Eurosurveillance

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

Impact factor **4.659**

Vol. 20 | Weekly issue 10 | 12 March 2015

ecdc
EUROPEAN CENTRE FOR
DISEASE PREVENTION
AND CONTROL

www.eurosurveillance.org

Two cases of acute severe flaccid myelitis associated with enterovirus D68 infection in children, Norway, autumn 2014

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Enterovirus D68 (EV-D68), phylogenetic clade B was identified in nasopharyngeal specimens of two cases of severe acute flaccid myelitis. The cases were six and five years-old and occurred in September and November 2014. EV-D68 is increasingly associated with acute flaccid myelitis in children, most cases being reported in the United States. Awareness of this possible neurological complication of enterovirus D68 infection is needed.

An unexpectedly high proportion of children were admitted for severe respiratory infections at the Oslo University Hospital, Ullevål, Norway, during September and October, 2014 [1]. Enterovirus was detected in 66 (22%) of 303 samples from children hospitalised with acute respiratory infection, and in five of 51 samples received from outpatient clinics. Enterovirus D68 (EV-D68) was verified in 33 of the enterovirus-positive samples from hospitalised patients, and in one of the outpatients.

We report two cases of severe acute flaccid myelitis (AFM) associated with EV-D68 infection that occurred in September and November 2014 in Norway.

Case 1

A six year-old girl was referred to a paediatric department in the Oslo area, Norway, on 20 September 2014. She was previously healthy and fully vaccinated according to the Norwegian child vaccination programme, including polio vaccine. She reported a sore throat, neck pain, headache and occasional vomiting for two days. Fever (38.6 °C) occurred on the second day and she became increasingly tired with tachypnoea, coughing and abdominal pain. She presented with a faint voice, reduced general condition, neck pain, but no nuchal rigidity. General and neurological examination was normal. Leucocyte count was 12.9×10^9 cells/L (norm: 5.0–15.5) with neutrophilocytes accounting for 74%. C-reactive protein (CRP) was 4 mg/L (norm: 0.0–4.0). Viral upper airway infection was suspected, but PCR analysis of a nasopharyngeal specimen was negative for common respiratory viruses, and the patient was discharged. She was readmitted two days later with further deteriorated general condition, general muscle weakness, mainly proximal and more severe in both upper extremities and neck, and weak/ absent deep tendon reflexes. A chest X-ray showed an atelectasis in the left lower lobe but a lower respiratory specimen was not secured. Severe respiratory failure, due to diaphragmatic paresis, resulted in respirator treatment. Meningitis treatment with cefotaxime, ampicillin and aciclovir was started. Acute disseminated encephalomyelitis (ADEM) was suspected, and methylprednisolone instituted.

Cerebrospinal fluid (CSF) pleocytosis (173×10^6 cells/L (norm: 0–4), 94% mononuclear) and a slightly increased CSF protein concentration (0.45 g/L (norm: 0.000–0.450)) were found on Day 4. On Day 6, before intravenous immunoglobulin infusion or plasmapheresis, only a slight pleocytosis (23×10^6 cells/L) was found, however, an increased IgG index indicated intrathecal IgG production but oligoclonal bands were not detected. CSF cell count had normalised on Day 44, with CSF protein still slightly increased (0.648 g/L).

PCR analyses of the CSF revealed no intrathecal herpes simplex virus, human herpes virus 6 or 7, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, human parechovirus or enterovirus. Bacterial culture, including for *Listeria monocytogenes*, was negative. No intrathecal antibodies against *Borrelia burgdorferi* were detected. Serological analysis showed no infection with herpes simplex virus, varizella-zoster virus, Epstein-Barr virus, cytomegalovirus, *Mycoplasma*

pneumoniae, Borrelia burgdorferi, Cryptococcus neoformans or *Mycobacterium tuberculosis*. Two faecal specimens, collected on Days 21 and 22, showed no growth of enterovirus. Antibodies associated with autoimmune encephalitis could not be detected.

Magnetic resonance imaging (MRI) on Day 4 showed oedema of mainly grey matter in a longitudinal, transverse pattern, extending from the pedunculi cerebri to the thoracolumbar level. Day 8 there was regression of the oedema, and MRI was normal on Day 44. Electroencephalography (EEG) was normal on Day 3 and Day 16, but compatible with encephalitis/ encephalopathy on Day 5.

Following reports from the United States (US) and Canada on EV-D68, a nasopharyngeal specimen taken on Day 2 was tested with a generic real-time RT-PCR for enteroviruses targeting the 5' non-coding region, and was positive. Sequencing of the PCR product suggested EV-D68, which was confirmed by a real-time RT-PCR EV-D68 assay (ct value < 30 cycles). PCR for EV-D68 was negative in serum (Days 4 and 12), faeces (Day 9) and CSF (Days 4 and 44), and in tracheal secretion (Day 30).

The patient now walks steadily, but head control is poor. Motor impairment is worse proximally and still pronounced in the proximal upper extremities and neck where muscular atrophy is evident. She is partly fed through a gastrostomy tube and speaks with a thin voice.

Case 2

A five year-old girl was referred to a paediatric department in the Oslo area, Norway, on 13 November 2014. She was previously healthy and fully vaccinated according to the Norwegian child-vaccination programme, including polio vaccine. She had a history of upper airway infection, poor feeding and drinking, and fever up to 39.4 °C for 12 days. On Day 4 of the illness she complained of neck and back pain. On Day 5, headaches, abdominal pain and vomiting occurred. On Day 6, she complained of stiffness of the neck. The family doctor found a reduced general condition, mild dehydration, but no nuchal rigidity. On Day 7, weakness occurred in the lower extremities, impairing gait, and the pain and general condition had worsened.

Upon examination at the hospital she was awake and alert, with panting tachypnoea. She was tachycardic (pulse: 161/min) and febrile (38.4 °C), other vital signs were normal. Neurological examination was normal. White blood cell count was 12.8×10^9 cells/L with 77% neutrophilocytes. CRP was 10 mg/L. A chest X-ray showed a pneumonic infiltrate. PCR analysis of a nasopharyngeal specimen was negative for common respiratory viruses. Erythromycin was instituted and the patient was discharged. PCR of a nasopharyngeal aspirate was negative for *Mycoplasma pneumoniae* and bacterial culture showed significant growth

of *Haemophilus influenzae*. Lower respiratory samples were not secured. On Day 12, the patient was readmitted with increasing weakness of the left arm, gait difficulties, pain in the neck, left shoulder and both legs, most severely when extended. She had dyspnoea, tachypnoea and panting. Brudzinski's sign was positive and she presented flaccid paralysis grade 1–2 of the left arm as well as weakness of neck muscles, she could not hold her head or walk unsupported. The patient hypoventilated since the diaphragm was partly paretic. Continuous positive airway pressure (CPAP) treatment and cough assist machine were instituted and intravenous immunoglobulin infusion was given.

A lumbar puncture revealed 14×10^6 cells/L, a protein level of 0.88 g/L and glucose of 4.4 mmol/L (norm: 2.5–4.4). There was no serological evidence of autoimmune encephalitis. EV-D68 was detected in a nasopharyngeal specimen collected at Day 7, using a generic real-time RT-PCR (ct value < 30 cycles), however, culture was negative. EV-D68 was not found in stool (Day 18), serum (Day 15), or CSF (Day 15). CSF cultures (virus and bacteria) (Day 15) were negative. An MRI scan on Day 15 showed cervical central medullary oedema and on Day 20 grey matter oedema at cervical and thoracic level, representing myelitis, as well as radiculitis in the lumbar region. Neurography supported the diagnosis of acute anterior myelitis.

The outcome is with severe paresis and atrophy in the proximal left arm and left upper limb girdle.

Sequence analysis for Case 1

PCR for sequencing was performed as described in Nix et al. [2]. Sequencing was not possible in for Case 2, due to low amounts of virus and insufficient sample material. The sequence from Case 1 was aligned with the sequences of one reported AFM case from 2014 in France as well as other European strains, including the majority of the 16 Norwegian EV-D68 cases identified in autumn 2014 (Figure) [1].

Discussion

EV-D68 is mainly associated with respiratory disease and was first isolated in California in 1962 from children with airway infection [3,4]. Until 2009, EV-D68 was rarely isolated [5]. Since then, outbreaks of respiratory disease with EV-D68 have occurred worldwide [6-9]. EV-D68 infections in the Dutch population is increasing [10]. In autumn 2014, EV-D68 was found in 11% of children hospitalised for airway infection in the Oslo region [1]. Of the 34 patients, 32 were younger than seven years.

During an outbreak of respiratory EV-D68-disease in the US in autumn 2014, nine children presented with AFM; EV-D68 was detected in four of eight nasopharyngeal specimens [11]. From 2 August 2014 to 2 March 2015, the American Centers for Disease Control and Prevention (CDC) verified reports of 115 children in 34 US states who developed AFM. These cases are

Phylogenetic cluster analysis of enterovirus D68 partial VP1 sequences (207 nt) from an acute flaccid myelitis case, Norway, September 2014
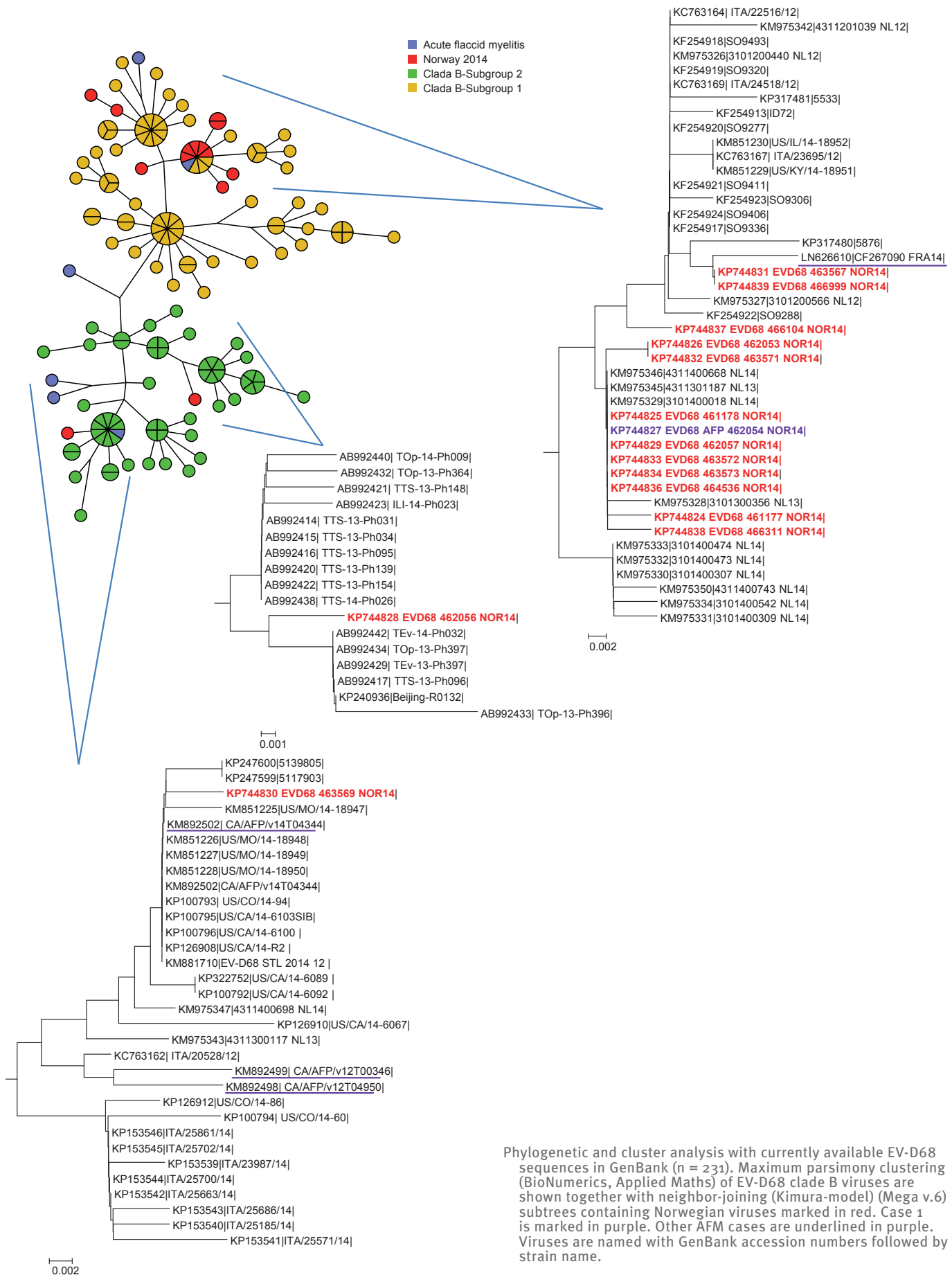
Phylogenetic and cluster analysis with currently available EV-D68 sequences in GenBank (n = 231). Maximum parsimony clustering (BioNumerics, Applied Maths) of EV-D68 clade B viruses are shown together with neighbor-joining (Kimura-model) (Mega v.6) subtrees containing Norwegian viruses marked in red. Case 1 is marked in purple. Other AFM cases are underlined in purple. Viruses are named with GenBank accession numbers followed by strain name.

currently being investigated [12]. A single European case of EV-D68-associated AFM is reported [13]. This virus was of Clade B, closely related to Dutch, Spanish and Italian types from 2009 to 2014. From outbreaks in Asia and Australia, enterovirus-71 (EV-A species) is known to cause AFM [14,15]. However EV-D68 has rarely been reported to cause AFM, and almost never been isolated from CSF [5,14].

In the two AFM cases we report here, the virus was only detected in the early nasopharyngeal specimens, highlighting the importance of obtaining this specimen early. Pleocytosis, increased intrathecal IgG index, increased CFS-proteins, spinal grey matter-oedema and denervation on neurophysiology may indicate direct CNS infection and neuron destruction. A causal relation is however not proven since no direct evidence of EV-D68 infection in the CNS has been found. Detection of EV-D68-virus may have been coincidental and due to the high incidence of the virus [9]. However, the two cases share striking clinical and imaging similarities [16] and no other pathogen has been detected. Intrathecal EV-D68 antibody detection would be indicative of EV-D68 being the neurotrophic pathogen, however this analysis is not available in our hospital and was therefore not performed.

Sequencing of the VP1 gene obtained from Norwegian cases showed a similar genotype in the AFM case and non-AFM cases. While a statistical increase is difficult to prove for such a rare disease, the authors are of the opinion that there is an increase in AFM cases associated with enterovirus D-68 as has been reported from the US [17]. On basis of our findings we can speculate that this may not be caused by increased neurotrophy in a single genotype, but perhaps due to an increased number of EV-D68-infected individuals. It is possible, however, that mutations in non-VP1 regions are responsible for increased neurotrophy.

As we found an identical VP1-sequence in Case 1 and in a child with solely respiratory disease, host-related factors are likely to be of importance for the individual risk of developing AFM. At least three different EV-D68 strains were circulating in Norway in the autumn of 2014, suggesting three separate introductions. No differences in the partial sequence of the VP1 region were observed between the AFM case and non-AFM cases. International collaboration is needed to confirm the association between EV-D68 and AFM, and to achieve knowledge on treatment and outcome. It is important to recognise this disease, which may become life-threatening.

Conflict of interest

None declared.

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Self-sampling for community respiratory illness: a new tool for national virological surveillance

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This report aims to evaluate the usefulness of self-sampling as an approach for future national surveillance of emerging respiratory infections by comparing virological data from two parallel surveillance schemes in England. Nasal swabs were obtained via self-administered sampling from consenting adults (≥16 years-old) with influenza symptoms who had contacted the National Pandemic Flu Service (NPFS) health line during the 2009 influenza pandemic. Equivalent samples submitted by sentinel general practitioners participating in the national influenza surveillance scheme run jointly by the Royal College of General Practitioners (RCGP) and Health Protection Agency were also obtained. When comparable samples were analysed there was no significant difference in results obtained from self-sampling and clinician-led sampling schemes. These results demonstrate that self-sampling can be applied in a responsive and flexible manner, to supplement sentinel clinician-based sampling, to achieve a wide spread and geographically representative way of assessing community transmission of a known organism.

