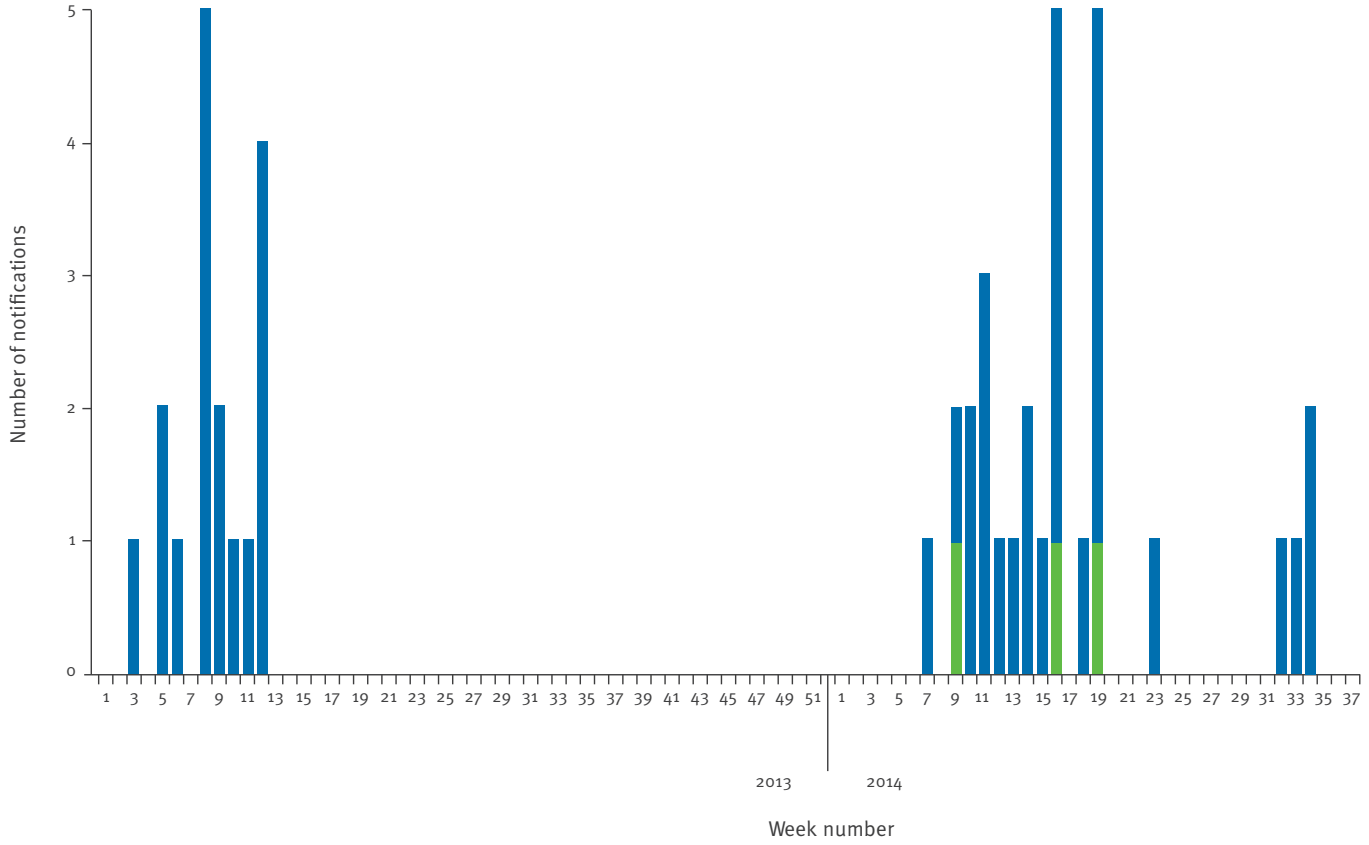
In Denmark, measles is a notifiable disease, and national measles surveillance is conducted through close collaboration between the WHO National Reference Laboratory for Measles and Rubella at the Department of Microbiological Diagnostics and Virology at the Statens Serum Institut (SSI), the Department of Infectious Disease Epidemiology, SSI, the Danish Health and Medicines Authority, general practitioners (GPs), hospital clinicians, regional hospital microbiology laboratories and public health medical officers. The public health medical officers perform contact tracing and give advice to hospitals and GPs on post-exposure prophylactic therapeutics.

In Denmark, the first dose of MMR vaccine has been given at the age of 15 months since its introduction in 1987. In 2008, the Danish Expanded Programme on Immunisation (EPI) schedule was changed and the timing of the second MMR vaccine dose adjusted from 12 years to 4 years of age. The rationale for the change was to increase herd immunity by increasing the immunity of children aged under 12 years [6].

Since 1996, MMR vaccination status in Denmark has been recorded in a national vaccination database, in which data are automatically entered when GPs use specific codes for reimbursement for performing the

Figure 1

New measles notifications by week in Denmark, 2013 and 2014 (n = 44)



For the 41 laboratory-confirmed cases (shown in blue), the notification date is based on first sampling day. For the three probable cases, whose infection was not laboratory confirmed but diagnosed based on clinical criteria and epidemiological link (shown in green), GP diagnosis date is used.

For 2014, only weeks 1–37 are shown, but no cases were notified during the rest of the year.

vaccinations. Since the introduction of this database, MMR vaccination coverage in Denmark has been estimated to be 88–90% for the first dose and 83–88% for the second [7]. That is until 2013, when coverage decreased to 87% for the first dose and 80% for the second, underlining the challenge facing the Danish EPI programme when trying to meet the goal of 95% coverage, as recommended by WHO [8]. However, a study in which parents of children not registered in the national database were interviewed about the vaccination status of their children documented under-reporting of coverage in the database, to the magnitude of 3–4 percentage points [7]. Given this, and the fact that the vaccination of some of children will be delayed [7], coverage may be higher in reality.

The advantage of molecular characterisation of measles virus followed by phylogenetic analysis during outbreaks has been demonstrated previously [9,10]. In our current study, we combine phylogenetic analyses of viruses from confirmed measles cases in Denmark during 2013 and 2014 with classical epidemiological investigations in order to investigate the consequences of imported cases of measles and the associated potential public health challenges in the attempt to keep a nation free of endemic transmission of measles virus.

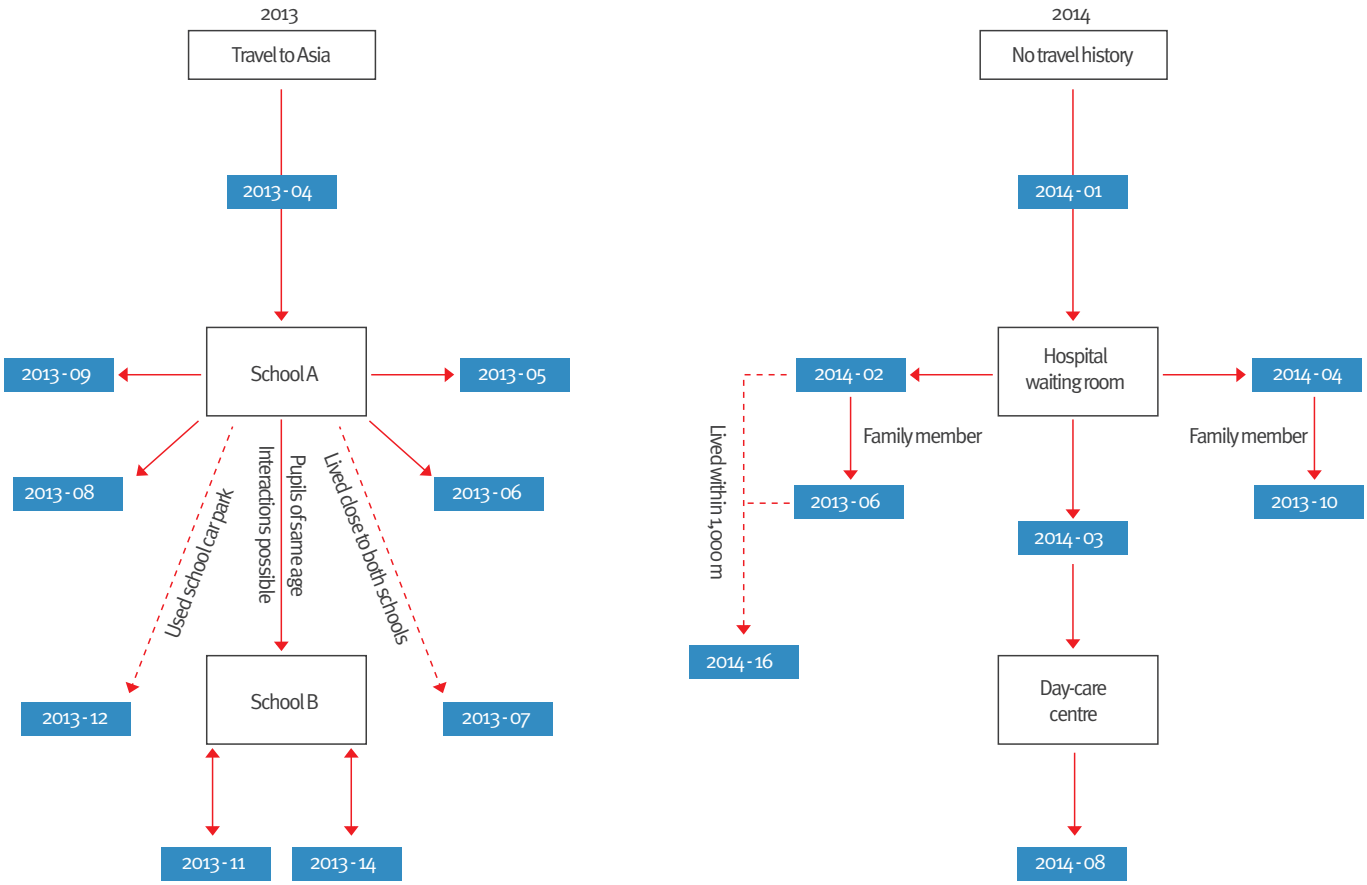
Methods

We used the European Union measles case definition, as described by ECDC [11]. We defined a cluster as having a minimum of three persons with measles, for whom an epidemiological link to an identified index case, either directly or by secondary infection, was established.

For any measles case, the local GP takes the lead on collecting all available information on the patient that is necessary for public health action, such as the likely source of exposure and contacts of the case. This information is recorded in a standardised notification Form (National Board of Health form 1515), which is sent to both the Department of Infectious Disease Epidemiology at SSI and to the Regional State Epidemiologist (RSE). After receiving the official notification form, the RSE decides on the appropriate public health action, e.g. tracing unvaccinated contacts and the extent of administration of prophylactic vaccination or immunoglobulin.

Figure 2

Possible transmission routes in clusters of measles cases in Denmark, 2013 and 2014 (n = 17)



For 2013, only the large cluster is shown. Solid lines show epidemiological links involving known close contact with a measles case, whereas dotted lines indicate possible contact. The arrows show the direction of transmission. Double-headed arrows indicate a possible connection between the cases but the direction of the transmission cannot be established.

Analysis of specimens

During 2013 to 2014, specimens from suspected measles cases – primarily urine, throat swabs and serum – were sent to the WHO National Reference Laboratory for Measles and Rubella, SSI, for laboratory confirmation and further characterisation of measles virus-positive samples. In most cases, multiple specimens were submitted simultaneously. Serum specimens were tested for IgG and IgM antibodies using a BEP 2000 Advance system (Siemens Healthcare) with an in-house ELISA (unpublished data). Measles virus RNA was purified using the MagNA pure LC system (Roche), using the Total NA kit (Roche).

All samples (serum, throat swabs and urine) were further tested for the presence of measles virus RNA by real-time reverse transcription-PCR (RT-PCR) according to the procedures described by Mubarak et al. [12]. Positive samples were further characterised by RT-PCR followed by Sanger sequencing on an ABI 3100 (Thermo Fischer) using primers and running conditions as recommended by WHO [13]. Measles virus sequences were genotyped online using the 450 bp fragment of the N gene recommended for genotyping by WHO by the shared member state sequence database, the Measles Nucleotide Surveillance (MeaNS) tool [14].

Phylogenetic analyses were performed by alignment and neighbour-joining tree-building using ClustalW [15]. Bootstrapping was performed using 1,000 replicates. Sequences shorter than the recommended 450 bp were excluded from the phylogenetic analysis.

Vaccination status

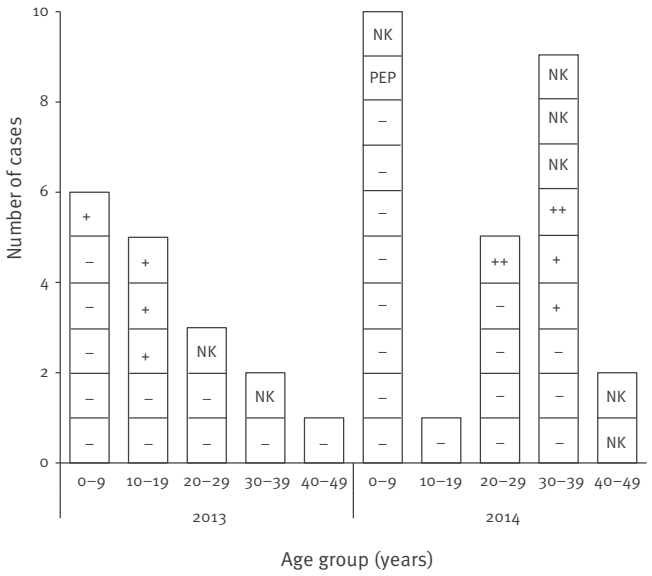
Vaccination status was based on data available in the national vaccination database, which was initiated in 1996; status before this was based on statements from the patient and/or the GP. Thus, vaccination status was only fully available in the database for persons up to 17 years and 18 years of age, in 2013 and 2014, respectively.

Nomenclature

Confirmed cases were recorded using the year and chronological order in which they were laboratory confirmed (e.g. the first case in 2013 was given the ID 2013-01). All viral sequences were named in accordance with the WHO standards implemented by MeaNS [14].

Figure 3

Measles cases by age group, Demark, 2013 and 2014 (n = 44)



MMR: measles-mumps-rubella; NK: unknown; PEP: post-exposure prophylaxis; RT-PCR: real-time reverse transcription-PCR.

Vaccination status: – indicates that the person was not vaccinated with a measles virus-containing vaccine; + indicates that the person had received the first dose of MMR vaccine; ++ indicates that the person had received both doses of MMR vaccine.

Results

Epidemiology of measles cases in Denmark in 2013 and 2014

In 2013 and 2014, 17 and 27 measles cases were notified, respectively. In 2013, all cases were laboratory confirmed, whereas in 2014, 24 cases were laboratory confirmed, while GPs (one in France and two in Denmark) diagnosed the remaining three probable cases, based on clinical and epidemiological criteria.

In 2013, the cases were notified during weeks 3 to 12, whereas in 2014, the first notification came slightly later, but the cases were notified over a longer period (weeks 7–34) (Figure 1). In the latter cases, diagnosis was based on clinical signs and epidemiological links to known laboratory confirmed cases. For a detailed overview of all cases from both years, see the Table.

In 2013, there was a large cluster consisting of nine epidemiologically linked cases (case ID 2013-04 to 2013-09, 2013-11, 2013-12 and 2013-14) and a small cluster of four cases (2013-13 and 2013-15 to 2013-17). An additional four unlinked cases were detected: three of these cases (2013-01 to 2013-03) were notified before the first cluster was identified. One had travelled from the United Kingdom and one from Asia during the incubation period; the source of the infection of the third was unknown. The only other unlinked case (2013-10), occurred during the same time as the cases in the large cluster and was of the same age group as the majority in the cluster (7–11 years), but no epidemiological link could be established.

During 2014, a single cluster was detected, consisting of eight cases (2014-01 to 2014-04, 2014-06, 2014-08, 2014-10 and 2014-16). In addition, five imported cases each resulted in one secondary case (2014-05 linked to 2014-07, 2014-09 linked to 2014-11, 2014-12 linked to 2014-14, 2014-15 linked to 2014-20, and 2014-19 linked to 2014-22) and one importation (2014-24) resulted in two secondary cases (2014-25 and 2014-27). The remaining six cases were regarded as individual importations for whom no epidemiological links could be established. The cases for whom the travel history was known showed travel to Asia (2014-05 and 2014-12), the Netherlands and Germany (2014-13) and Turkey (2014-24). One case, an asylum seeker (2014-26), had been travelling through Europe, but the sequence of the measles virus from that case grouped together with that from the case who had travelled to Turkey (see below).

In 2013, the first measles case was identified in mid-January as an imported case from the United Kingdom. Within two weeks, two other unconnected cases were confirmed, one imported from Asia and one with no travel history. The index case of the 2013 large cluster was diagnosed with measles during the first week of March. The patient (2013-04), aged 10–19 years, had returned to School A after a trip to Asia. In the same school, four other pupils were also diagnosed with measles within the incubation period for the disease.

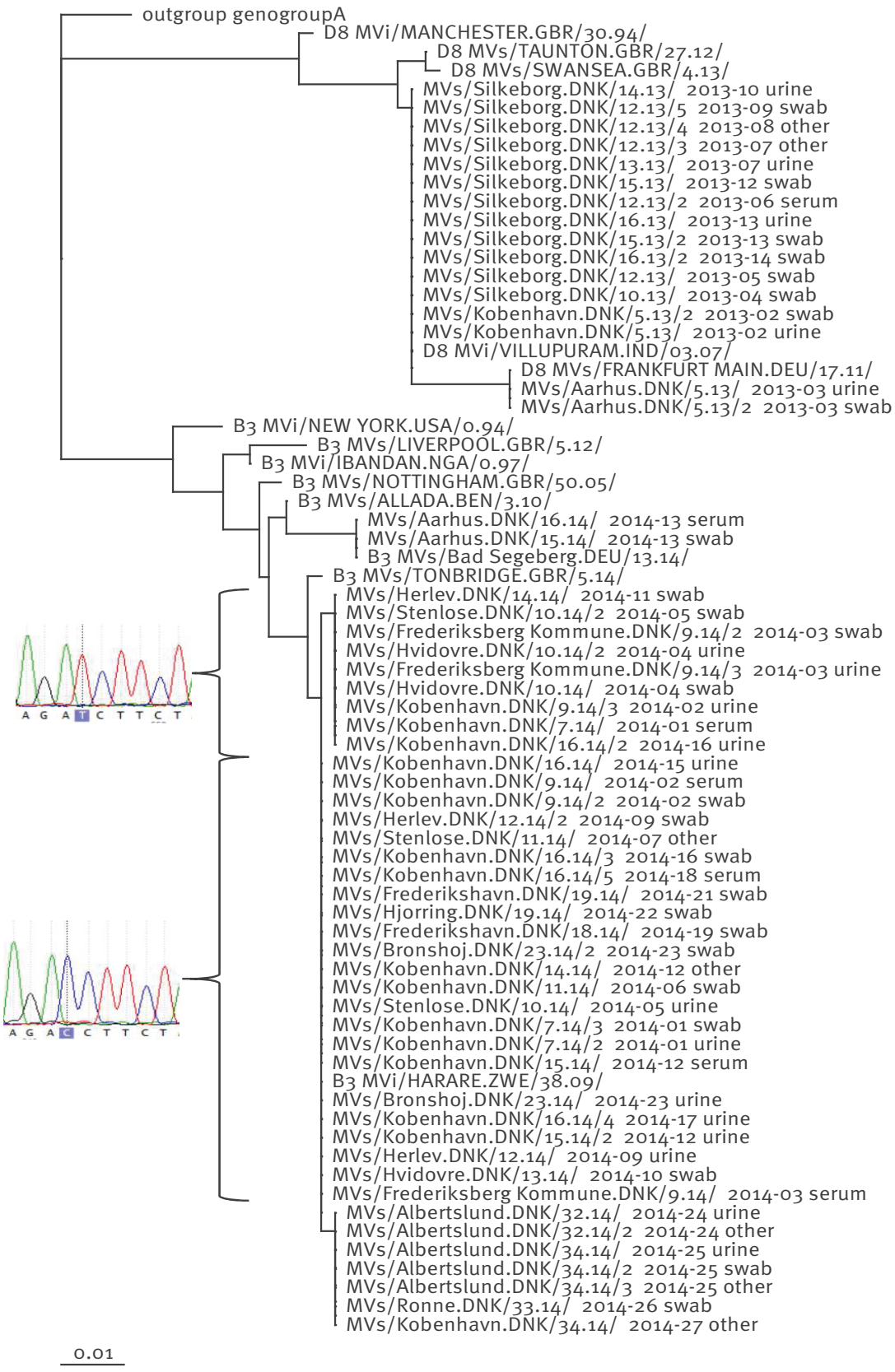
Two weeks after the introduction of measles at School A, a child at another school (School B), comprising pupils of the same age group, located less than 200 metres from School A, was diagnosed with measles and two weeks later, another child was diagnosed with measles at School B. In addition to the seven pupils from the two schools, this cluster also contained two individuals with a possible epidemiological link to the cluster: a person in their 30s who used the car park at School A and a person in their 40s who lived close to both schools (Figure 2).