Introduction

The 2009 A(H1N1) influenza global pandemic presented major challenges for health systems around the world in both developed and resource limited countries. Accurate recognition of viral transmission in the community and predictive assessment of trends in clinical morbidity were required to optimise specific interventions such as antiviral prophylaxis and vaccination of risk groups and more general social distancing measures such as school closures. Novel and flexible approaches to surveillance were required during these periods of rapidly changing disease indicators and fluctuating demand for healthcare delivery.

During the initial phase of the pandemic in England (May to June 2009), laboratory testing focused on patients who fulfilled the national algorithm [1]. The case definition targeted travellers returning to the United Kingdom (UK) from high risk countries (e.g. Mexico) presenting for treatment, mainly in secondary care settings. In parallel, general practitioner (GP) virological surveillance was enhanced to provide an estimate of community morbidity due to the newly emerged influenza A(H1N1)pdm09 virus. Aggregation of data from laboratory-confirmed cases provided a reasonable estimate of growth and spread of the pandemic but as the pandemic progressed, the pattern of healthcare provision shifted.

Rather than contact GPs or visit emergency departments, patients were encouraged to use the national telephone helpline (NHS Direct (NHSD); note that NHSD ceased operations on 31 March 2014). In July 2009, amid the first wave of the pandemic, an influenza-specific telephone and web-based health service was launched (National Pandemic Flu Service (NPFS)) that authorised the collection of oseltamivir for those patients over the age of one year, with no respiratory complications and with no underlying medical conditions who were suspected of having pandemic influenza A(H1N1). NPFS continued through the second wave of the pandemic until early in 2010. Virological surveillance of those in the community seeking access to medical care through these alternative routes was undertaken through self-sampling. The feasibility of this approach had been previously demonstrated during seasonal influenza [2] and was instigated during May 2009 to assist the provision of accurate estimates of number of cases [3].

Here, virological surveillance data, including semi-quantitative analysis of viral load, obtained from patient self-sampling is compared with GP (clinician) sampling to assess the usefulness of this approach for future national surveillance of emerging respiratory infections.

Methods

Community self-sampling

Self-sampling was based upon previously validated methodologies for both logistics and laboratory analyses [2,3]. In brief, symptomatic members of the public who had used either the national telephone service (NHSD or NPFS) and/or the website interface, who were symptomatic for 'cold/influenza' symptoms and issued a prescription/voucher for oseltamivir, were selected for the self-sampling scheme. Each day, equal numbers of eligible participants in England were randomly selected per region based on Strategic Health Authority (SHA) boundaries. A sampling kit similar to those provided to sentinel GPs but modified to fit through a standard letterbox, was sent to the participant's home address. Each kit included a personalised introductory letter, a patient information leaflet explaining the scheme, an instructional sheet on how to take a nasal swab sample, a dry swab, a vial of virus transport medium (VTM) and a short epidemiological questionnaire requesting information on basic patient demographics (age/sex), presenting symptoms and date of onset, date of swabbing and antiviral treatment (start date and number of doses of antivirals, if taken). Pre-paid packaging (which complied with the UN 3373 regulation) was also provided with instructions to return specimens by the postal system (at no cost to the patient) to the Health Protection Agency (HPA; the HPA became part of Public Health England on 1 April 2013) Colindale laboratory. Self-sampling was operational from 28 May 2009 through to 18 March 2010 (week 22 2009 to week 11 2010) through either NHSD (28 May to 2 August 2009 and 18 February to 18 March 2010) or NPFS (3 August 2009 to 12 February 2010). Data presented here were from 6 August to 18 November 2009, when self-sampling through NPFS was operational for those aged ≥ 16 years.

Clinician-based sampling through sentinel general practitioners

Sentinel GPs participating in the clinical surveillance network run by the Royal College of General Practitioners (RCGP) Weekly Returns Service (WRS) took a combined nose and throat swab from patients presenting with influenza-like-illness (ILI) [4]. Swabs were placed in VTM and returned to the HPA Colindale laboratory either by post or a hospital courier system (as previously described) [5]. This scheme was operational continuously between October 2008 and June 2010.

Study period

The clinician- and self-sampling schemes were compared on samples from those aged ≥ 16 years, returned within the 15 week period between 6 August 2009 and 18 November 2009, inclusive. During this period of time both schemes were fully operational across England, and were analysed in an identical manner at the HPA Colindale laboratory.

Virological testing by reverse transcription real-time polymerase chain reaction

Returned samples from both surveillance schemes were analysed by real-time polymerase chain reaction (PCR) at the HPA Colindale laboratory as described previously [6-9], testing for A(H1N1)pdm09 as well as seasonal influenza viruses (influenza A(H1N1), A(H3N2) and influenza B). Positive results from all schemes were released to GPs who arranged further clinical management as appropriate.

Statistical analysis

Linear regression models were used to assess viral shedding post onset of symptoms. Cycle threshold (Ct) values generated by the real-time PCR assays were used as the outcome variable with delay, scheme and age as predictor variables. The Ct values provided a qualitative positive or negative result that was then used in a logistic regression modelling analysis to compare results obtained from both self-sampling and clinician-sampling schemes. Swabs from the RCGP sentinel virological scheme were taken from patients at the point of presentation to medical services with illness while those taking part in the self-sampling scheme had an inevitable delay due to the posting of the sampling kit to the patient. Any swab obtained where the sampling date was seven or more days post-symptom onset was excluded, as were any swabs for which either the date of swabbing or the date of symptom onset was not recorded. Only adults aged ≥16 years were eligible for self-sampling during the study period, thus data from children were excluded from the RCGP dataset in order to derive an accurate assessment of comparability between the schemes.

The results from the remaining swabs were included in a mixed-effects logistic regression model where the outcome was the binary variable of whether the swab was positive for A(H1N1)pdm09 virus. The following variables were included in the model as fixed effects; centred sequential week number (week), age group (16–24, 25–44, 45–64, ≥65 years), scheme (self-vs clinician-sampling), and delay (day between symptom onset and swabbing). A composite factor for week and English region (London, West Midlands, North, and South) was included as a random effect to enable the temporal trends to vary between regions. The distribution of the random effects was assumed to be Gaussian. The models were fitted using the xtmelogit command in Stata 11, which utilises adaptive Gaussian quadrature to approximate the log-likelihood.

Ethical approval

Self-sampling was undertaken as part of a public health surveillance programme in response to the 2009 influenza pandemic and was carried out under NHS Act 2006 (section 251) which provides statutory support for disclosure of such data by the NHS, and the processing by the HPA for communicable disease control. As such, no explicit ethical approval was necessary or sought.

FIGURE 1

Comparison of cycle threshold (Ct) values between A) self-sampled and B) clinician-sampled influenza A(H1N1) pdm09 polymerase chain reaction positive swabs in adults ≥ 16 years

A

B

PCR: polymerase chain reaction.

obtained from PCR positive samples collected through both schemes (Figure 1).

There was no evidence of a difference in Ct values between clinician-based sampling and community self-sampling (p=0.93). There was also no difference between the schemes after the addition of age as a continuous variable for community-based self-sampling (p=0.15) or for clinician-based sampling (p=0.20). Age was also looked at as a categorical variable and there was no impact on the overall model (self-sampling p=0.45; clinician sampling p=0.38). The models were also not affected by adding time/week of swab (p=0.26 for both schemes) nor was there evidence of a regional effect (self-sampling p=0.32; clinician-sampling p=0.067). The results shown in Figure 1 indicate that when comparable samples were analysed there was no significant difference in Ct values obtained between self-sampling and clinician-led samples.

The sampling of individuals through the community self-sampling scheme was, however, invariably subject to greater delay post-illness onset, because of the time taken for swab kits to be delivered to the patient. There was a clear difference in the delay between onset of symptoms and swabbing in the two schemes, with around half the swabs in the clinician-led scheme taken within two days of symptom onset compared with the four days in the self-sampling scheme (Figure 2). Increasing time post-illness onset is known to correlate with reduced virus shedding in both seasonal and pandemic influenza [8,10].

A mixed-effects logistic regression model incorporating the swabbing results from both schemes was fitted. Differences between PCR positivity over time in the schemes were explored by incorporating an interaction

Results

A total of 26,237 swabs (20,722 self-sampled, and 5,515 clinician-sampled) were received during the pandemic between 28 May 2009 and 18 March 2010, of which 9,292 and 1,949 were within the 15 week study period, respectively. Exclusions included: swabs taken outside England; taken seven or more days after symptom onset; unknown date of swabbing, or symptom onset; contaminated sample (e.g. bacterial or fungal contamination) therefore unsuitable for testing; missing antiviral information (total exclusions: 3,249 (35.0%) self-sampled, and 803 (41.2%) GP-sampled swabs). The remaining swabs (6,043 self-sampled and 1,146 GP-sampled) were analysed as part of this study with a similar PCR positivity rate for influenza A(H1N1)pdm09 of 19.3%, and 25.9% respectively. To compare the submitted samples from self-sampling and clinician-based sampling, trend analysis of viral load was carried out through analysis of the semiquantitative Ct values

FIGURE 2

Empirical cumulative distribution function for the delay between onset of symptoms and date of swabbing in self-sampling (n=6,043 samples) and clinician-sampling (n=1,146 samples) schemes
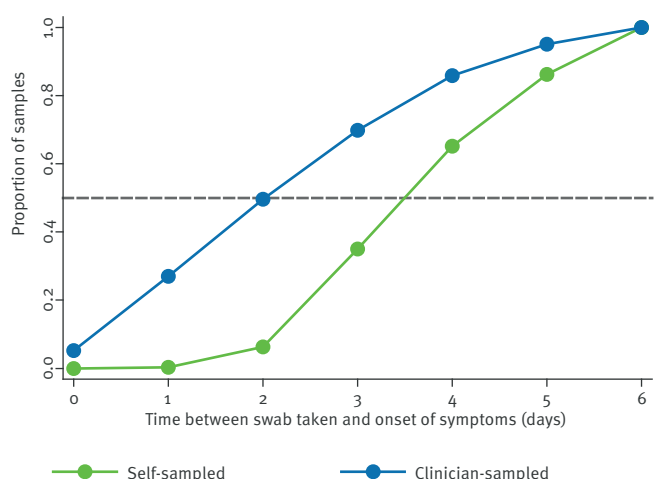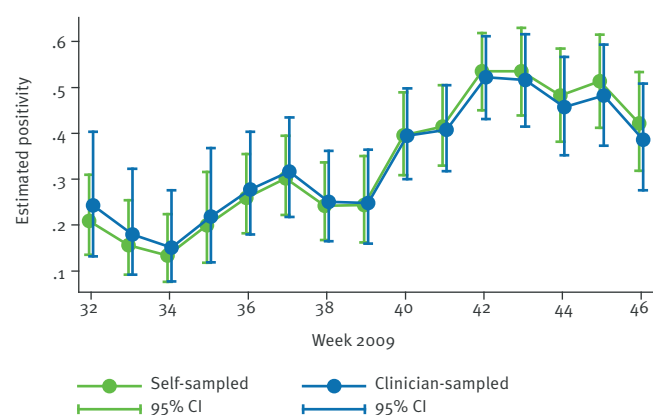
CI: confidence interval.

term into the regression model. There was no evidence that the association between positivity and the age group differed between the two schemes, p = 0.3 (Wald test statistic for interaction 4.89, 4 degrees of freedom (df)). After allowing for the delay in swabbing and age groups there was no evidence that positivity differed systematically between the schemes over time, p = 0.23 (Wald test statistic for interaction 1.39, 1 df). Representative results of the regression model are presented in Figure 3 for the 16 to 24 year-old age group, and the northern SHA grouping (North East, North West, Yorkshire and the Humber, and East Midlands) and a three day delay in swabbing (Figure 3). Other age groups and regions exhibited the same temporal differences between the schemes (data not shown).

Discussion

During the early stages of the pandemic, diagnosis and national level statistics were obtained through accumulation of individual case based diagnostic testing in returning travellers [1]. The transition from individual case based laboratory diagnosis and contact prophylaxis in the initial 'containment phase' to the clinical assessment 'treatment phase' of later stages of the pandemic was managed by using sentinel primary care linked clinical virological surveillance to provide estimates of case numbers. Such monitoring is normally used during the winter months and provides gold standards of surveillance with an extensive historical dataset allowing comparative analysis, but limited predictions [4].

Our work here sought to compare virological surveillance data generated through a self-sampling approach

with our gold standard surveillance data generated through clinician led sentinel swabbing practices. The application of this approach during the pandemic supplemented available knowledge in real time [11] and was used for periods of time in parallel with existing RCGP virological surveillance activities.

Self-sampling for near-real time virological surveillance has not been used previously in large scale assessment of respiratory illness in the community during an influenza pandemic. The deployment of this capability was based on our successful pilot scheme in 2008 [2] and was an innovation within the UK health service sector during the global pandemic of 2009/10 [3]. Surveillance was secondary to the provision of patient care, therefore the use of clinical data already available in the health system provided a unique opportunity to evaluate the applicability of the self-sampling approach and assess whether to embed this capability as part of any future national level emergency response strategy.

Self-sampling and clinician-based sampling both provided meaningful semiquantitative data. The analysis of Ct values demonstrated no difference in viral load trends between the schemes when corrections were made for differences in the timing of swabbing and the delays in sampling. This is encouraging as it suggests significant value in obtaining swabs from patients, to test to influenza, later during infection, at a time when it is usually considered that there is limited opportunity to obtain virological information, thus further improving the potential to use self-sampling where delays in obtaining samples might occur.

The results from this work illustrate that self-sampling for virological surveillance can be used to supplement sentinel clinician-based sampling.

There is a general increasing trend towards self-sampling for illness surveillance, to reduce healthcare costs but also deliver innovative surveillance and screening programmes e.g. the use of self-sampling faecal occult bloods largely for detection of colonic cancer, self-sampling of urines for Chlamydia screening in young persons, and now respiratory illness (both viral and bacterial) sampling [12-18]. These methods may be particularly useful for responding to future influenza epidemics or pandemics which affect younger populations, however, as elderly patients are less likely to use internet or phone health services and therefore may not be included in a self-sampling cohort, this method might be less useful when responding to influenza subtypes that impact on older age groups. In addition, the resources required to develop the infrastructure needed to facilitate the systematic collection of patient data (including: the assembly and dissemination of sampling kits; the collation, analysis and interpretation of data; and the laboratory capacity to test samples) potentially limits the usefulness of self-sampling for responding to small scale or short-lived incidents. However, it may be advantageous to establish

self-sampling running on a continuous basis, at a low background level, which could contribute to national seasonal influenza surveillance programmes, but also be scaled up to rapidly response to an emerging threat.

It was difficult to get the evidence for community transmission in those going to GPs for testing as they were predominantly in risk groups at the early stages (e.g. returning travellers). Therefore it was hard to objectively assess the necessity for a change in response phase to the pandemic when only a few areas were particularly affected against a background of relatively low numbers of cases in the general population. In aiming to complement sentinel surveillance, our work here clearly demonstrates that respiratory self-sampling can be applied in a responsive and flexible manner to achieve a wide spread and geographically representative way of assessing community transmission of a known organism. Such a scheme could also be invaluable for targeting specific populations in response to public health threats during specific events.

Conflict of interest

DM Fleming has received funding to attend influenza related meetings and has received consultancy fees from influenza vaccine manufacturers. All other authors: none to declare.

Authors' contributions

All authors contributed to the design of the study. AJE, CP, DF, EP, GES all contributed to the extraction and processing of NHS Direct call data and the selection of self-sampling participants. AB, JE, CS, PS, AL, DB, MZ contributed to the laboratory testing of samples. AC and CP contributed to the statistical analysis. AB and AJE collaborated to write the manuscript. All authors contributed to drafting and have seen and approved the final version of the manuscript.