The second smaller measles cluster occurred in a day-care centre, in the same town as the larger cluster, involving three children aged under 4 years and a person in their 20s (Table). Even though direct epidemiological links between this and the large cluster could not be established, sequence homology of the measles viruses and the close proximity in time and location suggest a link between the two clusters. The remaining case from 2013 (2013-10), even though no epidemiological link could be established, were connected to the same town; thus 14 of the 17 cases in 2013 were relatively closely linked geographically.

The index case of the 2014 cluster (2014-01) was an unvaccinated child aged under four years with no known travel history outside Denmark. Before being

Figure 4

Neighbour-joining tree showing the phylogenetic relationship of measles virus sequences from cases in Denmark, 2013 and 2014



Only sequences consisting of the recommended 450 bp of the measles virus N region were included in the analysis (57 sequences from 36 cases). Bootstrapping was performed using 1,000 replicates (for clarity, bootstrap values are omitted). The names of the sequences consist of the name assigned by MeaNS followed by the case ID and the type of specimen. Reference strains show the genotype followed by the name of the reference strain in capital letters.

The chromatograms showing the conserved single base mutation between the two B3 clades is included to show the unambiguity of the sequencing results.

Table

Overview of all cases of measles in Denmark, 2013 and 2014 (n = 44)

Case ID	Age group in years	RT-PCR-positive sample				Vaccination status	First sampling date[b]	Epidemiological link
		Serum	Urine	Throat swab	Unknown[a]			
2013-01	20–29					–	17 Jan 2013	Travel to United Kingdom
2013-02	30–39					+	31 Jan 2013	Travel to Asia
2013-03	20–29					–	1 Feb 2013	Unknown
2013-04	10–19					+	8 Mar 2013	Index case of large cluster (stay in Asia)
2013-05	10–19					+	18 Mar 2013	2013-04 (attended School A)
2013-06	10–19					–	19 Mar 2013	2013-04 (attended School A)
2013-07	40–49					–	21 Mar 2013	Lived close to Schools A and B
2013-08	5–9					–	21 Mar 2013	2013-04 (attended School A)
2013-09	5–9					+	22 Mar 2013	2013-04 (attended School A)
2013-10	5–9					–	2 Apr 2013	Unknown
2013-11	10–19					+	2 Apr 2013	Classmate of 2013-14 at School B
2013-12	30–39					Unknown	8 Apr 2013	Used car park at School A
2013-13	0–4					–	14 Apr 2013	Index case of small cluster (source of infection unknown)
2013-14	10–19					–	18 Apr 2013	Classmate of 2013-11 at School B
2013-15	0–4					–	19 Apr 2013	2013-13 (attended Day-care centre A)
2013-16	0–4					–	20 Apr 2013	2013-13 (attended Day-care centre A)
2013-17	20–29					Unknown	22 Apr 2013	Worked at Day-care centre A
2014-01	0–4					–	12 Feb 2014	Index case of single cluster (source of infection unknown)
2014-02	0–4					–	25 Feb 2014	2014-01 (in hospital waiting room when 2014-01 present)
2014-03	0–4					–	28 Feb 2014	2014-01 (in hospital waiting room when 2014-01 present)
2014-04	0–4					–	4 Mar 2014	2014-01 (in hospital waiting room when 2014-01 present)
2014-05	30–39					+	5 Mar 2014	Travel to Asia with 2014-07
2014-06	0–4					PEP	10 Mar 2014	2014-02 (family member)
2014-07	30–39					+	10 Mar 2014	Travel to Asia with 2014-05
2014-08	0–4	Not sampled				–	14 Mar 2014[b]	2104-03, attended same day-care centre as 2104-03. Diagnosed in France.
2014-09	20–29					–	20 Mar 2014	Unknown source of infection
2014-10	20–29					++	26 Mar 2014	2014-04 (family member)
2014-11	30–39					++	1 Apr 2014	2014–09 (family member)
2014-12	40–49					Unknown	5 Apr 2014	Travel to Asia
2014-13	20–29					–	12 Apr 2014	Travel to Netherlands and Germany
2014-14	0–4	Not sampled				–	16 Apr 2014[b]	2014-12 (relative)
2014-15	30–39					–	20 Apr 2014	Unknown source of infection
2014-16	30–39					–	18 Apr 2014	Lived near 2014-02 and 2014-06
2014-17	20–29					–	16 Apr 2014	Unknown source of infection
2014-18	10–19					–	17 Apr 2014	Unknown source of infection
2014-19	20–29					–	28 Apr 2014	Unknown source of infection
2014-20	1–5	Not sampled				–	5 May 2014[b]	2014-15 (family member)
2014-21	30–39					Unknown	9 May 2014	Unknown source of infection
2014-22	30–39					–	9 May 2014	Worked at hospital where 2014-19 was hospitalised
2014-23	0–4					–	3 Jun 2014	Unknown source of infection
2014-24	30–39					Unknown	4 Aug 2014	Travel to Turkey
2014-25	30–39					Unknown	19 Aug 2014	2014-24 (family member)
2014-26	0–4					Unknown	16 Aug 2014	Sought asylum in Denmark
2014-27	40–49					Unknown	20 Aug 2014	2014-24 (visited 2014-24 in hospital)

MMR: measles-mumps-rubella; PEP: post-exposure prophylaxis; RT-PCR: real-time reverse transcription-PCR.

Green cells in the column 'RT-PCR-positive sample' indicate a positive diagnostic real-time RT-PCR from the specimen specified. The grey cells in this column indicate that the particular sample type was not submitted.

Vaccination status: – indicates that the person was not vaccinated with a measles virus-containing vaccine; + indicates that the person had received the first dose of MMR vaccine; ++ indicates that the person had received both doses of MMR vaccine.

The colour coding in the column 'Epidemiological link' indicates the possible links.

[a] Type of specimen unknown (not specified or information unobtainable for other reasons).

[b] Or date of diagnosis for probable cases, based on clinical signs and epidemiological link.

admitted to the paediatric ward of a hospital, the child had stayed in two different waiting rooms: first at the GP's practice and subsequently at the hospital. The public health medical officer later determined via contact tracing that about 60 children had been in the same waiting rooms at some point during the presence of the infected child. Information about post-exposure prophylaxis to non-vaccinated contacts was sent out by post by the public health medical officer, but for some contacts, the information was received too late to prevent infection (e.g. more than 3–6 days post exposure). Subsequently, three (2014-02 to 2014-04) of the 60 children (attack rate: 5%), were diagnosed with measles, which was confirmed both serologically and by RT-PCR. Two of the cases infected in the hospital waiting room transmitted the virus to family members, one of whom was in their 20s. Moreover, before being diagnosed with measles, one of the children who had been in the waiting room attended a day-care centre, where at least one other child was infected. All but one of these cases were aged under 4 years. Additionally, a person in their 30s (2014-16) living less than 1,000 metres from cases 2014-02 and 2014-06 was considered part of this cluster due to geographical proximity, giving a total of eight cases in this cluster.

Three of the four later cases in 2014 (2014-24, 2014-25 and 2014-27) were likely connected, in which 2014-25, a member of 2014-24's family, as well as an acquaintance (2014-27) were infected, likely while visiting 2014-24 during their hospital admission for measles.

Age distribution and vaccination status

The age groups affected differed markedly between the two years. In 2013, the majority of the measles cases (11/17) were younger than 12 years (Figure 3), whereas the majority of cases (16/27) in 2014 were older than 19 years.

Most of the 2013 and 2014 cases were unvaccinated (10/17 and 17/27, respectively). In Denmark, the two-dose MMR vaccination programme was implemented in 1987. Thus, vaccination can be expected only in individuals from 15 months to 27 years of age. In this study, 13 of the 27 unvaccinated cases were outside this age group.

In seven cases, only administration of the first dose of MMR vaccine could be documented (n = 5 and n = 2 in 2013 and 2014, respectively). In 2014, two persons (2014-10 and 2014-11, in their 20s and 30s, respectively) were infected in spite of having followed the full vaccination programme (Figure 3). In both cases, measles was diagnosed based on clinical symptoms and virus detection by RT-PCR from throat swabs. In neither of these cases could antibody levels or IgG avidity be tested since serum was not submitted.

For the remaining cases in both years, no vaccination history could be identified in the records of the national vaccination database and/or of the GP (n = 2 and n = 6, respectively) (Figure 3).

In 2014, a child aged under four years (2014-06, relative of 2014–02) received post-exposure vaccination, but became symptomatic two weeks after the initial symptoms of 2014-02.

Genotyping and phylogenetic analysis

In 2013, the only measles virus genotype identified was D8. Phylogenetic analysis showed that all sequences analysed (16 from 12 cases), except for two from the same patient (2013-03), were 100% identical to the D8 reference strain MVi/Villupuram.IND/03.07/. The two virus sequences from 2013-03 (from urine and swab samples) matched 100% to the D8 reference strain MVs/Frankfurt Main.DEU/17.11 (Figure 4).

In 2014, again only a single genotype was found: B3 (41 sequences from 24 cases). The majority of sequences (23/41) grouped with the B3 reference strain MVi/Harare.ZWE/38.09/, showing 100% sequence identity. Another cluster contained nine 100% identical sequences from seven cases (Figure 4). The sequences in this cluster differed from the Harare strain by only a single conserved point mutation (Figure 4). In five cases (2014-01, 2014-02, 2014-03, 2014-05 and 2014-16), the sequence variants were observed in different specimens collected from the same patient, indicating that virus representing both sequences was transferred simultaneously. In all cases, only one of the two variants was detected in individual specimens (Figure 4). Analysis revealed that this is a silent mutation, not altering the amino acid sequence.

One sequence (from case 2014-13) clustered at some distance from all known B3 strains. This case was a person in their 30s who had travelled from the Netherlands via Germany to Denmark and became symptomatic after arriving home. The viral sequences from this case matched 100% with a sequence found in the MeaNS database from a measles case in Germany in 2014 (Bad Segeberg/DEU/13.14, GenBank accession number: KJ769091).

Another interesting observation is that the last four cases in 2014 (weeks 32–34, 4–20 August) grouped together. Case 2014-24 is thought to have imported measles from Turkey and probably infected two secondary cases (2014-25 and 2014-27). The last case in this sequence group was a child seeking asylum in Denmark after travelling through Europe. The sequences in this group also differ from the B3 MVi/Harare.ZWE/38.09/ reference strain by a single silent base mutation.

Discussion

In 2012, there were two measles cases reported in Denmark [16], whereas in 2013 and 2014, 17 and 27 measles cases were notified, respectively. In these two years, only a single genotype was detected: D8 and B3, respectively, which corresponds well with the

genotypes dominating in Europe during these years [17,18].

The pattern of spread of measles in Denmark was very similar in the two years described here. In both years, a single case resulted in a large cluster. In 2013, an additional small cluster of four cases was observed in a day-care centre, and even though no epidemiological link could be established to the large cluster, the geographical and timely coincidence between the two clusters indicates that they could be a result of the same importation. If this is true, all cases in 2013 arose from just four importations, of which three were without secondary cases. Our study demonstrates that the importation of measles virus only sometimes leads to a cluster, most likely if the virus is imported by children of pre-vaccination age.