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Household transmissibility of avian influenza A (H7N9) virus, China, February to May 2013 and October 2013 to March 2014

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To study human-to-human transmissibility of the avian influenza A (H7N9) virus in China, household contact information was collected for 125 index cases during the spring wave (February to May 2013), and for 187 index cases during the winter wave (October 2013 to March 2014). Using a statistical model, we found evidence for human-to-human transmission, but such transmission is not sustainable. Under plausible assumptions about the natural history of disease and the relative transmission frequencies in settings other than household, we estimate the household secondary attack rate (SAR) among humans to be 1.4% (95% CI: 0.8 to 2.3), and the basic reproductive number R_0 to be 0.08 (95% CI: 0.05 to 0.13). The estimates range from 1.3% to 2.2% for SAR and from 0.07 to 0.12 for R_0 with reasonable changes in the assumptions. There was no significant change in the human-to-human transmissibility of the virus between the two waves, although a minor increase was observed in the winter wave. No sex or age difference in the risk of infection from a human source was found. Human-to-human transmissibility of H7N9 continues to be limited, but it needs to be closely monitored for potential increase via genetic reassortment or mutation.

Introduction

Influenza A (H7N9) was first detected among humans in eastern China in February 2013 and, as of 7 December 2014, had caused 453 laboratory-confirmed clinical human infections with 178 deaths in China, according to the Chinese Center for Disease Control and Prevention (Chinese CDC). The virus is a reassortant of avian H7, N9 and H9N2 strains [1] with evidence of the capacity to bind to mammalian cells [2,3] and limited airborne transmission in animal models [4,5]. Currently, the virus is not pathogenic in birds, but highly pathogenic and virulent in humans [6-9]. Normally, the influenza season in humans in the northern hemisphere runs from October to April with some variation in timing [10,11]. After a relative hiatus in the number of human H7N9 cases over the summer of 2013 in China (e.g. only two cases reported in July 2013), a second large wave of cases appeared, starting in October 2013 [12].

While the ongoing sporadic reporting of cases (per communication with the Chinese CDC) implies the H7N9 virus has not yet reached the stage of efficient human-to-human transmission, an animal model has shown another H7 virus was able to transmit among co-housed ferrets without much loss of virulence, with a few mutations obtained after 10 serial passages [13]. It is therefore highly relevant to assess the risks of human-to-human transmission using available data.

The household is a setting well suited to investigating human-to-human transmission of many infectious agents, including influenza viruses [14]. So far, no quantitative analysis of household transmission of H7N9 virus has been reported, though qualitative epidemiological descriptions of possible transmissions in a few families with more than one laboratory-confirmed case have been presented [15-18]. We use household information on laboratory-confirmed cases collected by the Chinese CDC to estimate the household secondary attack rate (SAR), i.e. the probability that a typical index case infects a given household member. In addition, the basic reproductive number, R_0, defined as the number of people a typical infected person would infect in a completely susceptible population, is estimated from the SAR in conjunction with assumptions about the contribution of schools and the general community relative to households in future epidemics. Other investigators estimated R_0 to be 0.10 using the sequence of reported cases during the spring outbreak of 2013 [19]. We provide the first rigorous evaluation of R_0 based on transmissibility in the household setting.

TABLE 1

Assumptions about probability distributions for the incubation period and relative infectiousness since symptom onset (day 1) for the infectious period for avian influenza A(H7N9) in human cases

Duration	Probability distributions for the incubation period in days												Mean
	1	2	3	4	5	6	7	8	9	10	11	12	
Short	0.1	0.2	0.3	0.25	0.1	0.05	–	–	–	–	–	–	3.2
Medium	–	0.05	0.1	0.2	0.3	0.2	0.1	0.05	–	–	–	–	5
Long	–	0.025	0.025	0.05	0.1	0.15	0.3	0.15	0.1	0.05	0.025	0.025	7
Duration	Relative infectiousness since symptom onset (day 1) for the infectious period in days												–
	1–3	4	5	6	7	8	9	10	11	12	13	14	
Short	1	0.8	0.6	0.3	0.1	–	–	–	–	–	–	–	–
Medium	1	1	0.9	0.8	0.7	0.5	0.3	0.1	–	–	–	–	–
Long	1	1	1	1	0.9	0.8	0.7	0.6	0.5	0.3	0.2	0.1	–

These assumptions are used in all our subsequent analyses.

Methods

Household study

Contact data were collected by the Chinese CDC on households with laboratory-confirmed symptomatic cases over the two distinct waves of the epidemic, 125 households during the first wave in the spring of 2013, and another 187 households from the second wave in the winter and spring of 2013–14, offering a unique opportunity to examine the potential change in human-to-human transmissibility of the virus. This investigation was part of the public health emergency response of Chinese CDC, and therefore no informed consent of the household members was required.

A household in this study was defined as a group of related family members living in the same building structure and in daily close contact with each other (our definition of close contact is given below). The case definitions for clinical and confirmed human infections with H7N9 were similar to the H5N1 case definitions suggested by the World Health Organization in 2006. Data of both laboratory-confirmed clinical cases and their close contacts, including household members, were obtained from a review of medical records and interviews with relatives, contacts and health-care providers. Close contacts of a confirmed case were monitored for seven days for symptoms, and throat swabs were collected from contacts with respiratory symptoms for laboratory testing. Throat swabs were also collected from some close contacts without symptoms. Details of the case definition, laboratory methods and data collection were described previously [18].

Households of laboratory-confirmed clinical cases from all 10 provinces (Shanghai, Zhejiang, Jiangsu, Anhui, Jiangxi, Shandong, Beijing, Henan, Hunan and Fujian) affected by the first wave were included. Households from three provinces (Zhejiang, Hunan and Guangdong) affected by the second wave during October 2013 to March, 2014, were added. Age, sex and dates of symptom onset, hospitalisation, death and recovery (if known) were collected for all family members. In addition, the following types of contact with the index case were recorded for each family member: (i) dining together; (ii) living in the same housing unit; (iii) sharing utensils, towels, toys, etc.; (iv) having contact with excreta (excrement and urine) of cases; (v) providing care; (vi) visiting; and (vii) other contacts. We define close contact as all contact types except for (vi) and (vii). Non-close contacts were excluded from our analysis.

Natural history of avian influenza A(H7N9) in human cases

There is limited information on the natural history of infection in humans caused by the H7N9 virus, as the exposure dates cannot be clearly identified for most cases. However, to estimate transmission probabilities, distributional assumptions often have to be made about the natural history, in particular the distributions of the incubation period (i.e. time from infection to the onset of influenza symptoms), the latent period (i.e. time from infection to the onset of infectiousness) and the infectious period (i.e. time that an infected person is infectious to others). However, there are little data about these periods.

Incubation and latent periods

Cowling et al. estimated the mean incubation period of H7N9 to be 3.1 days [6]. Chen et al. reported a 3-, 6- and 8-day lag between last exposure to live poultry and symptom onset in three patients, an average of 5.8 days [20]. Huang et al. found even longer incubation periods, a median of six days (range: 2–10) among 10 patients with a single self-reported exposure day and 7.5 days among 12 patients with multiple self-reported exposure days [21]. In contrast, former results about historical seasonal influenza suggested average incubation periods of those viruses of around two to three days [22].

We consider three possible distributions for our analysis (upper panel of Table 1). For the primary analysis, we adopt a minimum (d_{min}) of two days and a maximum (d_{max}) of eight days, with a probability mass distribution of $\theta = (\theta_{d_{min}}, \cdots, \theta_{d_{max}}) = (0.05, 0.1, 0.2, 0.3, 0.2, 0.1, 0.05)$. For influenza, the latent period and the incubation period are similar [23], and we assume the two periods overlap.

Infectious period

While the average time from symptom onset to recovery could be as long as 15 days (based on the surveillance data provided by the Chinese CDC), the actual duration of the infectious period is likely to be much shorter. For seasonal influenza, symptoms usually resolve in a week, and previous studies on seasonal and pandemic influenza A (H1N1) suggested a likely range of 7–10 days after symptom onset for the viral load to become undetectable [24-26]. Information on pathological and immunological response regarding the avian H7N9 is scarce. In a study of mice infected with H7N9 virus isolated from humans and ducks, viral titres were not detectable after day 8 post infection [27].

We explore three settings for the infectious period, each setting given by a fixed duration with decaying relative infectivity (lower panel of Table 1). We assume the infectious period starts from the day of symptom onset (day 1). The medium setting is adopted for the primary analysis, i.e. the duration of the infectious period is $D = 10$ days, and the relative infectivity levels are $\eta = (\eta_1, \cdots, \eta_{10}) = (1, 1, 1, 1, 0.9, 0.8, 0.7, 0.5, 0.3, 0.1)$. This setting gives a mean serial interval of approximately 9.4 days, slightly longer than the crude estimate of 7.5 days (see Results).

Human-to-human transmissibility

A likelihood-based statistical transmission model is used to test the hypothesis of no human-to-human transmission and to estimate the SAR and R_o [22,28]. Suppose we observe an epidemic from day 1 to day T among a population of N individuals in H households. Person-to-person transmissibility of the virus is measured by the probability that an infectious person infects his or her susceptible household contact in one day, denoted by p. The transmission probability can be adjusted for covariates via a logistic regression model given by $\text{logit}(p_{ijt}) = \text{logit}(p) + X'_{ijt}\beta$, where X_{ijt} is the vector of covariates associated with an infectious person j and a susceptible person i on day t. We let β be the vector of coefficients, where e^β is interpreted as the odds ratios. To account for exposure to an environmental reservoir such as poultry and to unknown casual contacts with human sources outside of the household, we assume that a susceptible person is infected by external sources during one day with probability b. Suppose that we have observed households ascertained by index cases. Let $h(i)$ be the collection of members of the household of individual i, and let $\tilde{t}_i$ be the symptom onset day of individual i. Then, the probability that susceptible individual i escapes infection during day t is

$$e_i(t) = (1 - b) \prod_{j \in h(i), j \neq i} [1 - p_{ijt}\,\eta(t - \tilde{t}_j + 1)],$$

Where $\eta(k)$ is the relative infectiousness of day k of the infectious period. We define $\tilde{t}_{h(i)}$ to be the collection of observed symptom onset days of all members in the household of individual i. Let $\theta(k)$ be the probability that the incubation period is k days. The likelihood contribution of individual i is

$$L_i(b, p \mid \tilde{t}_{h(i)}) = \begin{cases} \prod_{t=1}^{T} e_i(t), & \text{escaped,} \\ \sum_t \theta(\tilde{t}_i - t)[1 - e_i(t)] \prod_{\tau=1}^{t-1} e_i(\tau), & \text{infected.} \end{cases}$$

We embed the same likelihood structure in a resampling-based approach to test the null hypothesis $H_o: p = 0$ [29].

The SAR over an infectious period of D days is

$$\text{SAR} = 1 - \prod_{d=1}^{D} 1 - p\,\eta(d).$$

The SAR specific to a covariate value X is given by replacing p with $\text{logit}^{-1}[\text{logit}(p) + X'\beta]$. To estimate R_o, the fact that the probability of becoming an index case differs across age groups has to be considered, as it is known that older people have a higher chance of visiting high-risk areas such as live poultry markets [6,30]. For a population partitioned into three age groups: 0–19, 20–59 and ≥60 years, the basic reproductive number is given by

$$R_0 = SAR[\pi_1(n_1 - 1)(1 + \rho_S + \rho_C) + \pi_2(n_2 - 1)(1 + \rho_C) + \pi_3(n_3 - 1)(1 + \rho_C)],$$

Where π_j is the probability that an index case belongs to age group j, n_j the average household size if the index case is from age group j, and ρ_S and ρ_C are the relative contact frequencies of an infectious person in school and the community compared with within household, respectively. The community refers to all places other than households and schools where transmission occurs. Lacking the data to determine the ρ_S and ρ_C specific to China, we use the information in a previous study of influenza A(H1N1)pdm09 in the United States [22], where the plausible ranges for the local reproductive numbers in households (R_H) and schools (R_S) were 0.6–0.9 and 2.0–2.5, respectively, and the corresponding range for $\rho_S = R_S/R_H$ was 2–4, which is used in our analysis. Another previous study of influenza pandemics in the United States showed that the community at large generally accounts for somewhat fewer transmissions of influenza virus than within households [14]. The values 0, 0.5 and 1.0 were used for ρ_C in the A(H1N1)pdm09 study [22]. In our analysis for H7N9, we use 0.5, 1.0 and 1.5 for ρ_C to reflect the higher population density in public places in China.

We estimate π_j using a conditional Poisson model, in which temporal and spatial heterogeneity are controlled for by aggregating the data by appropriate location and time, e.g. by prefecture and week. Let

FIGURE 1

Laboratory-confirmed avian influenza A(H7N9) cases in humans from (A) February–May 2013 (125 households, 130 cases) and (B) October 2013–March 2014 (187 households, 196 cases), household data, China

A. February–May 2013

B. October 2013–March 2014
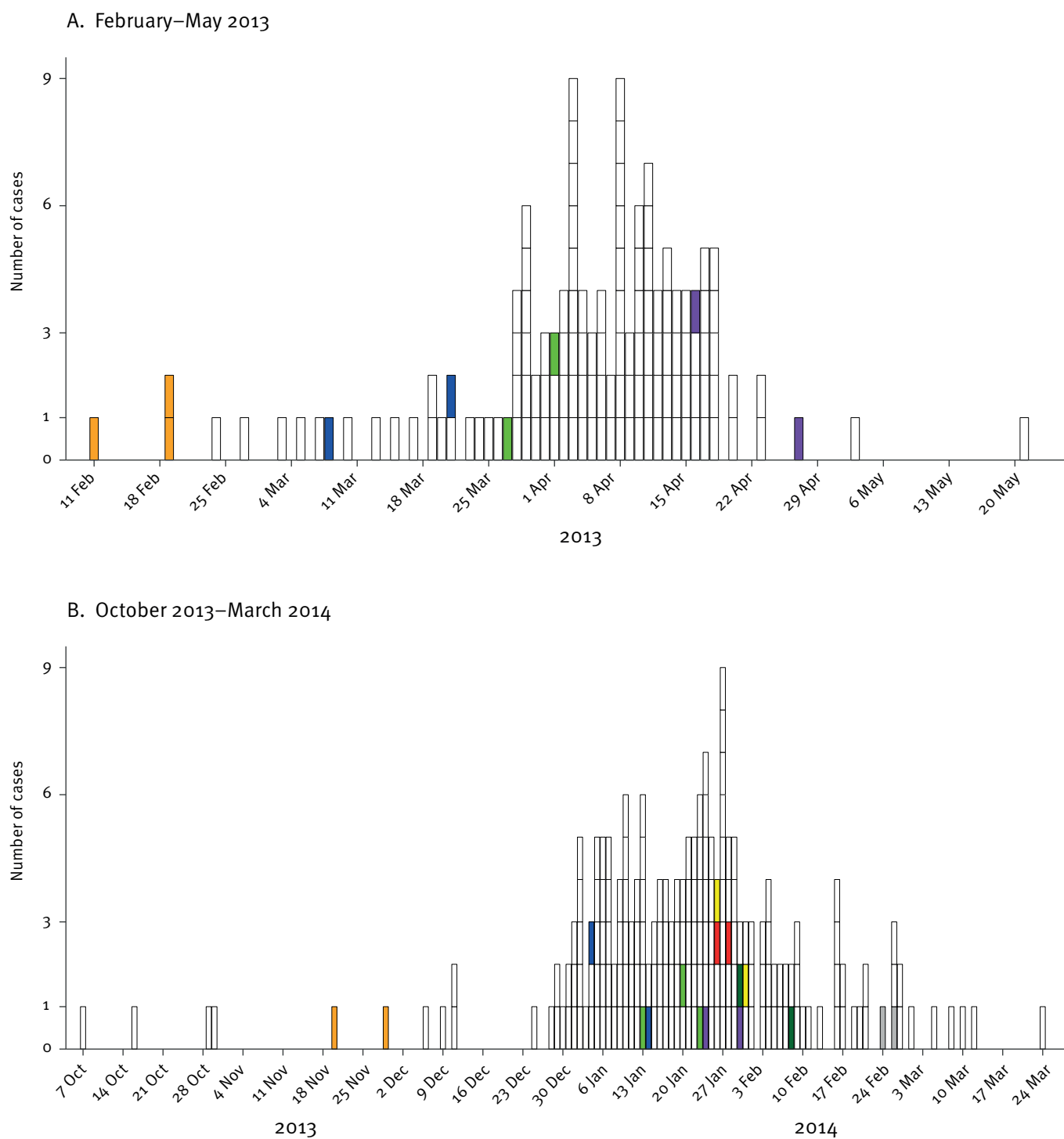
Cases from the same household are shown in the same colour; single index cases are left blank.

Table 2

Estimates of secondary attack rate and basic reproductive number based on household clusters of avian influenza A(H7N9) cases in humans, ignoring poultry-to-human transmission, China, February–May 2013 (113 households, 118 cases) and October 2013–March 2014 (181 households, 190 cases)

Incubation period	Infectious period	Value	Relative contact frequency within hospital vs within household, α		
			1.0	0.5	0.1
			Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Short	Short	SAR	1.3% (0.74 to 2.2)	1.4% (0.79 to 2.3)	1.4% (0.84 to 2.5)
		R_0	0.072 (0.042 to 0.12)	0.076 (0.044 to 0.13)	0.081 (0.047 to 0.14)
		p value	0.16	–	–
	Medium	SAR	1.4% (0.82 to 2.3)	1.6% (0.95 to 2.7)	1.8% (1.1 to 3.0)
		R_0	0.078 (0.046 to 0.13)	0.089 (0.053 to 0.15)	0.10 (0.060 to 0.17)
		p value	0.0005	–	–
	Long	SAR	1.4% (0.83 to 2.4)	1.8% (1.0 to 2.9)	2.2% (1.3 to 3.7)
		R_0	0.079 (0.047 to 0.13)	0.098 (0.058 to 0.17)	0.12 (0.073 to 0.21)
		p value	0.0015	–	–
Medium	Short	SAR	1.4% (0.82 to 2.3)	1.5% (0.87 to 2.5)	1.6% (0.92 to 2.6)
		R_0	0.077 (0.046 to 0.13)	0.082 (0.049 to 0.14)	0.087 (0.052 to 0.15)
		p value	0.0175	–	–
	Medium	SAR	1.4% (0.82 to 2.3)	1.6% (0.95 to 2.7)	1.8% (1.1 to 3.0)
		R_0	0.078 (0.046 to 0.13)	0.090 (0.053 to 0.15)	0.10 (0.060 to 0.17)
		p value	0.0035	–	–
	Long	SAR	1.4% (0.84 to 2.4)	1.8% (1.0 to 2.9)	2.2% (1.3 to 3.7)
		R_0	0.079 (0.047 to 0.13)	0.098 (0.058 to 0.17)	0.12 (0.073 to 0.21)
		p value	0.020	–	–
Long	Short	SAR	1.4% (0.82 to 2.3)	1.5% (0.87 to 2.5)	1.6% (0.92 to 2.6)
		R_0	0.077 (0.046 to 0.13)	0.083 (0.049 to 0.14)	0.087 (0.052 to 0.15)
		p value	0.047	–	–
	Medium	SAR	1.4% (0.82 to 2.3)	1.6% (0.95 to 2.7)	1.8% (1.1 to 3.0)
		R_0	0.078 (0.046 to 0.13)	0.090 (0.053 to 0.15)	0.10 (0.061 to 0.17)
		p value	0.10	–	–
	Long	SAR	1.4% (0.84 to 2.4)	1.8% (1.0 to 2.9)	2.2% (1.3 to 3.7)
		R_0	0.079 (0.047 to 0.13)	0.099 (0.058 to 0.17)	0.12 (0.073 to 0.21)
		p value	0.44	–	–

b: daily probability of infection by external sources; CI: confidence interval; R_0: basic reproductive number; ρ_C: relative contact frequency in the community; ρ_S: relative contact frequency in schools; SAR: secondary attack rate.

Results are based on the assumption of $b = 0$ (i.e. ignoring poultry-to-human transmission) and stratified by the duration of the incubation and infectious periods (duration settings are given in Table 1) and the relative contact frequency between a case and their household members within a hospital compared with within the household (α). Relative contact frequencies in schools and the community are set to $\rho_S = 3$ and $\rho_C = 1$. The test of $p = 0$ (no human-to-human transmission) is performed when $\alpha = 1$.

Y_{ktj} be the number of cases in age group j in prefecture k with symptom onset during week t. We assume $Y_{ktj} \sim \text{Poisson}(\lambda_{ktj})$ with the intensity $\lambda_{ktj} = N_{kj}e^{\gamma_{kt}+\beta_j}$, where N_{kj} is the population size in age group j of prefecture k, and $\exp(\gamma_{kt})$ is the rate of a single person in the reference age group of prefecture k becoming a case during week t. For the parameters to be identifiable, we set $\beta_3 = 0$, i.e., setting the age group ≥ 60 years as the reference group. Conditioning on the total number of cases $Y_{kt} = \Sigma_j Y_{ktj}$, the vector ($Y_{kt1}$, Y_{kt2}, Y_{kt3}) follows a multinomial distribution with size Y_{kt} and probabilities

$$\pi_{kj} = \frac{N_{kj}e^{\beta_j}}{\sum_l N_{kl}e^{\beta_l}}, j=1,2,3.$$

The maximum likelihood estimates of β_1 and β_2, denoted by $\hat{\beta}_1$ and $\hat{\beta}_2$, are found by maximising the log-likelihood

$$\sum_{k,t}\sum_{j=1}^{3} Y_{ktj}\log(\pi_{kj}).$$

A reasonable estimate for π_j is

$$\hat{\pi}_j = \frac{N_j e^{\hat{\beta}_j}}{\sum_l N_l e^{\hat{\beta}_l}},$$

where $N_j = \sum_k N_{kj}$ and $\hat{\beta}_3 = 0$. Due to data availability, we used surveillance data up to 27 January 2014 to estimate π_j.

Results

We collected household data for 130 laboratory-confirmed clinical cases in 125 households in 10 provinces for February to May 2013 and for 196 laboratory-confirmed clinical cases in 187 households in Hunan, Zhejiang and Guangdong provinces for October 2013 to March 2014. The epidemic curves of the cases in these households during the two waves are shown in Figure 1 (panels A and B). Interestingly, multi-case households appeared from the very beginning of the 2013 wave. After excluding households without close contacts, 118 cases in 113 households in the first wave and 190 cases in 181 households in the second were used in this analysis. There was an asymptomatic infection identified in one household in the first wave, but this person was considered a non-case in our analysis; i.e., we only focus on laboratory-confirmed infections with clinical symptoms, because (i) it is not clear whether asymptomatic hosts can transmit the virus or not, and (ii) probably not all asymptomatic infections were detected.

Under the assumption that all non-index cases were infected by the index cases, an estimate of the mean serial interval is 7.5 days (95% confidence interval (CI): 4.9 to 9.0). There was no difference in the distribution of the serial interval between the two waves (p value = 0.31 based on the two-sample Wilcoxon test). Using the transmission model, we first test the hypothesis of no human-to-human transmission, and we then estimate the SAR and R_o without adjusting for any covariate (Table 2). The estimation of R_o is based on the estimates of

$\hat{\pi}_1$ = 0.044 (95% CI: 0.023 to 0.083),

$\hat{\pi}_2$ = 0.44 (95% CI: 0.39 to 0.51)

and

$\hat{\pi}_3$ = 0.51 (95% CI: 0.45 to 0.58)

obtained from the conditional Poisson model. In addition to taking into account the uncertainty in the natural history of disease, the results are also stratified by the relative contact frequency between a case and his or her household members within a hospital compared with within the household, denoted by a. When a is assumed unknown, its estimate varies dramatically from well below one to well above one, depending on the assumption about the natural history of disease. This indicates a lack of information about a in the data. Consequently, we assume a is known and examine results at three levels of a: 1.0, 0.5 and 0.1, based on the rationale that a family member was more likely to be cautious in having contact with an infected person who was hospitalised. Hypothesis testing is performed only under $a = 1$. The statistical evidence for human-to-human transmission is significant for most plausible distributions of the incubation and infectious periods except when both of them are short or both are long. The daily probability of infection by external sources, b, is estimable only under the setting of both the incubation and infectious periods being short. In all other settings, the maximum likelihood estimate (MLE) of b is given by the boundary value 0. As a result, we set

$b = 0$ for estimating SAR and R_o in the primary analysis. Given $a = 1$, regardless of the assumption of the natural history, the estimates for SAR and R_o are generally low and stable. Using median incubation and infectious periods as the primary setting, we estimate SAR as 1.4% (95% CI: 0.8 to 2.3) and R_o as 0.08 (95% CI: 0.05 to 0.13), respectively, i.e. the probability that an H7N9 case infects another household contact is 0.014, and an H7N9 case infects an average of 0.08 other people. Lower values of a correspond to slightly higher estimates for SAR and R_o, and the estimates appear higher with longer infectious periods but are insensitive to the incubation period. With $a = 0.1$ and a long infectious period, the estimates reach 2.2% for SAR and 0.12 for R_o. The interpretation of the estimates for SAR and R_o under $a < 1$ is limited to the household setting, i.e. as if the infected person will not be hospitalised.

The above results assume the relative contact frequencies in schools and the community are fixed at $\rho_S = 3$ and $\rho_C = 1$. A sensitivity analysis for R_o with respect to ρ_S and ρ_C is shown in Figure 2, where three levels chosen from previous work [22] are considered for each parameter. These estimates are not sensitive to ρ_S, but are sensitive to ρ_C, which is likely due to the fact that ρ_S is only associated with children and children are the least exposed to the environmental reservoir as compared with other age groups. Under long incubation and infectious periods and $a = 0.1$, $\rho_C = 1.5$ could yield estimates as high as 0.15 for R_o. On the other hand, under short incubation and infectious periods, $a = 1$, and $\rho_C = 0.5$, the estimate for R_o could be as low as 0.05. Overall, R_o is well below one, so that sustained person-to-person transmission will not take place.

With limited data about secondary transmission, we examine whether the household transmissibility of the virus changed between the two waves. In addition, possible heterogeneity in the risk of infection from a human source between the sexes and between age groups (adults (18 years or older) versus children (under 18 years)) are tested using the logistic regression in the chain binomial model. The results are summarised in Table 3, suggesting that the estimated odds ratios of within-household secondary transmission are relatively robust to assumptions about the natural history of the disease and the relative contact frequency in the hospital versus in the household. None of these factors have a significant effect on secondary transmission, possibly due to the lack of a sufficient number of secondary transmissions. However, males appeared to have a somewhat higher risk of infection from a human source compared with females, the odds ratio being 2.24 (95% CI: 0.69 to 7.26), though not statistically significant, under a medium length of incubation and infectious periods and $a = 1$. There was no significant difference in the risk between adults and children, the odds ratio being 0.66 (95% CI: 0.20 to 2.19). The odds ratio between the second and the first waves was 1.3 (95% CI: 0.4 to 4.27), suggesting the virus did not

FIGURE 2

Stratified estimates of basic reproductive number based on household clusters of avian influenza A(H7N9) cases in humans, China, February–May 2013 (113 households, 118 cases) and October 2013–March 2014 (181 households, 190 cases)
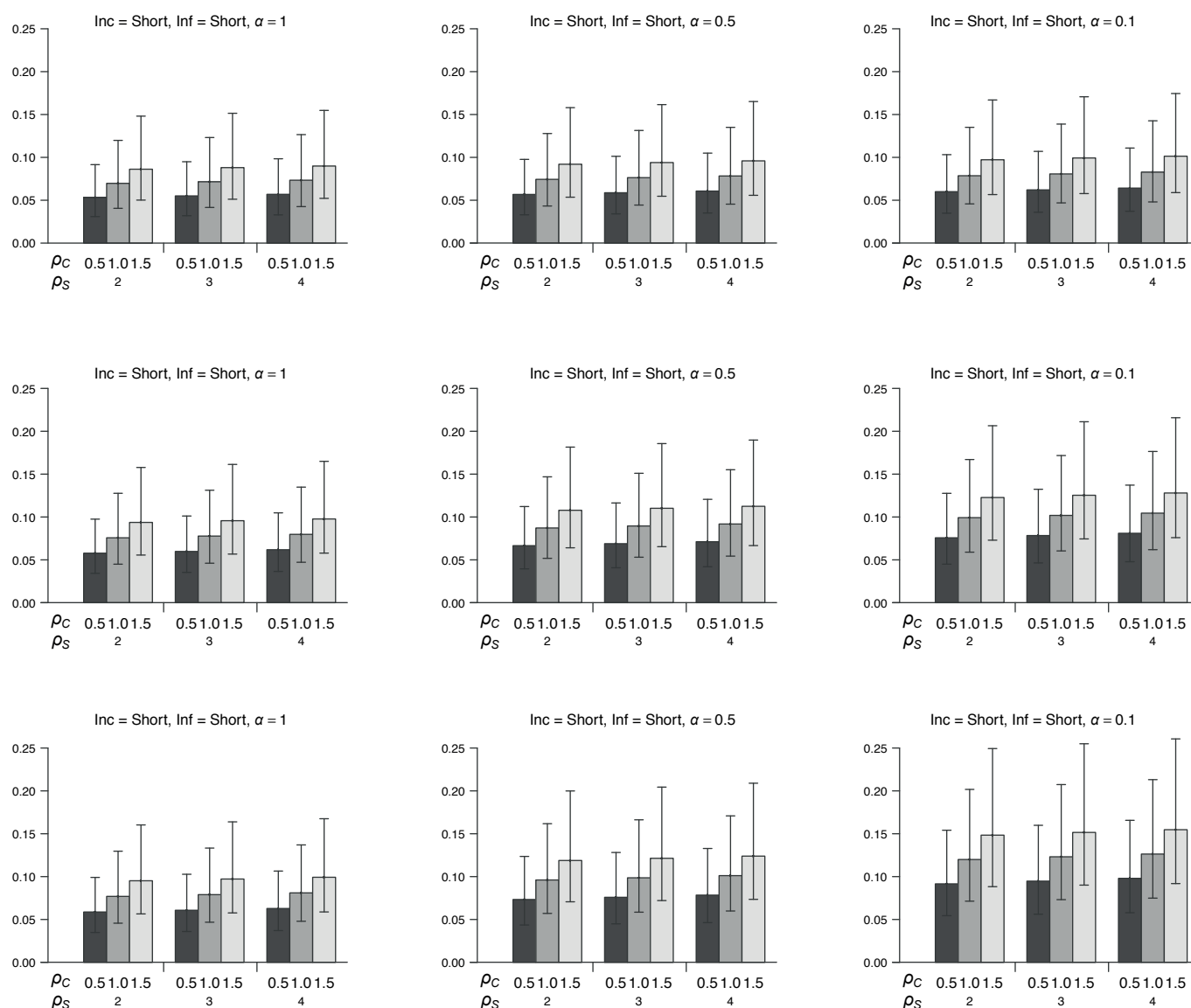
Inc: incubation period; Inf: infectious period.

The vertical axes are the estimates of basic reproductive number (R_o). The estimates are stratified by the duration of the incubation and infectious periods (duration settings are given in Table 1), the relative contact frequency between a case and their household members within a hospital compared with within the household (*a*), and the relative contact frequencies in schools and the community, ρ_S and ρ_C. Bars represent 95% confidence intervals.

gain much human-to-human transmissibility between the two waves.

Two of the families in the second wave may have had multiple index cases, since symptom onsets in the first and second cases were only two days apart, and both cases were believed to have had exposure to live poultry. A sensitivity analysis was performed assuming the first two cases in each of these two families were both index cases. This assumption of multiple index cases removes the short serial intervals, leading to a longer

mean serial interval of 8.4 days (95% CI: 6.8 to 9.6). In the setting of medium-length incubation and infectious periods and α=1, estimates for the SAR and R_o are slightly lowered, to 1.2% (95% CI: 0.68 to 2.1) and 0.067 (95% CI: 0.038 to 0.12), respectively. The differences in transmissibility between waves and in the risk of infection between adults and children further diminish with the odds ratios, to 1.0 (95% CI: 0.29 to 3.48) and 0.93 (95% CI: 0.25 to 3.51), respectively.