The period in which new measles cases were detected was more prolonged in 2014 and included 10 more cases than in 2013. This may be a result of the slightly different importation pattern observed during 2014, where at least eight independent importations were detected. Of these, one resulted in the large cluster of eight cases and five cases resulted in one or two secondary cases (Table).

The 2014 cluster shares many similarities with earlier reported outbreaks in Denmark during 2008 and 2009, which were believed to have started in GPs' waiting rooms [19,20]. This emphasises the need to rethink how to assemble potentially contagious people waiting to be examined and their relatives in small rooms – not only in hospital settings but throughout the entire healthcare system. In Denmark, there is pre-screening before patients are referred to the hospital emergency room, either by the GP or by telephone interview by specially trained nurses outside of GP opening hours. If patients with symptoms of contagious diseases are referred to an isolation waiting room at this stage, situations like that in 2014 may be prevented. Another lesson to be learned from the 2014 outbreak is that when trying to reach traced contacts who may have been exposed to the pathogen (e.g. measles virus in this instance), using postal mail services may not be the most appropriate approach. In this digitalised era, other methods, such as a bulk text message, could have been considered, since most people own and carry a mobile phone and text messages are independent of people being able to answer the phone at a specific time.

The phylogenetic analysis in our study showed that despite the fact that multiple importations occurred from different countries, the measles viruses detected were very closely related. This may be a result of very low mutation rates found in the genome of measles virus [21]. An interesting finding was that in 2014, measles virus sequences obtained from different specimens collected from the same patient showed conserved sequence differences in several cases. Of the seven cases infected with virus containing the mutation, five (2014-01 to 2014-04 and 2014-16) were epidemiologically connected to the large cluster (Figure 2), which supports the theory of simultaneous transfer of the two variants. This strongly indicates that different measles variants can co-exist and co-transfer simultaneously. How widespread this phenomenon is, is unknown since the MeaNS database only allows the uploading of one sequence per sample and does not allow the submission of ambiguous sequences. Furthermore, the result of Sanger sequencing is a consensus sequence: thus minority variants below the level of detection by Sanger sequencing (commonly estimated to be around 20%) are not reported. This finding could have important implications for the use of molecular data in cluster definitions. Cases might be discarded as not being epidemiologically linked if sequencing was only performed on one specimen. Deep sequencing using next-generation sequencing technology could be used to further elucidate the prevalence of co-existing sequence variants, and to epidemiologically link the transmission of such variants.

Our data from 2013 and 2014 clearly show that the vaccination coverage of the MMR vaccine in Denmark (in 2013, it was 87% and 80% for the first and the second vaccination dose, respectively) is not high enough to prevent importation of measles leading to an outbreak due to insufficient herd protection or immunity. The data also indicate that the age of the index patient is important, if a single case is to result in an outbreak.

In both years, the cluster index cases were children who had contact with many other children of the same age. Especially in 2014, very young children, 12 months of age or younger, led to the establishment of the outbreaks. It has been argued that earlier administration of the first dose of MMR vaccine would lessen the risk of this type of outbreak [22]. A previous study has shown that vaccine failure after the second dose is significantly higher if the first dose is given to children of naturally infected mothers at ≤ 15 months of age [23]. However, another study has shown that this may not be relevant in a population with a high vaccination coverage, since maternal antibodies from vaccinated mothers wane much faster than those from naturally infected mothers. Thus, infants are only covered for around three months by maternal antibodies from vaccinated mothers [24]. Our results show that the second vaccination dose is important for sustained protection against infection, since at least seven cases were patients of an age at which the second MMR dose should have been administered. During the two years, seven persons contracted measles after having received only the first MMR vaccine dose. This corresponds with other findings that receiving the second dose is more important than the interval between the two doses [25,26]. Even if the two-dose vaccination programme is adhered to, vaccine failure can be observed. In the present study, with the reservations mentioned in the results section, two cases (in their 20s and 30s) had received both MMR doses before infection. The probability of finding

cases among vaccinated individuals will increase with increasing vaccination coverage and others have also shown vaccine failure and even further transmission from persons with documented two-dose vaccinations [27]. Both cases in our study who had received both vaccination doses (2014-10 and 2014-11) were secondary cases and did not result in further transmission. Studies have shown that in areas with no endemic measles virus circulation, antibody titres will decline over time [28]. This might be one explanation for the occurrence of measles in two-dose vaccinated cases in our study. On the other hand, caution is needed when vaccination status is based on the memory of the individuals concerned.

The effect of a third dose of MMR vaccine given at the age of 11 to 17 years has so far only been evaluated for mumps, but with promising results [29]. Whether this is also true for measles needs to be investigated. Regardless, the two-dose vaccination programme has, from its onset in Denmark in 1987, had an enormous effect on the incidence of measles. Until 1987, between 20,000 and 70,000 cases per year were not uncommon, and the mean number of measles-related deaths were one per 300 cases from 1877 to 1986 [16]. The last recorded death in Denmark caused by measles was in 1989 [16].

Of the 44 cases during 2013 to 2014, only seven were of a pre-vaccination age, whereas 27 were unvaccinated. Seven cases received only the first dose but not the second. This shows that follow-up regarding missed vaccination, e.g. in the form of reminder letters, may be necessary in order to ensure sufficient vaccination coverage. If the WHO goal of elimination of the disease by the end of 2015 is to be achieved, new measures are needed to ensure sufficient vaccination coverage. These could include closer contact between parents and health authorities and clearer communication about vaccination, including risk–benefit and responsibility aspects. In Denmark, interviews of parents showed that the most common reason for failure to vaccinate is forgetfulness [7]. Initial experience in Denmark with reminder letters to parents of children not registered with one or more vaccinations and updated information to parents reluctant to let their children be vaccinated against MMR shows promising results [30].

Conflict of interest

None declared.

Authors' contributions

LKK, PHSA, KTF and TKF contributed to acquisition of data. JR and MWP performed laboratory work. LDR, JF, LKK, PHSA, JR, MWP and TKF analysed and interpreted the data. LDR drafted the manuscript. LDR, JF, LKK, PHSA, JR, MWP, KTF and TKF critically revised the manuscript. All authors approved the final version of the manuscript.

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Uptake and impact of vaccinating school age children against influenza during a season with circulation of drifted influenza A and B strains, England, 2014/15

RG Pebody [1], HK Green [1], N Andrews [1], NL Boddington [1], H Zhao [1], I Yonova [2,3], J Ellis [1], S Steinberger [1], M Donati [1], AJ Elliot [1], HE Hughes [1], S Pathirannehelage [2,3], D Mullett [2,3], GE Smith [1], S de Lusignan [2,3], M Zambon [1]

1. Public Health England (PHE), United Kingdom
2. Royal College of General Practitioners Research and Surveillance Unit, Birmingham, United Kingdom
3. University of Surrey, Guildford, United Kingdom

Correspondence: Richard G Pebody (richard.pebody@phe.gov.uk)

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The 2014/15 influenza season was the second season of roll-out of a live attenuated influenza vaccine (LAIV) programme for healthy children in England. During this season, besides offering LAIV to all two to four year olds, several areas piloted vaccination of primary (4–11 years) and secondary (11–13 years) age children. Influenza A(H3N2) circulated, with strains genetically and antigenically distinct from the 2014/15 A(H3N2) vaccine strain, followed by a drifted B strain. We assessed the overall and indirect impact of vaccinating school age children, comparing cumulative disease incidence in targeted and non-targeted age groups in vaccine pilot to non-pilot areas. Uptake levels were 56.8% and 49.8% in primary and secondary school pilot areas respectively. In primary school age pilot areas, cumulative primary care influenza-like consultation, emergency department respiratory attendance, respiratory swab positivity, hospitalisation and excess respiratory mortality were consistently lower in targeted and non-targeted age groups, though less for adults and more severe end-points, compared with non-pilot areas. There was no significant reduction for excess all-cause mortality. Little impact was seen in secondary school age pilot only areas compared with non-pilot areas. Vaccination of healthy primary school age children resulted in population-level impact despite circulation of drifted A and B influenza strains.

Background

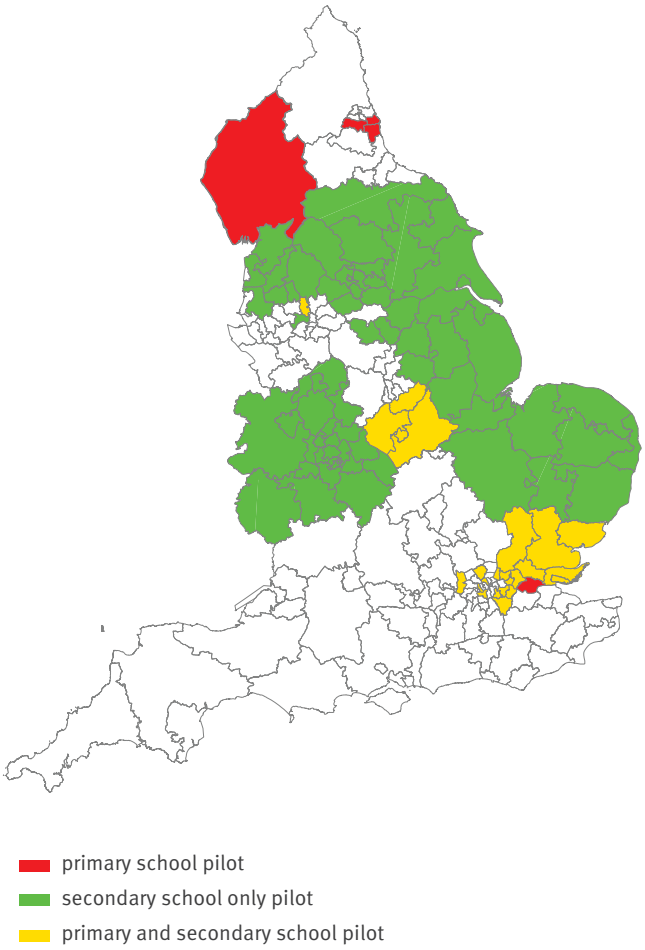
The United Kingdom (UK) started the phased introduction of a universal childhood influenza vaccination programme in the 2013/14 influenza season following the recommendation of the Joint Committee on Vaccination and Immunisation (JCVI) that all healthy children aged two to less than 17 years should be offered the newly licensed live attenuated influenza vaccine (LAIV) [1]. The decision was informed by transmission modelling using Bayesian evidence synthesis, which predicted that vaccination of healthy children would provide direct protection to the vaccinated children themselves and by reducing infection in this group, it would decrease transmission of influenza in the general population and thus provide indirect protection to groups at higher risk of severe disease such as the elderly and those with underlying clinical risk factors [2]. Although North America has a long-standing childhood influenza vaccination programme, there is only limited published observational evidence of whether such programmes produce such indirect population effects [3-5]. Questions also remain as to which paediatric age-groups to target to achieve optimal direct and indirect protection; is it preferable to either vaccinate all school age children or to focus on certain groups such as primary school age children alone?