Estimates of odds ratios of within-household secondary transmission with regard to the wave indicator (second wave to first wave), sex (men to women) and age group (adults to children)[a] based on household clusters of avian influenza A(H7N9) cases in humans, China, February–May 2013 (113 households, 118 cases) and October 2013–March 2014 (181 households, 190 cases)

| Incubation period | Infectious period | Value | Relative contact frequency within hospital vs within household, α | | |
| | | | 1.0 | 0.5 | 0.1 |
			Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Short	Short	Wave	1.56 (0.45 to 5.39)	1.57 (0.46 to 5.39)	1.57 (0.46 to 5.39)
		Sex	2.65 (0.78 to 9.0)	2.63 (0.78 to 8.89)	2.60 (0.77 to 8.80)
		Age	0.51 (0.15 to 1.74)	0.49 (0.15 to 1.69)	0.48 (0.14 to 1.65)
	Medium	Wave	1.30 (0.40 to 4.27)	1.31 (0.40 to 4.27)	1.31 (0.40 to 4.26)
		Sex	2.24 (0.69 to 7.24)	2.21 (0.69 to 7.12)	2.18 (0.68 to 7.0)
		Age	0.66 (0.20 to 2.18)	0.64 (0.19 to 2.10)	0.62 (0.19 to 2.01)
	Long	Wave	1.30 (0.40 to 4.26)	1.30 (0.40 to 4.25)	1.31 (0.40 to 4.23)
		Sex	2.23 (0.69 to 7.20)	2.20 (0.68 to 7.07)	2.17 (0.68 to 6.92)
		Age	0.66 (0.20 to 2.17)	0.64 (0.19 to 2.08)	0.61 (0.19 to 1.98)
Medium	Short	Wave	1.30 (0.40 to 4.29)	1.31 (0.40 to 4.31)	1.32 (0.40 to 4.32)
		Sex	2.25 (0.69 to 7.29)	2.23 (0.69 to 7.21)	2.21 (0.68 to 7.15)
		Age	0.66 (0.20 to 2.20)	0.64 (0.20 to 2.13)	0.63 (0.19 to 2.07)
	Medium	Wave	1.30 (0.40 to 4.27)	1.31 (0.40 to 4.27)	1.31 (0.40 to 4.26)
		Sex	2.24 (0.69 to 7.26)	2.22 (0.69 to 7.14)	2.19 (0.68 to 7.02)
		Age	0.66 (0.20 to 2.19)	0.64 (0.19 to 2.10)	0.62 (0.19 to 2.01)
	Long	Wave	1.30 (0.40 to 4.26)	1.30 (0.40 to 4.25)	1.30 (0.40 to 4.23)
		Sex	2.23 (0.69 to 7.22)	2.21 (0.69 to 7.09)	2.17 (0.68 to 6.94)
		Age	0.66 (0.20 to 2.17)	0.64 (0.19 to 2.08)	0.61 (0.19 to 1.98)
Long	Short	Wave	1.30 (0.40 to 4.29)	1.31 (0.40 to 4.30)	1.32 (0.40 to 4.31)
		Sex	2.26 (0.70 to 7.32)	2.24 (0.69 to 7.24)	2.22 (0.69 to 7.18)
		Age	0.66 (0.20 to 2.20)	0.65 (0.20 to 2.13)	0.63 (0.19 to 2.07)
	Medium	Wave	1.30 (0.39 to 4.27)	1.30 (0.40 to 4.26)	1.31 (0.40 to 4.25)
		Sex	2.25 (0.69 to 7.29)	2.22 (0.69 to 7.16)	2.19 (0.68 to 7.04)
		Age	0.66 (0.20 to 2.19)	0.64 (0.19 to 2.10)	0.62 (0.19 to 2.01)
	Long	Wave	1.30 (0.40 to 4.26)	1.30 (0.40 to 4.24)	1.30 (0.40 to 4.22)
		Sex	2.24 (0.69 to 7.24)	2.21 (0.69 to 7.11)	2.18 (0.68 to 6.96)
		Age	0.66 (0.20 to 2.17)	0.64 (0.19 to 2.09)	0.61 (0.19 to 1.98)

CI: confidence interval.

Results are based on the assumption that the daily probability of infection by external sources (b) = 0 (i.e. ignoring poultry-to-human transmission) and stratified by distributions of the incubation and infectious periods (duration settings are given in Table 1) and the relative contact frequency between a case and their household members within a hospital compared with within the household.

[a] Adults: 18 years or older; children: under 18 years.

Thus far, we have assumed $b = 0$, i.e. all secondary cases were infected by the index cases. To assess how sensitive the estimates of SAR and R_0 are to the value of b and to allow for the possibility of imported cases during each household outbreak, another set of analyses were conducted with $b = 9.67 \times 10^{-5}$, the only non-zero MLE obtained when both the incubation and infectious periods are short. As expected, the estimates of both the SAR and R_0 are slightly lowered (Table 4). The ranges of the point estimates are 1.1–2.0% for the SAR and 0.06–0.11 for R_0, compared with 1.3–2.2% and 0.07–0.12, respectively, when $b = 0$. Large values for b would further reduce the estimates of SAR and R_0 but may not be reasonable assumptions as b is generally much smaller than p and the estimates of p in our analyses are at the 10^{-3} scale.

Discussion

We found statistical evidence of human-to-human transmission of the avian influenza A (H7N9) virus, but it is clear that such transmission is not sustainable. Our estimate of the household SAR is below 2%, and the estimate of $R_0 = 0.08$ is way below the threshold 1. In contrast, for seasonal human influenza, the estimates for the household SAR mostly range from 10% to 30% [22], and the R_0 estimates vary from 1.1 to 1.6

TABLE 4

Estimates of secondary attack rate and basic reproductive number based on household clusters of avian H7N9 cases in humans, taking into account the probability of infection by external sources, China, February–May 2013 (113 households, 118 cases) and October 2013–March 2014 (181 households, 190 cases)

Incubation period	Infectious period	Value	Relative contact frequency within hospital vs within household, α		
			1.0	0.5	0.1
			Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Short	Short	SAR	1.1% (0.58 to 2.0)	1.1% (0.61 to 2.1)	1.2% (0.60 to 2.2)
		R_0	0.061 (0.033 to 0.11)	0.064 (0.034 to 0.12)	0.065 (0.034 to 0.12)
	Medium	SAR	1.3% (0.71 to 2.2)	1.4% (0.79 to 2.5)	1.5% (0.79 to 2.7)
		R_0	0.070 (0.040 to 0.12)	0.079 (0.044 to 0.14)	0.082 (0.044 to 0.15)
	Long	SAR	1.3% (0.74 to 2.3)	1.6% (0.90 to 2.8)	1.9% (1.0 to 3.4)
		R_0	0.073 (0.041 to 0.13)	0.090 (0.051 to 0.16)	0.10 (0.056 to 0.19)
Medium	Short	SAR	1.2% (0.69 to 2.2)	1.3% (0.72 to 2.3)	1.3% (0.75 to 2.4)
		R_0	0.069 (0.038 to 0.12)	0.072 (0.040 to 0.13)	0.075 (0.042 to 0.14)
	Medium	SAR	1.3% (0.73 to 2.2)	1.5% (0.83 to 2.6)	1.6% (0.90 to 2.9)
		R_0	0.072 (0.041 to 0.13)	0.082 (0.046 to 0.15)	0.090 (0.051 to 0.16)
	Long	SAR	1.3% (0.72 to 2.3)	1.6% (0.90 to 2.8)	2.0% (1.1 to 3.5)
		R_0	0.072 (0.040 to 0.13)	0.090 (0.051 to 0.16)	0.11 (0.061 to 0.20)
Long	Short	SAR	1.3% (0.75 to 2.3)	1.4% (0.80 to 2.4)	1.5% (0.84 to 2.5)
		R_0	0.073 (0.042 to 0.13)	0.078 (0.045 to 0.14)	0.082 (0.047 to 0.14)
	Medium	SAR	1.3% (0.73 to 2.3)	1.5% (0.85 to 2.6)	1.7% (0.97 to 3.0)
		R_0	0.072 (0.041 to 0.13)	0.084 (0.048 to 0.15)	0.095 (0.054 to 0.17)
	Long	SAR	1.3% (0.72 to 2.3)	1.6% (0.92 to 2.8)	2.0% (1.2 to 3.5)
		R_0	0.072 (0.040 to 0.13)	0.091 (0.051 to 0.16)	0.11 (0.065 to 0.20)

CI: confidence interval; R_0: basic reproductive number; SAR: secondary attack rate.

Results are based on the assumption that the daily probability of infection by external sources (b) = 9.67×10^{-5} and are stratified by the duration of the incubation and infectious periods (duration settings are given in Table 1) and the relative contact frequency between a case and their household members within a hospital compared with within the household (α). Relative contact frequencies in schools and the community are set respectively to $\rho_S = 3$ and $\rho_C = 1$.

[31]. For past pandemic influenza strains, R_0 estimates are somewhat higher, e.g. from 1.3 to 1.7 for A(H1N1) pdm09, 1.5 to 1.8 for H2N2 and H3N2 in the late 1950s and 60s, and 1.8 to 2.4 for H1N1 in 1918 [22,32]. Our R_0 estimate for H7N9 is similar to a previous estimate of 0.10 [19]. However, our estimate is based on comprehensive household data covering two epidemic waves and partially accounts for heterogeneity in clustering pattern across age groups, whereas the previous estimate was made using only data from the first wave under the simplified but unrealistic assumption of random mixing of the whole population. Our estimate is relatively robust to uncertainty in the natural history of the disease but is somewhat sensitive to the relative transmission intensity in the community at large. Obtained via a Bayesian approach, the previous estimate had a credible interval of 0.01 to 0.49, much wider than our CIs, even with the uncertainty in ρ_C and ρ_S factored in, and was sensitive to the prior distribution of R_0. In addition, we provide the first estimate for the household SAR, which could be useful in future simulation studies involving household transmission.

In the resampling-based testing of $p = 0$, we implicitly assume that b is constant over time. This assumption is unlikely to be true, as the exposure to zoonotic infection was substantially reduced when live poultry markets were closed in heavily affected areas from mid-April to June in 2013 and from late January to February in 2014 to control the spread [33]. Due to the difficulty of the resampling-based test in handling covariates, we used an asymptotic test [34] to address this issue. The effect of market closure on b has to be assumed known to implement this test, and we explored three values for the odds ratio (1.0, 0.5 and 0.1) for market closure versus without closure. The value of 0.1, or 90% reduction, is close to previous estimates [33]. The p values are all less than 0.001, consistent with the statistical significance of human-to-human transmission. However, the significance level should not be over-interpreted, as the asymptotic test may not be suitable for sparse data [29].

We did not find statistical evidence for sex or age differences in the risk of human-to-human transmission, which could be due to the lack of power to detect such differences. Higher risk of poultry-to-human transmission in males was previously noticed during the first wave [6] and was also reflected in the male-to-female ratio of index case numbers in our household data:

88/37 during the first wave and 124/65 during the second. One hypothesis for such a difference between the sexes is that in the Yangtze River Delta, in particular Shanghai, where the first wave originated from, old men more frequently visit live poultry markets than women. In our analysis, male household contacts appeared to have a somewhat higher risk of infection than female contacts. If this sex difference was true, it might imply that the majority of non-index cases were infected by zoonotic rather than human sources. On the other hand, if there were truly no age difference, it implies that most non-index household cases were infected by human sources, or otherwise more adult non-index cases would have been observed in households, because adults were believed to be more prone to infection by poultry due to increased exposure [6,30]. The lack of age difference in human-to-human transmission is also consistent with the fact that most people were naive to this novel virus [35]. More household data in future outbreaks should be collected to re-examine the sex and age effects on the human-to-human transmissibility of the virus.

Our analysis could be improved if we had genetic sequences and/or exposure and contact tracing information to help narrow the source of infection. Genetic linkage has been established in a couple of households included in our data [15-17]. However, similar viral genetic sequences do not necessarily imply human-to-human transmission, as the same virus could have been contracted from the same animal source. On the other hand, a certain level of heterogeneity in genetic sequences would imply impossible direct transmission pairs. Exposure and contact tracing information was obtained for some but not all cases in our study, but such data lack details and are subject to recall bias. Moreover, simultaneous testing of both human cases and possible animal sources would be helpful, but it is often difficult to trace the animal source, e.g. poultry purchased as food, or to coordinate between administrative units in charge of human health and those responsible for animal surveillance.

The human-to-human transmissibility of H7N9 remained not only limited but also temporally stable. We observed only a minor increase in the SAR estimates between the two successive waves, which is likely just a stochastic effect. However, some recent studies suggested genetic changes in the RNA segments of NS, NP and PB of the viral samples in Guangdong Province of southern China during the second wave as compared with sequences in eastern and central China from the first wave [36]. In addition, these segments are similar to the influenza A(H9N2) viruses circulating in the same province, giving rise to concern about reassortment of H7N9 with viruses that could lead to more efficient human-to-human transmission. Therefore, the efforts on collecting and analysing household transmission data should be continued in addition to the routine surveillance and contact tracing. Should human H7N9 transmissibility increase to the level of that of seasonal human influenza, it would be very important to deploy antivirals agents and vaccines in areas where human cases are occurring. Human H7N9-inactivated vaccines are being developed by government agencies and private pharmaceutical companies [37]. These vaccines are currently in phase I and II safety and immunogenicity trials. The use of antiviral agents and vaccines needs to be incorporated into a comprehensive programme for the assessment of transmissibility and intervention effectiveness.

Conflict of interest

None declared.

Authors' contributions

Yang Yang, Ira Longini, Zijian Feng and Wuchun Chao conceived the study and evaluated study results. Yang Yang, Liqun Fang, and Ira Longini designed analysis plan. Zijian Feng, Yanping Zhang, Enfu Chen, Jiayu Hu and Fenyang Tang collected data. Liqun Fang and Maijuan Ma provided further data support and verification. Yang Yang conducted data analysis. M. Elizabeth Halloran, Song Liang, Tom Britton and Eben Kenah evaluated study results. Yang Yang drafted the manuscript. Yang Yang, Ira Longini, Elizabeth Halloran, Zijian Feng, Wuchun Chao and Song Liang contributed to the final version. Ira Longini, Zijian Feng and Wuchun Cao share equal senior responsibility.

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Emergence and spread of predominantly community-onset *Clostridium difficile* PCR ribotype 244 infection in Australia, 2010 to 2012

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We describe an Australia-wide *Clostridium difficile* outbreak in 2011 and 2012 involving the previously uncommon ribotype 244. In Western Australia, 14 of 25 cases were community-associated, 11 were detected in patients younger than 65 years, 14 presented to emergency/outpatient departments, and 14 to non-tertiary/community hospitals. Using whole genome sequencing, we confirm ribotype 244 is from the same *C. difficile* clade as the epidemic ribotype 027. Like ribotype 027, it produces toxins A, B, and binary toxin, however it is fluoroquinolone-susceptible and thousands of single nucleotide variants distinct from ribotype 027. Fifteen outbreak isolates from across Australia were sequenced. Despite their geographic separation, all were genetically highly related without evidence of geographic clustering, consistent with a point source, for example affecting the national food chain. Comparison with reference laboratory strains revealed the outbreak clone shared a common ancestor with isolates from the United States and United Kingdom (UK). A strain obtained in the UK was phylogenetically related to our outbreak. Follow-up of that case revealed the patient had recently returned from Australia. Our data demonstrate new *C. difficile* strains are an on-going threat, with potential for rapid spread. Active surveillance is needed to identify and control emerging lineages.

Introduction

Clostridium difficile is the most common cause of infectious diarrhoea in hospitalised patients [1], in the United States (US) costing more than USD 3 billion (EUR 2.8 billion) annually [2]. Infection occurs following ingestion of *C. difficile*. Exposure to agents that alter the gut microflora, often antibiotics, [3] is normally required, but about half of the cases with community-onset *C. difficile* infection (CDI) may not have received recent antibiotics [4]. Since 2003, rates of healthcare-related CDI have escalated worldwide, with a new hyper-virulent strain of *C. difficile* (PCR ribotype 027) responsible for outbreaks of severe disease in North America and Europe [5]. Following the first detection of ribotype 027 in Australia in 2008 [6], there have been two known clusters of ribotype 027 infection that occurred in Melbourne and Sydney in 2010 [7]. However, the strain has not become endemic in Australia; it is unclear whether this is because of early recognition and subsequent prevention of large scale spread and/or because of relatively conservative antimicrobial prescribing policies, for example low use of fluoroquinolones to which ribotype 027 is resistant [5,8]. Rates of community-associated CDI have also increased in North America and Europe [9,10] and community-associated CDI is estimated to be responsible for more than one third of all CDI cases. Patients with community-associated CDI tend to be younger, less likely to have been exposed to antibiotics, and have fewer co-morbidities than patients with healthcare-acquired infection [11].

Australian States and Territories currently operate separate, primarily hospital-based, mandatory surveillance for CDI as part of hospital accreditation. All Australian states have seen a significant increase in hospital-identified CDI incidence since mid-2011, which is unlikely to be completely explained by recent changes to more sensitive molecular diagnostic methods or increased awareness of CDI and testing. The

proportion of recent CDI cases defined as community-associated has also increased throughout Australia [12]. A recent report on CDI in Tasmania concluded that the observed increase in CDI was most likely due to acquisition in the community [13].

In October and November 2011, a large cluster of an apparently new (to Australia) ribotype was identified by a reference laboratory in Western Australia (WA) in isolates from New South Wales (NSW). At about the same time, reports emerged from Victoria of cases of CDI initially identified as putative ribotype 027 with the GeneXpert system (Cepheid, Sunnyvale, CA), but actually matching the NSW ribotype [14]. Subsequent interrogation of the WA laboratory database identified further CDIs due to the same ribotype (eventually identified as ribotype 244).

Investigation of the 12 ribotype 244 cases in Victoria, demonstrated that the strain was associated with more severe disease, 58% had severe disease compared to 25% of cases with non-ribotype 244, non-ribotype-027 strains, and increased mortality, 42% 30-day mortality vs 0% [14]. Whole genome sequencing of one of the 12 ribotype 244 isolates showed it to be from the same clade as ribotype 027, but genetically distinct from ribotype 027 [14]. Ribotype 244 cases have also been identified in New Zealand: In a case–control study, 10 ribotype 244 CDI cases were more likely to develop severe colitis than 20 age- and sex-matched controls with CDI with other ribotypes [15].

In this report, we describe the secular trends in the prevalence of hospital-identified CDI in WA from 2010 to 2012, and in particular CDI due to ribotype 244. We use whole genome sequencing of ribotype 244 isolates recovered in WA and two other states to investigate strain clonality. Lastly, we report international spread of this ribotype.

Methods

Epidemiology
CDI incidence data were obtained from the WA hospital CDI surveillance programme from January 2010 to December 2012 inclusive. In WA, surveillance for CDI has been mandatory since January 2010 for all public metropolitan, regional and integrated district hospitals, and private hospitals funded to provide care to public patients. These hospitals were also encouraged to submit all *C. difficile*-positive faecal samples for PCR ribotyping from January 2010 onwards, with increasing numbers participating during 2010 and 2011, such that all hospitals were providing samples by October 2011.

A CDI case was defined as a case of diarrhoea, i.e. unformed stool taking the shape of its container, meeting the following criteria: the stool sample yielded a positive result in a laboratory assay for *C. difficile* toxin A and/or B, or a toxin-producing strain of *C. difficile* was detected in the stool sample by culture or other means. Cases were only included once in an eight-week period; repeat samples from the same patient after eight weeks were considered a new infection. Patients younger than two years were excluded.

CDI was reported from patients attending any area of a hospital, i.e. all inpatient wards and units (including psychiatric, rehabilitation and aged care admissions) and emergency and outpatient departments (including haemodialysis and day surgery units); as such, reporting reflected the total burden of CDI on a hospital and sampled the surrounding community, as the reported cases included disease with both healthcare- and community onset. Data on recent healthcare facility exposure were available on cases identified at metropolitan hospitals (accounting for the majority of cases), allowing these CDI cases to be classified according to the place of probable exposure as described by Kuijper et al. [16] and recent US guidelines [17]: CDI was classified by location of onset, as healthcare facility (HCF) onset or as community-onset, and by the timing relative to any previous healthcare exposure. CDI onset more than 48 hours after HCF admission and within four weeks of discharge was denoted HCF-associated, onset between four and 12 weeks post-discharge was denoted indeterminate/unknown (whether community-onset or HCF-onset), and onset more than 12 weeks following last HCF exposure was denoted community-associated. HCF-associated CDI rates are reported per 10,000 bed-days. As identified community-associated cases were only a subset of all community-associated cases (cases presenting to primary care facilities and smaller hospitals were not included), simple counts of community-associated cases per quarter are presented. Severe CDI was defined as an episode of CDI with one or more signs of severe colitis [18].

PCR ribotyping and toxin gene profiling
Crude bacterial template DNA for toxin profiling was prepared by resuspension of cells in a 5% (wt/vol) solution of Chelex-100 resin (Sigma-Aldrich, Castle Hill, NSW, Australia). All isolates were screened by PCR for the presence of the toxin A (*tcdA*) and toxin B (*tcdB*) genes [19] and the binary toxin (*cdtA* and *cdtB*) genes [20], and for changes in the repeating region of *tcdA* [21]. PCR ribotyping was performed as previously described [22]. PCR ribotyping banding patterns were identified by comparison with a reference library consisting of reference strains from the European Centre for Disease Prevention and Control (ECDC)-Brazier collection [23], a collection of the most prevalent PCR ribotypes currently circulating in Australia (B. Elliott, unpublished data), and a selection of binary toxin-positive strains. Ribotyping results were confirmed by the Reference Laboratory of the *Clostridium difficile* Ribotyping Network (CDRN) for England in Leeds, United Kingdom (UK). Antimicrobial susceptibility testing and breakpoints for metronidazole, vancomycin, clindamycin and moxifloxacin were determined by Clinical and Laboratory Standards Institute agar dilution [24].

Whole genome sequencing

Fifteen ribotype 244 isolates obtained between 16 July 2011 and 18 January 2012 and submitted to PathWest for ribotyping at the time of the study were selected for whole genome sequencing (WGS): seven samples from Victoria, four from NSW and four from WA. All four ribotype 244 strains held by the UK-based CDRN reference laboratory (three North American isolates (from New Jersey, 2004; Indiana, 2011; 2007) and a recent UK clinical isolate from November 2011) were also sequenced. Following subculture of a single colony on Columbia blood agar for 48 h, DNA was extracted using a commercial kit (QIAamp, Qiagen, Hilden, Germany). A combination of standard Illumina and adapted protocols was used to produce multiplexed paired-end libraries. Pools of eight samples were sequenced at the Wellcome Trust Centre for Human Genetics, Oxford, UK, on the Illumina MiSeq platform (Illumina Inc., San Diego, CA), generating 150 bp paired-end reads. The sequences reported in this paper have been deposited in the National Center for Biotechnology Information Sequence Read Archive under BioProject accession number PRJNA277962 and are available at http://www.ncbi.nlm.nih.gov/bioproject/277962.

Sequence reads were analysed and assembled using a pipeline developed specifically for bacterial genomes: Each isolate was mapped using Stampy v1.0.11 (without Burrows-Wheeler Aligner pre-mapping, using an expected substitution rate of 0.01) [25] to the *C. difficile* 630 reference genome (GenBank: AM180355.1), CD630 [26]. Single nucleotide variants (SNVs) were identified across all mapped non-repetitive core genome sites using the SAMtools (version 0.1.12–10) mpileup command with the extended base-alignment quality flag, after parameter tuning based on bacterial sequences. A consensus of at least 75% was required to support a SNV, and calls were required to be homozygous under a diploid model. Only SNVs supported by at least five reads, including one in each direction, were accepted. Sequence reads were also assembled de novo using Velvet [27], run with the Velvet Optimiser. De novo assemblies were used to determine in silico multilocus sequence types (MLST).

Sequences from the ribotype 244 isolates were compared with available fully-sequenced reference isolates, with sequences from a reference laboratory collection (CDRN), and a collection of clinical isolates from Oxfordshire, UK, representing all five *C. difficile* clades [28]. As ribotype 244 has been shown to be from Clade 2 [14], we included previously sequenced Clade 2 strains from the Oxfordshire clinical collection, including example ST1 strains (ribotype 027) and all available ST41, ST47, ST67, ST97 and ST114 strains.

Maximum likelihood phylogenetic trees were drawn based on variable sites called across all sequences, using PhyML [29] with a Hasegawa, Kishino and Yano (HKY) substitution model. Ribotype 244 sequences were analysed using BEAST 1.7.5 [30] to generate a
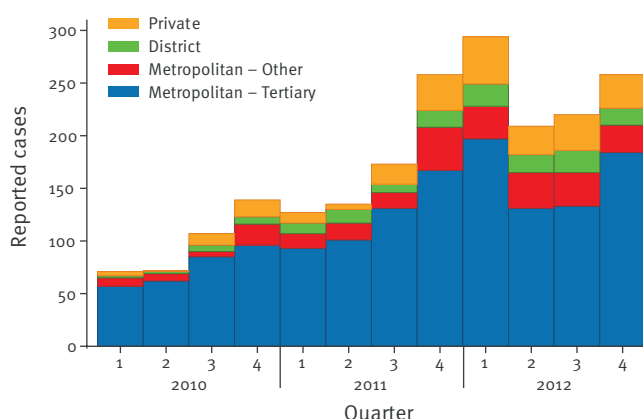
CDI: *Clostridium difficile* infection.

CDI was reported for patients attending any area of a hospital, i.e. all inpatient, emergency and outpatient departments. As such, reporting reflected the burden of CDI on a hospital and the surrounding community, as the reported cases included healthcare and community onset disease.

time-scaled phylogenetic tree and estimate the date of the most recent common ancestor of the outbreak strains. After identifying variable sites between the sequences, any variants clustered within 323 sites (the mean recombination insert size for Clade 2 isolates in a previous analysis [28]) were masked to remove these likely recombination events. To reduce the time for computation, any uncalled bases at otherwise invariant sites were assumed to be the same as the reference. We assumed a constant population size and a constant molecular clock at a previously estimated rate of 3.2×10^{-7} substitutions per site per year [28]. Data from two convergent chains, initiated from different starting values, each of 10,000,000 iterations, were combined after discarding the first 100,000 iterations as burn-in. A HKY substitution model was used with empiric base frequencies.

Results

Epidemiology of *Clostridium difficile* infection in Western Australia

During the three years of surveillance from January 2010 to December 2012 in Western Australia, there were a total of 2,061 hospital-identified CDI cases with a peak of 294 cases in quarter 1 2012 (Figure 1). Of 1,681 cases reported by metropolitan hospitals (where data on recent healthcare facility exposure was recorded), 1,086 (65%) cases were HCF-associated, 478 (28%) community-associated and 117 (7%) indeterminate/unknown. Overall HCF-associated CDI incidence increased markedly in mid-2011 from 2.5/10,000 bed-days in the second quarter (April–June) to a peak at over 4.5/10,000 bed-days in the first quarter of 2012 (January–March) before declining over the next two

Figure 2

Quarterly healthcare facility-associated and community-associated CDI rates in metropolitan hospitals, Western Australia, 2010–2012 (n = 1,681)
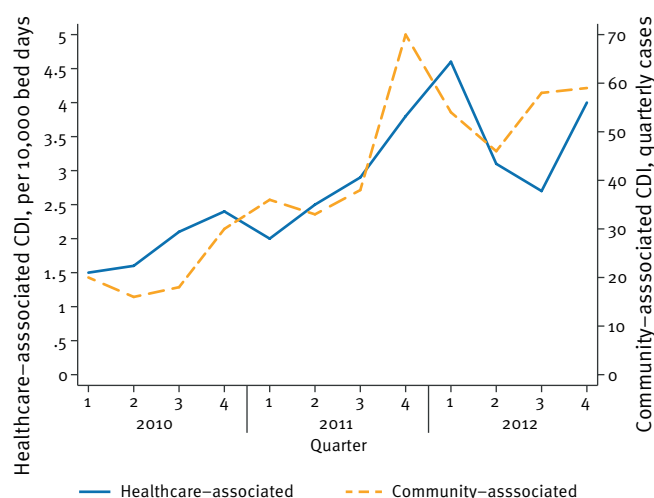
CDI: *Clostridium difficile* infection.

Rates are based on 1,681 (82%) cases identified at metropolitan hospitals where data on recent healthcare exposure was available. The remaining 380 cases (18% of total 2,061 cases) were identified in smaller district and private hospitals where data on recent healthcare exposure was incomplete. The bed-day denominator is based on bed-days in metropolitan hospitals only.

Table

Ribotypes causing CDI in Western Australia, October 2011–September 2012 (n = 657)

Ribotype	Number of isolates	Prevalence
014/020 group	264	35%
002	84	11%
056	40	5%
054	35	5%
052	32	4%
070	28	4%
015	24	3%
010	21	3%
244	21	3%
046	19	3%
017	19	3%
QX001	18	2%
005	18	2%
064	17	2%
QX077	17	2%
Total	657	88%

CDI: *Clostridium difficile* infection.

Ribotypes were available for 746 CDI cases. The 15 most common ribotypes are shown, accounting for 88% of all ribotyped cases. Ribotype 244 accounted for 3% of all cases. QX001 and QX077 were novel ribotypes.

quarters, and then increasing again in the last quarter of 2012 (Figure 2). Trends in community-associated CDI rates followed the rates of healthcare-associated cases closely.

Ribotype 244 epidemiology in Western Australia

In WA, between January 2010 and December 2012, 25 cases of ribotype 244 CDI were identified. During the main period of the outbreak, between 17 August 2011 and 23 April 2012, there were 19 cases of ribotype 244 CDI, with cases in each month except March. No further cases were recorded for over four months, until late August 2012, with six additional cases (≥1/month) up to December 2012 (Figure 3). Before August 2011, no ribotype 244 isolate had been obtained from a CDI case in WA since ribotyping of cases began as part of comprehensive surveillance in January 2010. Only five cases were identified in WA in 2013, and no ribotype 244 cases have been identified in the first five months of 2014.

The Table provides a breakdown of the 15 most commonly isolated ribotypes in WA in the first year for which complete ribotyping data were available from all hospitals, October 2011 to September 2012. Although ribotype 244 only accounted for 3% of cases, it was the ninth most commonly occurring ribotype, which represents a significant emergence of a novel ribotype.