In the first year of the LAIV programme in England, all healthy children aged two to three years were offered a single dose of LAIV, together with children of primary school age (4–11 years) in a series of geographically discrete pilot areas. Early results suggested that vaccinating primary school age children led to population-level reductions for a range of influenza indicators in pilot areas compared with non-pilot areas [6]. These results, however, were not significant, likely due to the low intensity of virus circulation in the 2013/14 influenza season and the relatively limited number of primary school age children vaccinated.

In 2014/15, the national LAIV programme was extended to all two to four year-olds in England [7]. In addition, the primary school age pilots continued with an increase in the size of the target populations where healthy children 4 to 11 years of age were offered a dose of LAIV, together with the recruitment of additional

Figure 1

Geographical distribution of school-age pilot areas, England, week 40 2014 to week 14 2015



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pilot areas where healthy secondary school children aged 11 to 13 years were offered LAIV. A range of delivery models were deployed specifically school-based or within the community via pharmacies and primary care. The 2014/15 influenza season was a moderately intense season dominated initially by the circulation of influenza A(H3N2) virus, which usually results in severe disease in the elderly, followed by influenza B virus [8]. Virological surveillance found that, as seen elsewhere, the dominant circulating influenza A(H3N2) and B strains were antigenically and genetically drifted against the relevant components of the 2014/15 seasonal influenza vaccine for the northern hemisphere [9].

The implementation of the primary and secondary school age pilots provided a unique opportunity to assess the level of population protection that vaccinating school age children with LAIV might provide over and above the vaccination of pre-school age children in a season when drifted strains circulated. The aim of this paper is thus to measure the uptake of the programme and evaluate the total and indirect impact of vaccinating healthy children of primary or secondary school age in England in 2014/15.

Methods

Most areas that undertook vaccination of primary school age children in the 2013/14 season (6/7) decided to continue this activity in 2014/15 [6]. Local National Health Service (NHS) England teams with an interest in running secondary school age pilot influenza immunisation programmes were selected by the national team. Different models of delivery, in particular, school-based and community-based through pharmacy and primary care, were undertaken in these pilots. Most were school-based, with the exception of two area teams following a pharmacy-based model and one local team following a community GP delivery model.

Measuring vaccine uptake

The target population for delivery was defined as children of primary school age (born between 2 Sep 2003 and 1 Sep 2010; 4 to 11 years old) resident in six pilot areas in England: Cumbria, Greater Manchester, Leicestershire and Lincolnshire, London and Essex, Northumberland, Tyne and Wear. The target population for children of secondary school age (born between 2 Sep 2001 and 1 Sep 2003; 11–13 years of age) were children resident in 12 selected pilot areas (Arden, Birmingham and Black Country, Greater Manchester, East Anglia, Essex, Herefordshire and Worcestershire, Lancashire, London, North Yorkshire and Humber, Shropshire and Staffordshire, South Yorkshire and Bassetlaw, West Yorkshire). Four of the latter sites also ran primary school age programmes. The geographical distribution of these sites is shown in Figure 1.

Local NHS teams responsible for the delivery of the LAIV programme in pilot areas gathered and reported data on vaccine administration to Public Health England (PHE) using a standard proforma through a web-based portal. End-of-season programme uptake was calculated based on the number of children in the target population who were reported to have received at least one dose of influenza vaccine during the campaign period (September 2014 until January 2015). Healthy children and at-risk children in whom the vaccine was not contraindicated were offered LAIV. Inactivated influenza vaccine was offered to at-risk children in whom LAIV was contraindicated.

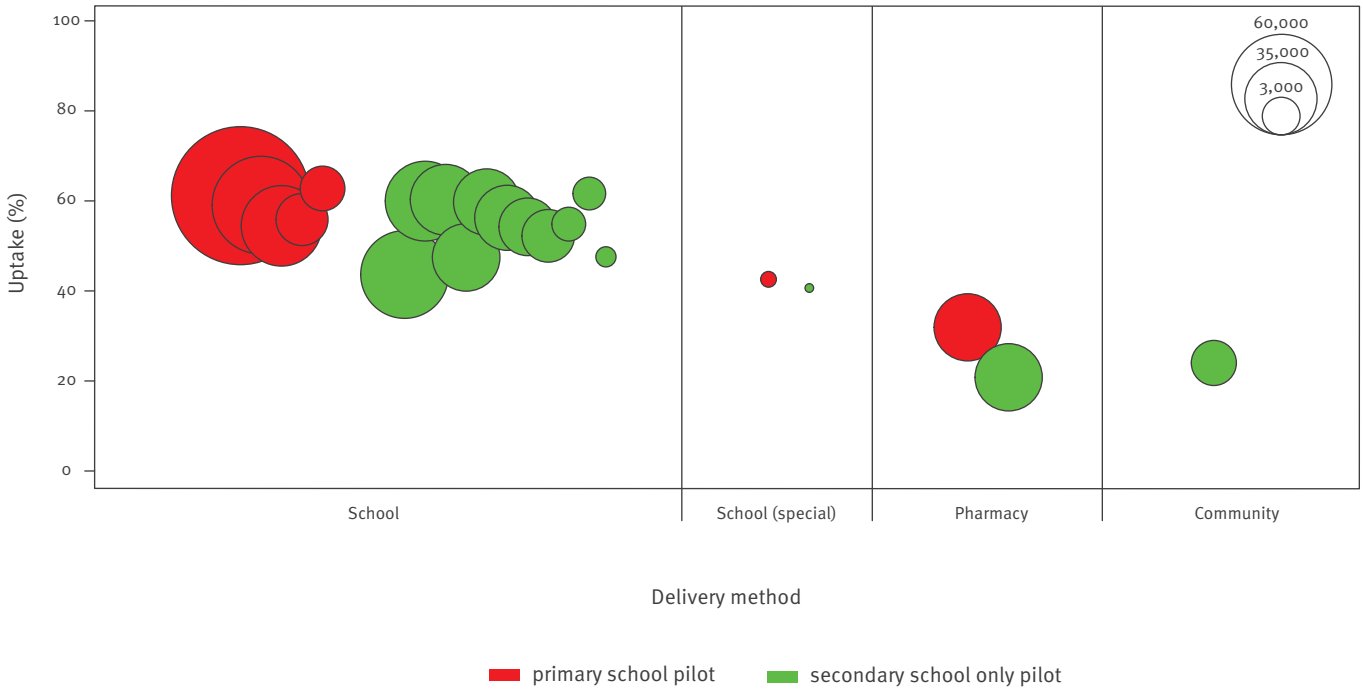
Measuring school age vaccine programme impact

The study period for the programme impact calculations was from week 40 2014 until week 14 2015, the end of notable influenza transmission in the community in the 2014/15 season [8].

LAIV programme impact was defined as the difference in cumulative disease incidence in school age pilots compared with non-pilot areas for the study period.

Figure 2

Uptake of primary and secondary school age influenza vaccination programme in pilot areas by type of delivery, England, week 40 2014 to week 14 2015



It was measured for a range of clinical and virological respiratory endpoints in primary and secondary care.

Primary school age only vaccine areas were pooled with primary and secondary school age vaccine areas to examine the impact of vaccinating primary school age children together with cohorts of two years of secondary school children in addition to the vaccination of pre-school age children. Secondary school age only pilot areas were compared with non-pilot areas to determine the impact of vaccinating the first two years of secondary school age children alone (i.e. in addition to vaccinating children two to four years of age).

Cumulative levels of activity in pilot versus non-pilot areas were compared for four age groups. To examine direct impact, the two targeted age groups for which surveillance data were available were primary school children (5–10 years old) and secondary school children (11–16 years old, where children aged 11–13 years were offered vaccine). To examine indirect impact, the non-targeted age groups compared were under 5 years old and 17 years old and older. Overall impact was assessed by comparing the disease incidence for all ages in pilot vaccination areas compared with non-pilot areas. Indirect impact was measured by comparing incidence in non-targeted age groups in pilot relative to non-pilot areas. To ensure appropriate geographical coverage for the sentinel surveillance schemes, additional sites (general practitioners (GPs), emergency departments and hospitals) were recruited in primary and secondary pilot areas where required.

Data sources

A range of surveillance systems were used to measure the impact of the school age vaccination programme.

Primary care

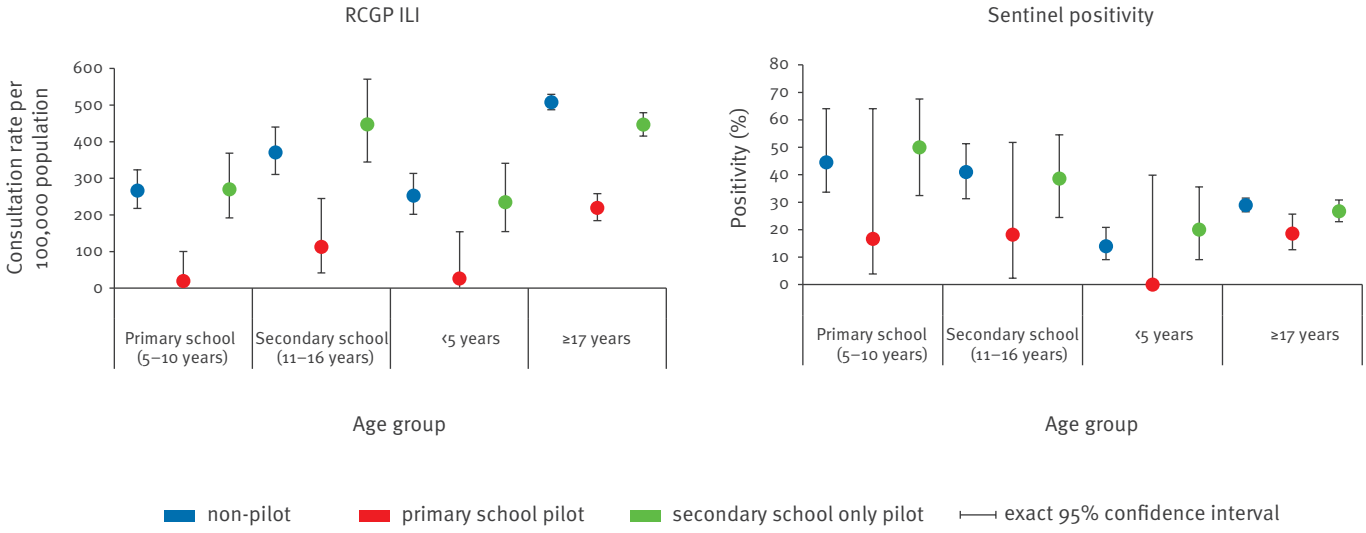
Surveillance in primary care was undertaken through monitoring the weekly influenza-like-illness (ILI) consultation rates through the Royal College of General Practitioners (RCGP) Research and Surveillance Centre (RSC) Weekly Returns Service sentinel GP network, with 29 general practices participating in pilot areas and 58 in non-pilot areas. A proportion of these practices, in conjunction with practices recruited through the PHE coordinated Sentinel Microbiology Network (SMN) scheme, undertook respiratory swabbing on patients under 18 years of age presenting with ILI, and a proportion of patients 18 years of age and older.