Of the 25 ribotype 244 cases, 13 were female, 14 were 65 years or older, 14 presented at hospital emergency departments or were outpatients, and 14 presented at non-tertiary or community hospitals. Using surveillance definitions, 18 were community-onset (14 were community-associated infections and four were both community-onset and HCFA), and seven were both

Figure 3

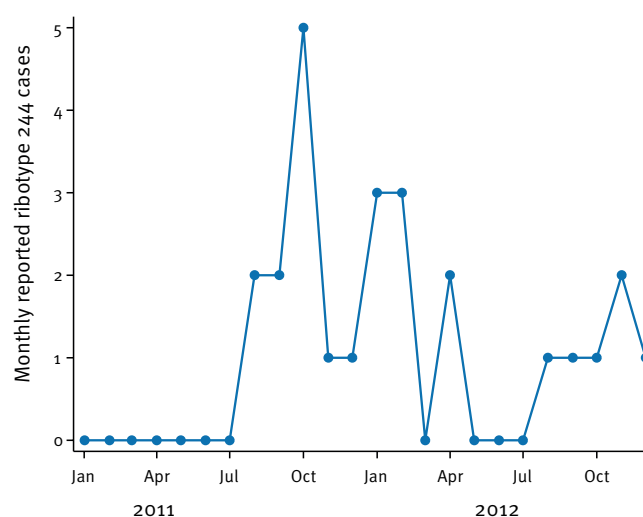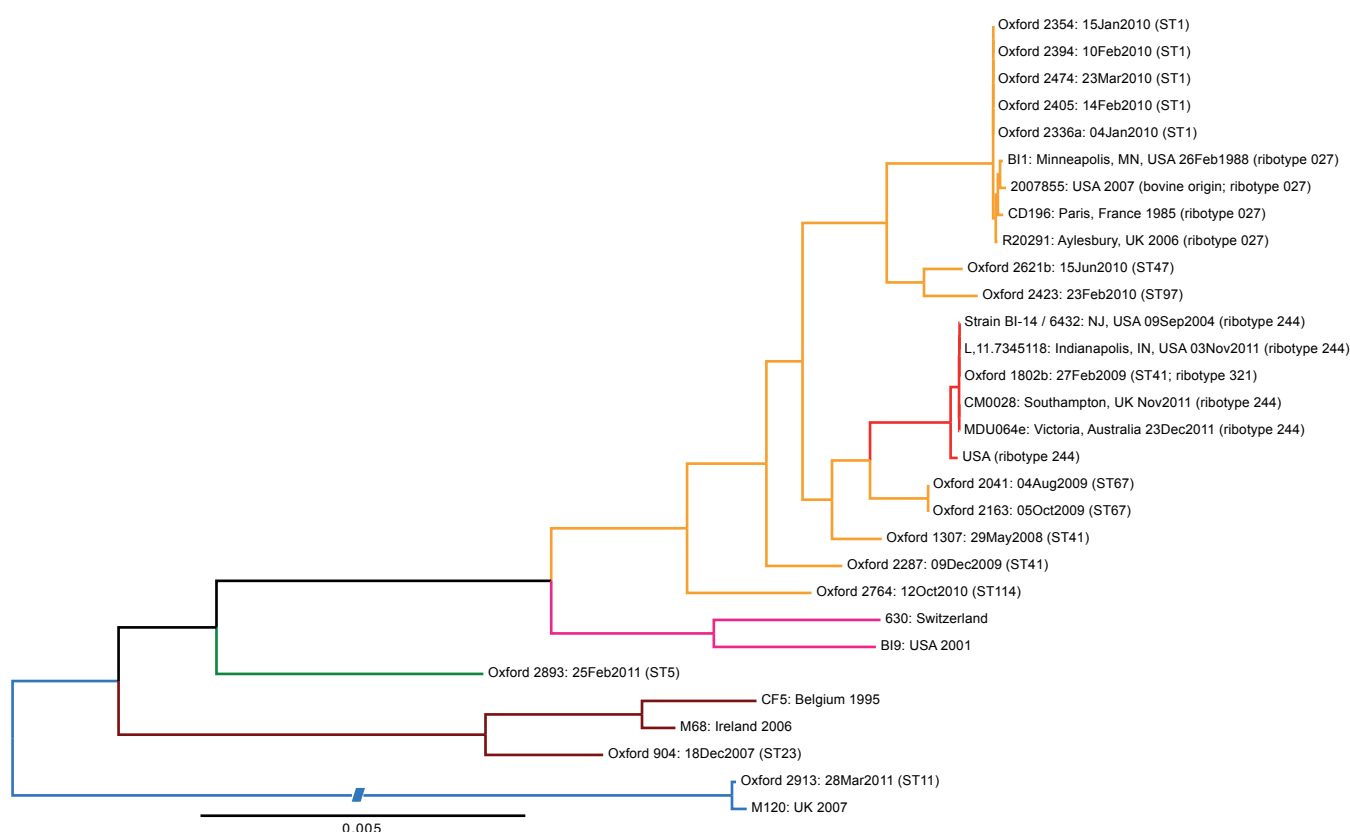Monthly ribotype 244 cases in Western Australia, 2011–2012 (n = 25)

FIGURE 4

Phylogenetic relationship of outbreak ribotype 244 lineage, Western Australia, October 2011–September 2012, to global *Clostridium difficile* diversity

Reference isolates from the *C. difficile* clades are shown. One example ribotype 244 outbreak strain, MDU-064e, is included, with four UK reference collection ribotype 244 isolates. Example clinical strains from each of the five clades from an Oxfordshire, UK collection are shown, as well as five example strains from ST1/ribotype 027/NAP1, and all available samples from other Clade 2 STs.

Pink: Clade 1; orange: Clade 2 (outbreak lineage, and a closely related Oxfordshire ST41, ribotype 321, sample in red); green: Clade 3; maroon: Clade 4; blue: Clade 5. Where available the date and location of isolation is shown.

Maximum likelihood tree based on 112,792 variable sites called across all sequences. Scale shows the number of substitutions per site, based on a whole genome length of 4 290 252 nt. The blue line depicting Clade 5 contains a break; the overall length of this branch is 0.028.

HCF-onset and HCF-associated. The median patient age was 68 years (interquartile range: 38–88 years). In WA between January 2010 and December 2012, the proportion of ribotype 244 cases that were community-associated, 14 of 25 (56%), was significantly greater than the proportion of non-ribotype 244 cases with available data on healthcare exposure, 464 of 1,656 (28%, p=0.006). No ward-based clustering of ribotype 244 HCF-associated cases was observed. Clinical data were available on 15 of the 25 ribotype 244 cases. Six cases had severe CDI as defined [18], but there were no deaths attributable to CDI.

All ribotype 244 isolates were positive for *tcdA, tcdB* and binary toxin and had a single nt deletion at position 117 in *tcdC* seen in ribotype 027, but had no other deletions. All were susceptible to metronidazole, vancomycin, clindamycin and moxifloxacin, with modal minimum inhibitory concentrations of 0.5mg/L, 2mg/L, 4 mg/L and 2 mg/L, respectively. We determined the in silico multilocus sequence type (ST) of the ribotype 244 strains as ST41.

Ribotype 244 evolution

We investigated the phylogenetic relationship between whole genome sequences obtained from 15 Australian ribotype 244 outbreak isolates, four ribotype 244 isolates held by a UK reference laboratory, and existing sequences obtained from the five major *C. difficile* clades (Figure 4). In keeping with a previous study showing 10,803 SNVs between ribotype 244 and ribotype 027 isolates [14], we demonstrate the ribotype 244 lineage diverged relatively early from other Clade 2 lineages. It only shares a relatively ancient common ancestor with ST1 (ribotype 027). For example, we observed 12,026 SNVs between CD196 (a published ribotype 027 reference) and outbreak strain MDU-064e. The observed SNVs differ from the earlier comparison by Lim et al. because of the different ribotype 027 strains studied and the different

FIGURE 5

Bayesian phylogeny of ribotype 244 outbreak isolates, Australia, July 2011–January 2012 (n = 15) and related strains
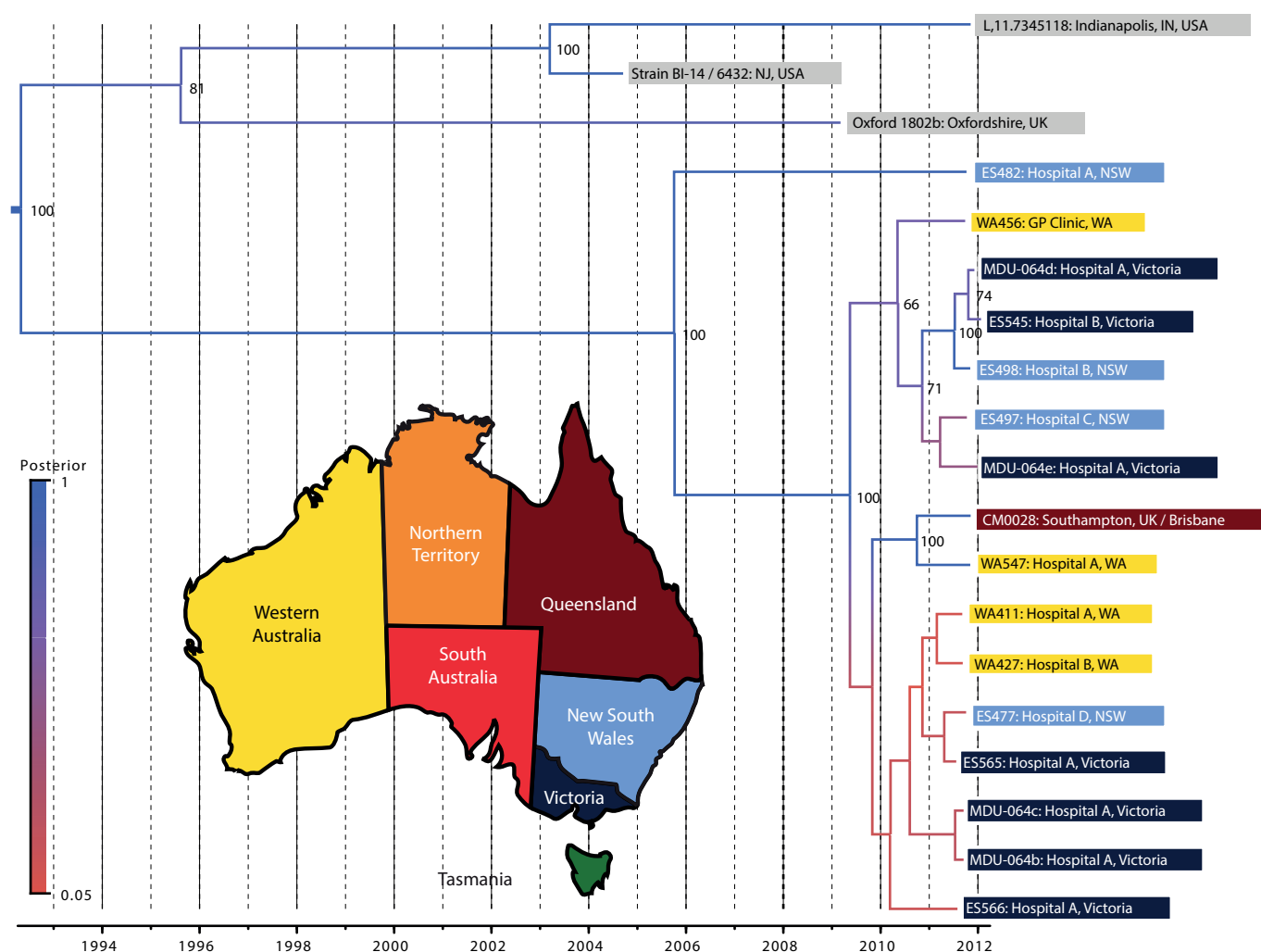
Tips are positioned by sampling date and coloured by Australian state of origin; grey: related UK and US strains; maroon: a closely related UK strain, as the patient had recently returned from Brisbane, Queensland. Tree branches are coloured by the posterior clade probability for each node. As indicated in the legend, blue branches are strongly supported by the sequence data and red branches more weakly supported. Nodes with posterior probabilities greater than 50% are annotated with the posterior support as a percentage. The terminal branches with low support at the bottom of the tree indicate that these isolates are similar (within four observed SNVs), consistent with a point source, but the exact ordering of their ancestry is not certain from the data.

sequence assembly and analysis approaches used. It is likely that, despite both belonging to the same clade, the current ribotype 244 is separated from ribotype 027 by hundreds/thousands of years given rates of *C. difficile* evolution [5,28,31].

Ribotype 244 outbreak phylogeny

All 15 Australian ribotype 244 isolates were within 16 observed SNVs of each other, and all but one isolate were within eight SNVs, including a cluster of seven cases from three different states which were within four SNVs of each other. We compared the phylogenetic relationship between the outbreak isolates using a time-scaled Bayesian phylogeny (Figure 5). Excluding the single outlying isolate, the estimated date of the most recent common ancestor of the outbreak isolates was April 2009 (95% credibility interval: March

2008–April 2010), and including all Australian ribotype 244 isolates, it was August 2005 (95% credibility interval: December 2002–February 2008). Despite sequenced isolates originating from Australian towns and cities separated by thousands of kilometres, the isolates were closely genetically related, and without evidence of geographical intra-state clustering of similar isolates.

Interestingly, a ribotype 244 isolate held in the CDRN reference laboratory, from a patient in Southampton, UK, fell within the diversity of the Australian outbreak strains. Further epidemiological investigation initiated as a result of the sequencing data revealed that the UK patient had returned from Australia (Brisbane, Queensland) three weeks before the onset of diarrhoeal symptoms; this person had spent ca three

months in Australia in late 2011. The patient had not made any visits to hospitals or GPs while in Australia nor stayed with anyone who was ill or on antibiotics. One relative they visited worked in a care home. The patient remained well until they required antibiotics for an unrelated complaint after returning to the UK. They developed diarrhoea shortly thereafter and were diagnosed with CDI within three weeks of return to the UK. The only risk factors for CDI identified in the UK patient were age over 65 years and recent antibiotic use.

Two ribotype 244 isolates from the US, one previously identified as ribotype 244 in [32], and a ribotype 321 isolate from Oxfordshire, UK were closely related to, but distinct from, the Australian ribotype 244 outbreak isolates (Figure 5). There are no reports of ribotype 321 in Australia. Only two ribotype 321 isolates have been identified by the UK CDRN reference laboratory to date, the Oxfordshire clinical strain and a food research centre isolate from Ireland in 2010.

Discussion

Here we describe a clonal outbreak of ribotype 244 throughout Australia, against a background of rising CDI incidence. Isolates originating from four states across Australia were genetically closely related without any evidence of geographical or temporal structure in their phylogenetic relationships, and over half of cases occurred without recent healthcare exposure, suggesting a possible community-based point source dispersed nationally. The majority of ribotype 244 cases were community-onset and many were detected in patients younger than 65 years and in those who presented to hospital emergency departments, rather than in hospital inpatients. Notably, by using the discriminatory power of WGS, we were also able to identify spread of the outbreak strain to the UK via a returning traveller. Since the end of 2012 there has been a decline in new cases of ribotype 244 in WA, supporting the suggestion that the cases in 2011/12 represented an outbreak followed by some ongoing transmission.

In contrast to Europe and North America, Australia has not seen large epidemics with *C. difficile* ribotype 027 despite at least two introductions [6,7]; it is possible that relatively conservative antimicrobial prescribing practices [8] and/or the geographical isolation of Australia have been responsible [33]. Despite the limited numbers of ribotype 244 cases seen, this outbreak does demonstrate that there is potential for epidemic spread in Australia too. Although ribotype 244 only accounted for 3% of all cases in WA, the outbreak occurred against a background of rising CDI incidence. Since mandatory reporting was introduced three years ago, rates of CDI in WA have more than doubled (Figure 2). Australia-wide increases in CDI incidence could be due to a number of factors. Increased awareness of CDI may have increased testing [34]; however, a 25% increase in laboratory testing in WA is very unlikely to account for the observed rise in CDI cases. Similarly, more sensitive testing methods could have resulted in increased reported incidence, with many laboratories

changing from enzyme immunoassay testing, with ca 60–85% sensitivity, to nucleic acid amplification tests, with more than 95% sensitivity (but decreased specificity) [35]. Changes in laboratory testing during late 2010 may have accounted for some of the increase in measured CDI rates in WA in quarters 3 and 4 of 2010. However, such potential laboratory ascertainment bias does not explain the increases in CDI rates recorded from quarter 3 onwards in 2011.

The introduction of new *C. difficile* strains, including ribotype 244, alongside rises in the incidence of established strains, underlies the observed increased incidence of CDI overall. Two snap-shot surveys from the Australian Commission on Safety and Quality in Healthcare, the first in October and November 2010, and the second in October and November 2012, described the ribotypes of *C. difficile* circulating in Australia. Representative samples of 330 and 556 isolates, respectively, predominantly from hospital laboratories, were collected from all Australian states and ribotyped. The most common ribotypes in 2010 were 014/020 (30%), followed by 002 (11%); a ribotype could not be assigned to 53% of isolates. By 2012, ribotypes 014/020 and 002 still accounted for 25% and 10% of CDI, respectively, but significant increases in the proportion of cases due to ribotypes 056, 070, 054, 015, 017, 053 and 244 were seen compared with 2010, with each of these ribotypes accounting for between 3% and 6% of cases (data not shown).