Secondary care

The UK Severe Influenza Sentinel Surveillance System (USISS sentinel) consists of a network of 30 NHS hospital trusts (15 in pilot areas and 15 in non-pilot areas in 2014/15) who report the weekly number of laboratory-confirmed influenza hospital admissions [10]. Confirmed influenza hospitalisation rates by age group and pilot area were calculated using estimated hospital catchment populations [11]. As age grouping of populations was not consistent with this analysis, age-specific denominator data were estimated using population age-distributions by Strategic Health Authority from the Office for National Statistics [12].

Figure 3

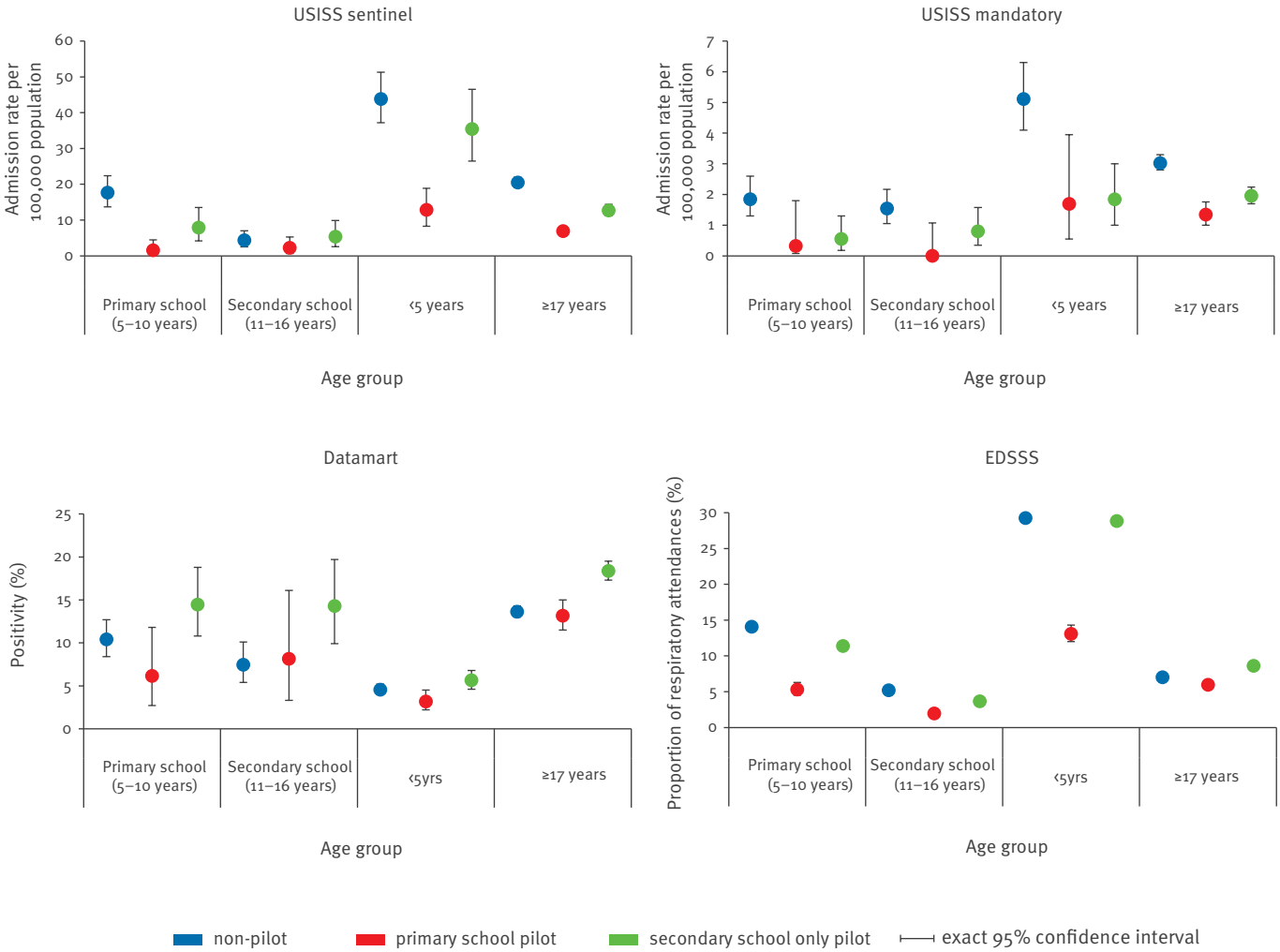
Cumulative primary care indicators in primary school pilot, secondary school pilot and non-pilot areas, England, week 40 2014 to week 14 2015



ILI: influenza-like illness; RCGP: Royal College of General Practitioners.

Figure 4

Cumulative secondary care indicators in primary school pilot, secondary school pilot and non-pilot areas, England, week 40 2014 to week 14 2015



EDSSS: Emergency Department Syndromic Surveillance System; USISS: Severe Influenza Sentinel Surveillance System.

Figure 5

Cumulative weekly all-cause and respiratory excess mortality in primary school pilot, secondary school only pilot and non-pilot areas, England, influenza season, week 40 2014 to week 14 2015

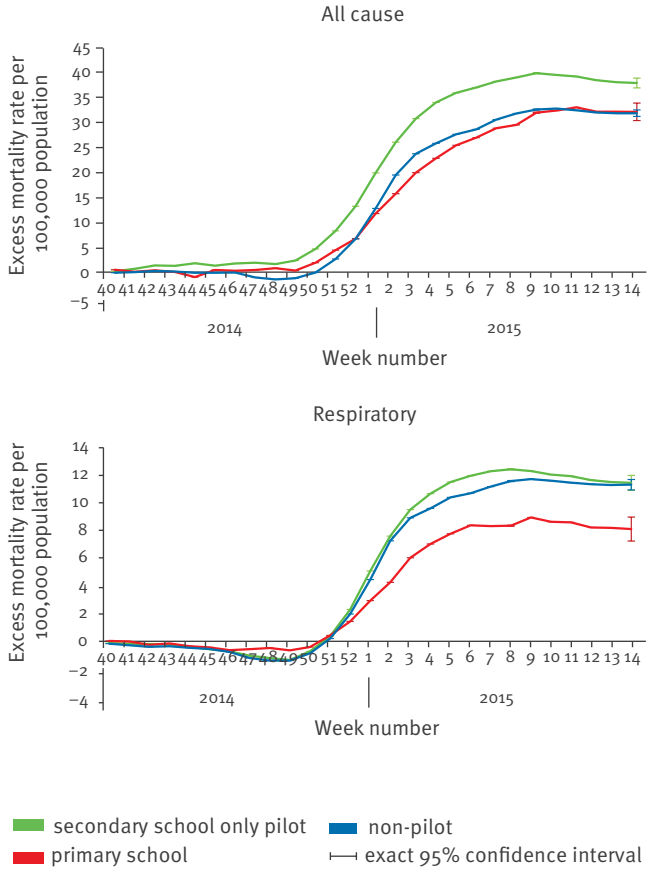
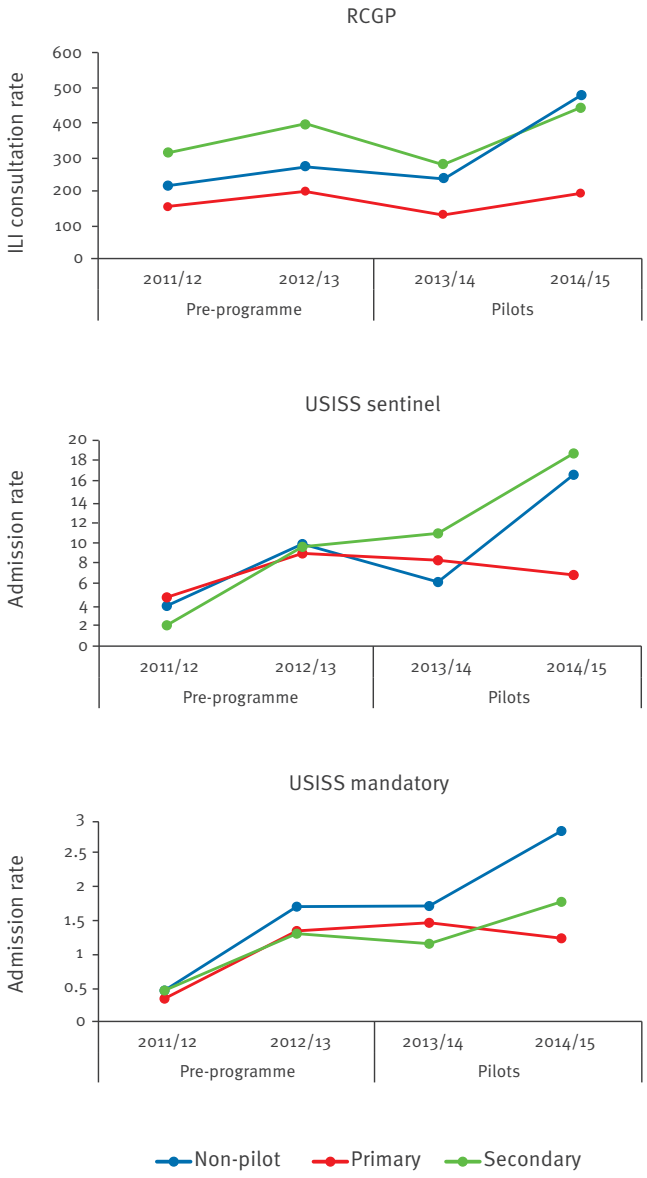


Figure 6

Cumulative, all-age influenza indicators in pilot and non-pilot areas before (2010/11 and 2012/13) and after (2012/13 and 2014/15) vaccine programme introduction, England



ILI: influenza-like illness; RCGP: Royal College of General Practitioners; USISS: Severe Influenza Sentinel Surveillance System.

Only sites with data available across all four seasons are included.

A mandatory scheme is also in operation in all NHS hospitals across England (UK Severe Influenza Surveillance System (USISS mandatory)), monitoring all influenza confirmed intensive care unit (ICU) / high dependency unit (HDU) admissions. Rates were calculated by pilot type and age group as for USISS sentinel [11].

The Respiratory DataMart scheme (RDMS) reports all influenza reverse-transcription-polymerase chain reaction (RT-PCR) respiratory swab results (both positive and negative) from a network of PHE and NHS laboratories in England, with the majority of samples (› 90%) taken from patients in secondary care [13]. Postcode of residence was used to allocate patients to pilot and non-pilot areas. Influenza swab positivity rates in pilot and non-pilot areas were compared by age group.

The Emergency Department Syndromic Surveillance System (EDSSS) monitors routine syndromic surveillance data, in real-time, using anonymised emergency department attendances, across a sentinel network of emergency departments [14]. Attendances monitored include those for respiratory illness. The proportion of all EDSSS attendances for respiratory illness in pilot and non-pilot areas (three emergency departments in primary school age pilot areas, eight in secondary and 18 in non-pilot areas) was compared by age group.

Excess mortality

Weekly excess mortality was estimated in pilot and non-pilot areas based on place of residence using routine death registration data from the Office for National Statistics. The European Monitoring of Excess Mortality for Public Health Action (EuroMOMO) standard algorithm was used to calculate the number of deaths expected for a given week in the year [15]. The number

Table 1

Impact of vaccinating primary and/or secondary school age children on selected primary care influenza surveillance indicators, England, influenza season, week 40 2014 to week 14 2015

Age group		Measure Non-pilot	RCGP (per 100,000 population)			Sentinel swab positivity (%)		
			Primary[a]	Secondary	Non-pilot	Primary[a]	Secondary	
Primary school	5–10 years	Rate/proportion	266.9	19.7	269.7	44.6	16.7	50
		(n/N)	(104/38,969)	(1/5,086)	(39/14,459)	37/83	1/6	17/34
		Risk difference		−247	3		−28	5
		OR (95% CI)		0.06 (0.01 to 0.62)	0.81 (0.39 to 1.69)		0.25 (0.03 to 2.22)	1.24 (0.56 to 2.77)
		p value		0.018	0.573		0.213	0.594
Secondary school	11–16 years	Rate/proportion	371.22	112.6	447.1	41	18.2	38.6
		(n/N)	(133/35,830)	(6/5,330)	(64/14,315)	41/100	2/11	17/44
		Risk difference		−259	76		−23	−2
		OR (95% CI)		0.31 (0.10 to 0.95)	0.96 (0.53 to 1.73)		0.32 (0.07 to 1.56)	0.91 (0.44 to 1.87)
		p value		0.04	0.882		0.158	0.79
Other age groups	<5 years	Rate/proportion	253.1	26.1	34.6	14	0	20
		(n/N)	(84/33,192)	(1/3,826)	(27/11,511)	21/150	0/7	8/40
		Risk difference		−227	−219		−14	6
		OR (95% CI)		0.08 (0.01 to 1.02)	0.65 (0.25 to 1.67)		1	1.54 (0.62 to 3.78)
		p value		0.052	0.367		NA	0.351
	≥17 years	Rate/proportion	508.1	219.1	446.6	29	18.5	26.7
		(n/N)	(2,299/452,461)	(143/65,260)	(767/171,735)	378/1,305	28/151	135/506
		Risk difference		-289	-62		−10	−2
		OR (95% CI)		0.41 (0.19 to 0.86)	0.67 (0.42 to 1.07)		0.68 (0.37 to 1.24)	1.23 (0.72 to 2.11)
		p value		0.018	0.092		0.206	0.455

CI: confidence interval; NA: not available; OR: odds ratio; RCGP: Royal College of General Practitioners.

[a] Includes primary and secondary school pilot areas.

of observed deaths (corrected for reporting delay) was compared with the modelled number expected each week to determine if statistically significant excess mortality was seen in pilot and non-pilot areas [16]. This was applied to all-cause deaths and deaths where the primary cause of death was coded as respiratory applying the International Classification of Disease version 10 (ICD 10) code "J" [17]).

Statistical methods

For the RCGP, USISS sentinel and USISS mandatory schemes, cumulative disease incidence rates per 100,000 population by age and pilot group were calculated by summing the number of disease episodes each week from week 40 2014 to week 14 2015 relative to the average weekly population at risk, with exact Poisson confidence intervals (CIs) calculated.

For the RCGP swabbing and EDSSS schemes, cumulative influenza swab positivity and proportion of emergency department attendances coded as respiratory were calculated by age and pilot group by summing the number of positive samples/patients attending with respiratory symptoms and the number of samples tested each week/total number of attendances from week 40 2014 to week 14 2015 with exact binomial CIs calculated.

To determine the impact in primary and secondary school age pilot areas, odds ratios and corresponding 95% CIs were calculated by age group and scheme, with non-pilot areas set as the reference group. Data were converted to binomial individual level across schemes and random effects logistic regression carried out, adjusting for clustering at the level of reporting unit (e.g. GP, trust, laboratory).

For the all-cause and respiratory coded deaths, the difference between the observed and expected weekly deaths was summed from week 40 2014 to week 14 2015 to obtain the cumulative number of excess deaths. Excess mortality rates were then calculated per 100,000 population.

Laboratory methods

Influenza laboratory confirmation for samples from primary and secondary care was undertaken using RT-PCR assays capable of detecting circulating influenza A viruses, influenza B viruses and other respiratory viruses. Samples in England were sent to the PHE

Table 2

Impact of vaccinating primary and/or secondary school age children on selected secondary care influenza surveillance indicators, England, influenza season, week 40 2014 to week 14 2015

Age group		Measure Non-pilot	USISS sentinel (per 100,000 population)			USISS mandatory (per 100,000 population)			Datamart (%)			Emergency department respiratory attendances (%)		
			Primary[a]	Secondary	Non-pilot	Primary[a]	Secondary	Non-pilot	Primary [a]	Secondary	Non-pilot	Primary[a]	Secondary	
Primary school	5–10 years	Rate/proportion	17.7	1.5	7.9	1.8	0.3	0.6	10.2	5.6	13.8	14.1	5.3	11.4
		(n/N)	69/ 390,100	3/ 194,944	13/ 165,028	36/ 1,948,613	1/ 309,643	5/ 901,855	90/ 882	8/ 143	48/ 348	(5,665/ 40,221)	(134/ 2,524)	(1,213/ 10,662)
		Risk difference		−16	−10		−2	−1		−5	4		−9	−3
		OR		0.07	0.25		0.24	0.44		0.58	1.08		0.26	1.03
				(0.01 to 0.55)	(0.05 to 1.39)		(0.02 to 3.01)	(0.12 to 1.69)		(0.25 to 1.31)	(0.55 to 2.12)		(0.08 to 0.91)	(0.93 to 1.12)
		p value		0.011	0.107		0.271	0.234		0.187	0.831		0.035	0.596
Secondary school	11–16 years	Rate/proportion	4.3	2.3	5.4	1.5	0	0.8	7.2	7.8	13.2	5.2	2.0	3.7
		(n/N)	17/ 391,330	5/ 218,765	10/ 185,372	32/ 2,077,854	0/ 344,510	8/ 995,408	40/ 558	7/ 90	31/ 234	(2,063/ 39,744)	(73/ 3,692)	(514/ 14,066)
		Risk difference		−2	1		−2	−1		1	6		−3	−2
		OR		0.62	0.86		1	0.74		1.05	1.81		0.2	0.65
				(0.11 to 3.38)	(0.11 to 3.38)			(0.20 to 2.67)		(0.43 to 2.56)	(0.97 to 3.37)		(0.05 to 0.82)	(0.56 to 0.75)
		p value		0.577	0.858		NA	0.642		0.921	0.063		0.024	‹0.001
Other age groups	‹5 years	Rate/proportion	43.8	12.8	35.4	5.1	1.7	1.8	4.5	3.3	5.4	29.3	13.1	28.8
		(n/N)	156/ 355,909	25/ 194,622	52/ 146,746	91/ 1,779,661	5/ 295,617	15/ 813,881	257/ 5,682	33/ 1,001	102/ 1,899	(24,237/ 82,859)	(441/ 3,374)	(5,615/19,469)
		Risk difference		−31	−8		−3	−3		−1	1		−16	0
		OR		0.38	0.69		0.39	0.63		0.76	1.29		0.35	1.02
				(0.11 to 1.32)	(0.20 to 2.44)		(0.06 to 2.55)	(0.21 to 1.87)		(0.50 to 1.14)	(0.94 to 1.76)		(0.04 to 2.94)	(0.98 to 1.06)
		p value		0.128	0.566		0.323	0.401		0.186	0.113		0.333	0.27
	≥17 years	Rate/proportion	20.5	6.9	12.7	3	1.3	2	13.1	12.5	17.4	7.0	6.0	8.6
		(n/N)	807/ 3,934,175	172/ 2,484,783	258/ 2,031,142	692/ 22,920,752	52/ 3,863,731	213/ 10,875,340	1,591/ 12,182	201/ 1,608	884/ 5,085	(34,738/ 495,828)	(2,878/ 48,300)	(14,966/173,781)
		Risk difference		−14	−8		-2	−1		−1	4		−1	2
		OR		0.6565 (0.2222 to 11.93)	0.7676 (0.2525 to 2.37)		0.54 (0.25 to 1.16)	0.62 (0.38 to 1.01)		0.91	1.16		0.7878	00.84
										(0.76 to 1.10)	(1.03 to 1.31)		(0.3737 to 11.66)	(00.50 to 1.4141)
		p value		0.434434	0.640640		0.115	0.056		0.327	0.014		0.518	0.503

CI: confidence interval; OR: odds ratio; RCGP: Royal College of General Practitioners; USISS: Severe Influenza Sentinel Surveillance System.

[a] Includes primary and secondary school pilot areas.

Microbiology Services, Colindale (RCGP scheme), one of the network of specialist PHE microbiology laboratories (SMN scheme) or NHS laboratories elsewhere in England.

Results

Uptake

The total target population for the pilots was estimated to be 346,962 primary school children and 371,109 for secondary school children aged 11 to13 years. Five of the six primary school pilot areas chose to deliver the programme through a school-based approach, while one, Cumbria, delivered through community pharmacies. Of the 12 secondary school pilots, 10 delivered the programme through schools only; one through community pharmacies and one through both schools and primary care.

An estimated 196,994 primary school age children received at least one dose of influenza vaccine resulting in an overall uptake of 56.8%. This ranged from 32.3% to 63.1% at pilot-site level (Figure 2). An estimated 184,975 secondary school age children received at least one dose of influenza vaccine, an overall uptake of 49.8%. Uptake ranged from 21.2% to 62.0% at pilot level (Figure 2).

In the primary school age programme, uptake in all the school-based areas was in excess of 50%, compared with coverage of around 30% in the area delivering the programme through a pharmacy-based model. These findings were mirrored by the secondary school age programme, where uptake through school-based models was close to or well in excess of 50%, with an uptake of less than 30% in the areas using community-based delivery models (Figure 2).

Influenza vaccine uptake achieved through primary care in two to four year olds in primary school pilot areas only was 44.1% (224 practices) compared with 39.4% (2,361 practices) in secondary school age vaccine pilot areas; 35.1% (1,012 practices) in primary and secondary school pilot areas and 38.1% (4,176 practices) in non-pilot areas.

Programme impact

Patterns of activity

The cumulative ILI consultation rate and swab positivity in primary care, emergency department respiratory attendances, cumulative hospitalisation incidence rate, RDMS influenza positivity and ICU/HDU rates from week 40 2014 to week 14 2015 were generally lower in pilot areas where primary school age children were vaccinated compared with non-pilot areas across both targeted and non-targeted age groups (Figures 3 and 4). These differences were less marked for the cumulative ICU/HDU and RDMS indicators, particularly in the older non-targeted age group (≥17 years of age).

A large excess all-cause and respiratory mortality was observed in both pilot and non-pilot areas. No significant reduction in all-cause mortality was observed in primary school pilot areas compared with non-pilot areas for all ages, whereas a significant reduction was observed for respiratory excess mortality (Figure 5).

Overall when comparing the various cumulative influenza indicators for secondary school pilot areas (11-13 year olds vaccinated only) to non-pilot areas, no such differences were observed, in both targeted and non-targeted age groups (Figures 3, 4 and 5).

Examination of pre-vaccination data for those indicators for which data were available, provided a mixed pattern, with evidence of similar activity in primary school areas compared to others in one season, whereas it was lower in another (Figure 6).

Impact

Vaccinating primary school age children resulted in significant reductions in cumulative incidence/laboratory-confirmed positivity in the targeted age group (5–10 years) in pilot compared with non-pilot areas for GP ILI consultations (94% reduction, $p=0.018$); emergency department respiratory attendances (74% reduction, $p=0.035$), confirmed influenza hospital admissions (93% reduction, $p=0.012$); with non-significant reductions in GP swabbing positivity (75% reduction, $p=0.213$), confirmed influenza ICU admissions (76% reduction, $p=0.271$) and DataMart influenza positivity (42% reduction. $p=0.187$) (Tables 1,2).

Vaccinating primary school age children also resulted in an indirect non-significant reduction in under five year-olds, in pilot compared with non-pilot areas, for GP ILI consultations (92% reduction, $p=0.052$); emergency department respiratory attendances (65%, $p=0.33$); confirmed influenza hospital admissions (61% reduction, $p=0.128$); confirmed influenza ICU/HDU admissions (61% reduction, $p=0.324$) and DataMart influenza positivity (24%. $p=0.186$) (Tables 1 and 2).

Significant indirect reductions were also seen in individuals ≥17 years of age when comparing GP ILI consultations in primary school pilot to non-pilot areas (59% reduction, $p=0.018$) and non-significant reductions in sentinel GP swabbing (32% reduction, $p=0.206$); emergency department respiratory attendances (21% reduction, $p=0.518$); influenza confirmed hospital admissions (34% reduction, $p=0.434$); influenza confirmed ICU/HDU admissions (46% reduction, $p=0.115$) and DataMart influenza positivity (9% reduction; $p=0.327$)(Tables 1 and 2).

Vaccinating secondary school age-children aged 11–13 years of age alone did not result in a significant reduction in cumulative incidence/positivity for any surveillance indicator when comparing secondary school pilot areas to non-pilot areas, for both targeted and

non-targeted age groups, with the exception of emergency department attendances in the target age group and confirmed influenza hospital admissions in adults (Table 2).

Through determining cumulative risk difference between pilot and non-pilot areas by age group and indicator, it is estimated that 16 primary school age children needed to be vaccinated to prevent one GP ILI consultation in the pilot population; 317 children to prevent one confirmed influenza hospitalisation and 2,205 children to prevent one confirmed influenza ICU/HDU admission.

Discussion

This study assesses the uptake and evaluates the impact of the second season of the new UK LAIV programme for children in England. Piloting the LAIV programme in primary and now secondary school age children in the 2014/15 influenza season, resulted in similar or higher levels of uptake compared with the first. Pilot areas that chose to deliver the programme through school settings achieved higher uptake than those delivered through community settings, such as pharmacies. Despite the circulation of drifted A(H3N2) and B influenza strains, our results demonstrate that vaccinating children of primary school age resulted in a significant reduction in incidence for a range of surveillance indicators. This effect was evident in targeted and non-targeted age groups compared with populations where primary school age children were not vaccinated. The size of the effect was less for more severe endpoints, in particular excess mortality. Vaccination of secondary school age children alone (11–13 years of age) failed to show conclusive evidence of such reductions in disease incidence in either targeted or non-targeted age-groups.

The study has a series of strengths; it builds on approaches developed in 2013/14 to evaluate the uptake and impact of the newly established childhood LAIV programme, population-level data sources are used and the findings are consistent with the 2013/14 findings in terms of uptake, and an impact seen across a range of indicators when primary school children only were targeted [6]. There are, however, also some limitations. Firstly, examination of historical data suggests some caution is needed. The apparent effect sizes should not be overestimated. Our results suggest that the level of activity was lower for some indicators in the previous season (2012/13) in primary school age pilot areas, although the observation was less apparent in the season before that (2011/12) (Figure 6). Secondly, uptake in two to four year-olds in primary school age only pilot areas was slightly different compared with non-pilot areas, which has the potential to affect effect sizes.

The uptake achieved in school age children this season builds on that reached in 2013/14, with coverage now in excess of 50% in almost all pilot areas delivering the programme through a school-based approach (for either primary or secondary school age children). Earlier modelling work had suggested that at these levels of uptake for all school-age children, indirect benefits through reduction in population transmission of influenza are likely to occur [2]. Our findings also highlight the lower uptake among school age children achieved in areas deploying non-school based delivery approaches. This mirrors observations in relation to human papillomavirus (HPV) adolescent vaccine programmes, where countries using school-based delivery models achieved consistently higher uptake compared with other approaches [18]. In countries such as England, with its very high school attendance levels, there seem to be clear advantages to this delivery approach for a paediatric influenza vaccine programme for children of school age, although there was still variation in uptake, particularly in relation to factors such as deprivation and ethnicity in 2013/14 [19]. Further work is still required to refine the optimal delivery model, particularly from an equity and efficiency perspective.

The finding that vaccinating children of primary school age, in addition to the pre-school vaccination programme, led to reductions in disease incidence in both targeted and non-targeted age groups for a range of influenza indicators builds on observations from the first year of the programme when only primary school age children were vaccinated in pilot areas [6]. This season we observed in these areas decreases in influenza disease not only in the primary school age children themselves, but also indirectly in children under five years of age, where the burden of influenza is recognised to be highest, together with smaller indirect reductions in adults, where influenza disease burden is also high (in particular in the elderly and clinical risk groups). The indirect impact of vaccinating primary school age children under five years old is over and above any direct impact that might have been due to the pre-school LAIV programme itself which operated across the whole of England in both pilot and non-pilot areas. These indirect reductions were consistently seen for primary care consultations (for both clinical and virological end-points) and laboratory confirmed hospitalisations and ICU/HDU admissions. As seen in 2013/14 though, the effect sizes became less as the end-points become more severe. These findings are supported by publications from elsewhere [3-5], including a recent article by Tran et al. showing that vaccination of school age children (with ca 50% uptake) led to large reductions in ILI emergency department visits across all ages in the local community [20]. In addition, the differential roll-out of the LAIV programme across the countries of the UK, with Scotland and Northern Ireland vaccinating primary school age children and Wales secondary school age children in 2014/15 has shown some early encouraging signs in relation to reductions in primary care consultations for those countries vaccinating primary school age children. This will provide further important opportunities to understand the population

level impacts of the universal paediatric influenza vaccination programme [8].

The 2014/15 season was characterised by significant excess all-cause mortality across Europe, particularly in the elderly, an observation consistent with the circulation of influenza A(H3N2) [8]. Although there was reduction in respiratory excess mortality in primary compared with non-pilot areas, we found no evidence of a significant reduction in excess all-cause mortality. The reduction in excess respiratory mortality in those areas where primary school age children were vaccinated is encouraging, though the reasons for the lack of visibility of an indirect effect for excess all-cause mortality, which is a more non-specific indicator, are not totally clear, but could well be linked to lack of study power and is consistent with the smaller reductions we saw for the more severe end-points in the older age-groups. Further work is planned to understand these differences, as this is where much of the health economic benefits of a school-age influenza vaccination programme will be derived [2].

Despite looking at a range of indicators, we were unable to demonstrate evidence that vaccinating a cohort of children of secondary school age (albeit the first two years of secondary school) alone led to any consistent reduction in disease incidence in either targeted or non-targeted age groups in pilot areas. Although other studies have shown reductions in rates of respiratory illness in secondary school populations that undertake universal vaccination [21], no other studies, to our knowledge have shown population level benefits of vaccinating secondary school age children alone, although it is important to note that only two cohorts of secondary school age pupils were offered vaccination in the present pilot.

It is also important to note that the reductions we observed occurred in a season in which an antigenically and genetically drifted A(H3N2) strain was the predominant circulating strain [8,9], that had earlier resulted in low overall vaccine effectiveness against influenza A(H3N2), albeit with some evidence of effectiveness for LAIV in children [22]. Of further note, is that the dominant circulating influenza B viruses at the end of the 2014/15 season also showed evidence of drift from the influenza B/Yamagata lineage vaccine strain [8]. These population-level impact findings of LAIV vaccination of school-age children suggests LAIV may have cross-protective effects against drifted strains, as reported previously [23].

In conclusion, our findings support the on-going roll out of the national LAIV programme for children of primary school age. The added benefit of vaccinating secondary school age children needs further careful consideration. Further work will need to continue to evaluate the impact of the LAIV programme against a range of end-points, in particular mortality related end-points.

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Conflict of interest

None declared.

Authors' contributions

RP led the design of the study and study group; all co-authors were members of the study group and involved in data collection, management and analyses; HG, NA and RP led the data analysis; HG undertook the summary analyses; HZ was responsible for the RDMS system, data management and analysis; NB was responsible for the USISS system, data management and analysis; HH, AE and GS were responsible for the EDSSS data system management and analysis; IY worked directly with the practices and SP, DM and SdeL were responsible for the RCGP data system management and analysis; JE, MD and MZ were responsible for virological testing schemes; SS and NB were responsible for monitoring of vaccine uptake in the pilot sites; RP drafted the initial manuscript; all co-authors reviewed and commented including approval of the final version

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