There now appears to be seasonality to CDI incidence in Australia; this has been demonstrated in other countries [36], but has been assumed to be due to healthcare-associated CDI following increased antibiotic treatment of winter-associated severe respiratory tract infections. By contrast, much of the increase in Australian CDI incidence has been due to community-associated CDI cases with no previous contact with the hospital system [12,13]. The most likely explanation for this is the combination of community-associated strains, and the seasonal peak in antimicrobial prescriptions in the community, following respiratory tract infections [37].

The detection of ribotype 244 in Australia is a recent occurrence. Retrospectively, only one of the unidentified isolates (from Queensland) from the 2010 snapshot survey was found to be ribotype 244. In addition to our data showing the emergence of ribotype 244 in WA where it had not been previously detected, recent surveys by other researchers suggest it is also present in other states. In a point prevalence survey in April 2012, 83 CDI isolates from Brisbane, Queensland were ribotyped and seven (8%) of them were ribotype 244 (personal communication, David Patterson, July 2013). Between June 2011 and August 2013, ribotype 244 accounted for 56 of 3,111 (2%) CDI cases in the North Sydney healthcare region. No ribotype 244 cases were seen in the region after January 2013 (data not shown), in keeping with the decline in ribotype 244 cases seen

in WA from 2013 onwards. A limitation of surveillance data used in our study and the surveys described above, is that they were hospital-based. Although cases tested as outpatients were included, patients tested by general practitioners working outside of hospitals were not. As such the surveillance may underestimate the proportion of total CDI accounted for by strains predominantly causing community-associated disease.

The recent emergence of ribotype 244 in surveillance data is in keeping with our estimated date (2009) for the most recent common ancestor of the majority of the outbreak strains. If a point source was the explanation for the outbreak we might have expected the most recent common ancestor to be closer in time to the beginning of the outbreak, i.e. around 2011, and fewer than the observed eight SNVs separating the majority of the outbreak strains. However it is also possible that some within-host/within-reservoir diversity existed in the source of the outbreak before the first cases, analogous to the within-host diversity seen in some CDI [28,38]. A single strain from NSW was more diverse, and may represent a separate introduction, or pre-existing diversity in the source of the outbreak.

Although ribotype 244 strains share some features with ribotype 027, WGS suggests these strains are substantially different at a whole genome level [14], confirmed in this study and in keeping with a previous description based on microarray data [39]. Ribotype 244 strains produce toxins A, B and binary toxin, are susceptible to fluoroquinolones and associated with more severe disease [14,15].

We have demonstrated that ribotype 244 CDI was significantly more likely to be community-associated than other ribotypes, 56% of ribotype 244 cases were community-associated, compared with 28% of other ribotypes. In keeping with our data, there is also some evidence that cases from New Zealand were more likely to be community-associated than controls, 50% of cases vs 15% of controls (p=0.08) [15]. In another case–control study in Victoria, Australia, proportionally more ribotype 244 cases were community-associated (four of 12) than non-ribotype 244 controls (six of 24), however this was not statistically significant (p=0.72) [14]. The relative excess of community-associated ribotype 244 CDI suggests a possible community source or reservoir. The nature of this source is unknown but is unlikely to be spores shed from recently hospitalised patients following discharge as this strain is different from those circulating in Australian healthcare facilities (predominantly ribotypes 014/020 and 002). One potential reservoir of infection in the community is animals [40]. *C. difficile* is known to colonise or infect animals and outside of Australia, the predominant animal strain is ribotype 078, commonly isolated from pigs and cattle [41]. This strain is responsible for increasing rates of community-acquired CDI in Europe [42,43], suggesting that a zoonosis exists, however

transmission of ribotype 244 from animals has not been observed to date. Contaminated food represents another potential source. *C. difficile* contamination of food has been previously reported in retail meat products in the US [44,45], in ready-to-eat salads/vegetables in Scotland [46] and France [47], and in vegetables and seafood in Canada [48,49], but again ribotype 244 has not been isolated from food to date. The recent emergence of ribotype 244 in Australia suggests it may have been imported. The oldest ribotype 244 isolate in our collection, from 2004, was from New Jersey, US, suggesting the current outbreak may have originated from North America, however this may represent sampling bias only.

The numbers of ribotype 244 cases being seen in Australia currently, although high compared with previous surveys, do not completely account for the increased CDI rates seen over the past two years. Ribotypes circulating previously account for many cases, e.g. 014/020, 002, 056, and 054 (Table). However other new ribotypes of *C. difficile* may have been introduced into the country recently or emerged locally. We already have preliminary evidence of this in a significant number of infections with ribotype 251 *C. difficile*, another strain that has not been found in Australia previously (data not shown). Our data demonstrate that new strains causing CDI are an ongoing threat in Australia and worldwide, with rapid potential for spread as has been seen with ribotype 027 [5]. Active surveillance of CDI cases [50,51] alongside strain typing and rapid sequencing [52] is needed to identify and control emerging lineages.

Conflict of interest

The institution of DWC and TEAP received per-case funding from Optimer Pharmaceuticals to support fidaxomicin trial patient expenses. DWC and TEAP also received honoraria from Optimer Pharmaceuticals for participation in additional meetings related to investigative planning for fidaxomicin. MHW has received honoraria for consultancy work, financial support to attend meetings and research funding from Actelion, Alere, Astellas, Astra-Zeneca, bioMerieux, Cerexa, Durata, Cubist, Nabriva, Novacta, Pfizer, Roche, Sanofi-Pasteur, Summit, The Medicines Company and VH Squared. No other author has a conflict of interest.

Authors' contributions

DWE, LT, ASW, TEAP, DWC, MHW and TVR designed the study. DWE, LT, BE, CS, PGH, RLS, TMK, GK, RM, DG, WNF, PA, KED gathered the data. DWE, LT, BE, ASW, TEAP, TVR analysed the data. DWE and TVR wrote the first draft. All authors revised the manuscript and decided to publish. All authors vouch for the completeness and accuracy of the data and analysis.

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Impact of 10- and 13-valent pneumococcal conjugate vaccines on incidence of invasive pneumococcal disease in children aged under 16 years in Germany, 2009 to 2012

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We assessed the impact of 10-valent and 13-valent pneumococcal vaccines (PCV10 and PCV13), which were introduced in Germany in 2009, on the incidence of meningitis and non-meningitis invasive pneumococcal disease (IPD) in children aged under 16 years in a population previously vaccinated with a seven-valent vaccine (PCV7). Surveillance of IPD (isolation of *Streptococcus pneumonia* from a normally sterile body site) is based on data from two independent reporting sources: hospitals and laboratories. IPD incidence was estimated by capture–recapture analysis. Incidence rate ratios (IRRs) were calculated for 2009 and 2012, thus comparing pre- and post-PCV10 and PCV13 data. IPD incidence caused by serotypes included in PCV13 decreased in all age and diagnosis groups. A rise in non-vaccine serotype incidence was seen only in children aged under two years. The overall impact varied by age group and infection site: for meningitis IPD in children aged under 2, 2–4 and 5–15 years, incidence changed by 3% (95% CI: −31 to 52), −60% (95% CI: −81 to −17) and −9% (95% CI: −46 to 53), respectively. A more pronounced incidence reduction was observed for non-meningitis IPD: −30% (95% CI: −46 to −7), −39% (95% CI: −54 to −20) and −83% (95% CI: −89 to −73) in children aged under 2, 2–4 and 5–15 years, respectively. A higher tropism of the additional serotypes for non-meningitis IPD may be a potential explanation. The heterogeneous findings emphasise the need for rigorous surveillance.

Introduction

The German Standing Committee on Vaccination (STIKO) included seven-valent pneumococcal conjugate vaccine (PCV7) in the infant vaccination calendar for all infants as of July 2006, with a 3+1 schedule given at 2, 3, 4 and 11–14 months of age [1]. Between 2007 and 2009, vaccination with PCV7 prompted a sharp decrease in vaccine serotype incidence for all children aged under 16 years in Germany, whereas non-vaccine serotype incidence rose. Both effects together resulted in a net reduction in IPD incidence [2]. Rising non-PCV7 serotype incidences were also observed in other countries: the most recent and comprehensive meta-analysis of the impact of PCV7 in different populations showed a reduction of about 50% for the incidence of all IPD and about 60% for meningitis IPD [3]. After almost complete elimination of PCV7 serotypes in most populations, non-PCV7 serotypes accounted for a substantial proportion of remaining IPD cases, drawing attention to the need for higher-valent vaccines.

In Germany, the switch to higher-valent vaccines took place in April 2009 with the introduction of a 10-valent vaccine (PCV10) (market share in 2010–12: 9%) and in December 2009, when the 13-valent vaccine (PCV13) was introduced (market share in 2010–2012: 91%, internal sales figures provided by Pfizer Pharma GmbH). These higher-valent vaccines include all the serotypes in PCV7 and the most frequent non-PCV7 serotypes (PCV10: 1, 5, 7F; PCV13: 1, 3, 5, 6A, 7F and 19A). As 73.6% of all IPD cases in children aged under 16 years in Germany from 2007 to 2009 were caused by PCV13 serotypes [2] and given that the effectiveness of the six additional serotypes in PCV13 has been established using the Broome method [4], a considerable benefit from the switch to higher-valent pneumococcal vaccines was expected.

In Germany, sustained surveillance for IPD is based on two independent data sources: active surveillance in hospitals and passive sentinel surveillance through microbiological laboratories. The hospitals and laboratories report on a voluntary basis, and there is an option for laboratories to send all pneumococcal isolates from IPD cases for serotyping to the National Reference Center for Streptococci in Aachen free of

charge. Case ascertainment from these two independ-ent sources allows underreporting to be corrected for by capture–recapture analysis.

Since vaccination coverage of pneumococcal conjugate vaccines has been above 85% since 2007, as estimated by internal sales figures provided by Pfizer Pharma GmbH, our data provide a basis to assess the impact of higher-valent vaccination (PCV10 and PCV13) up to three years after its introduction (2009 to 2012). Due to the limited market share of PCV10 (< 10%), we focused our research on the overall impact of both vaccines: PCV10 and PCV13. The aim of our study was to assess whether the incidence in PCV13 serotypes decreased during this period and whether there was a similar decrease in overall (PCV13 and non-PCV13 serotype) IPD incidence.

Methods

Data sources

Hospital surveillance of IPD in children aged under 16 years comprises all paediatric hospitals as well as pae-diatric hospital wards in Germany (n > 400, response rate > 95%) [5]. It is managed by the German paediatric surveillance unit, Erhebungseinheit für seltene pädia-trische Erkrankungen (ESPED). In the laboratory sen-tinel surveillance, cases are reported through a web interface (PneumoWeb) hosted by the Robert Koch Institute [2,6-8].

Case definition

Cases are children under 16 years of age treated for IPD as inpatients in a paediatric hospital or paediatric ward in general hospitals in Germany. IPD was defined as *Streptococcus pneumoniae* being isolated from at least one culture of blood, cerebrospinal fluid or a sample from any other normally sterile body site. Isolates from middle ear fluid were not included. Both surveillance sources (hospitals and laboratories) applied the same case definition.

Definition of serotype coverage

Serotypes were grouped into vaccine type (VT) sero-types, namely PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) plus the additional serotypes included in PCV13 (1, 3, 5, 6A, 7F, 19A) and into non-vaccine type (NVT) serotypes including all other serotypes. For cases for which serotype data were not available – because iso-lates were not sent for serotyping – the same serotype distribution as for the serotyped cases was assumed.

Pneumococcal isolates were serotyped at the German National Reference Center for Streptococci, by Neufeld's Quellung reaction using type and factor sera provided by the Statens Serum Institut, Copenhagen, Denmark.

Statistical analysis

Capture–recapture calculation (CRC) allows for adjust-ment of incomplete reporting in (at least) two sources by identifying overlapping cases and applying Bayes'

probability theory to estimate the number of cases not included in either of the sources [9]. To avoid bias caused by small sample sizes, the Chapman estimator [10] was applied.

For the analysis of children too young to be vaccinated, we defined these as all children aged ≤2 months. Although children may already receive the first dose when two months-old, we aimed to take account of the tendency of belated vaccination in Germany [11] as well as of inaccuracy in the exact age of the children: Due to data protection, the day of birth was not provided in our study and it was therefore assumed that all chil-dren were born on the first day of the month.

In this age subgroup ≤ 2 months, CRC was not possible since in 2012, all cases from the laboratory surveillance were included in the hospital surveillance, which ren-ders CRC impossible. Therefore the analysis of the age group ≤ 2 months, and the comparison with children aged 3–23 months, was based on the cumulative num-ber of cases in either source (ESPED and PneumoWeb).

The population denominator to convert the IPD case number estimates into incidence rates (per 100,000 children) was based on age-specific (0–2, 2–4, 5–15 and 0–15 years) population figures provided by the German Federal Statistical Office [12]. To assess the impact of the higher-valent pneumococcal conjugate vaccines (PCV10 and PCV13), we calculated incidence rate ratios (IRRs): we compared the incidence during years when PCV10 and PCV13 were exclusively used (2010, 2011 and 2012) with that in 2009, the last year of PCV7 use in Germany. A rate ratio of 1 indicates no effect of the switch from PCV7 to PCV10 and PCV13. Rate ratios less than 1 show a declining incidence of IPD, rate ratios greater than 1 indicate rising incidence. The 95% confidence intervals for the IRRs were cal-culated using the method described by Armitage and Berry [13]. To compensate for chance fluctuations of the incidences in 2009 and 2012, we additionally cal-culated the mean values of the two last years of PCV7 vaccination (2008–09) as reference for the IRR calcula-tion and compared them with the mean values of the last two years of PCV13 vaccination (2011–12).

All analyses were performed separately for meningitis and non-meningitis IPD cases, using SAS 9.2 software.

Results

Impact on vaccine-type incidence of invasive pneumococcal disease

From 2009 to 2012, there was a decrease in the inci-dence of IPD caused by serotypes included in PCV13 in all age groups for non-meningitis IPD, whereas a decrease for meningitis IPD could be observed in one age group (2–4 years) only. The absolute change in incidence was five- to 10-fold higher for non-meningitis IPD than meningitis IPD. The relative decrease ranged between 33% and 91%, depending on age and clinical

Incidence and incidence changes of meningitis and non-meningitis invasive pneumococcal disease in children aged under 16 years caused by serotypes in the 13-valent pneumococcal conjugate vaccine, based on capture–recapture calculation[a], Germany, 2009 and 2012

| Age in years | Diagnosis | Vaccine type serotype incidence or change per 100,000 children | | | |
		Incidence (95% CI) 2009	Incidence (95% CI) 2012	Absolute incidence change (95% CI) 2009 vs 2012	Relative incidence change[b] % (95% CI) 2009 vs 2012
‹2	Meningitis	2.2 (1.3 to 3.1)	1.5 (1.0 to 1.9)	−0.7 (−1.8 to 0.3)	−33 (−62 to 18)
	Non-meningitis	7.3 (4.6 to 10.1)	1.1 (0.6 to 1.7)	**−6.2 (−7.9 to −4.7)**	**−85 (−91 to −74)**
2−4	Meningitis	0.5 (0.3 to 0.7)	0.1 (0.05 to 0.07)	**−0.5 (−0.9 to −0.2)**	**−91 (−99 to −30)**
	Non-meningitis	4.7 (2.8 to 6.7)	2.0 (0.6 to 3.3)	**−2.8 (−4.0 to −1.7)**	**−58 (−71 to −40)**
5−15	Meningitis	0.1 (0.1 to 0.2)	0.07 (0.05 to 0.10)	−0.1 (−0.18 to 0.04)	−50 (−81 to 36)
	Non-meningitis	1.3 (0.5 to 2.0)	0.2 (0.1 to 0.3)	**−1.1 (−1.3 to −0.8)**	**−84 (−90 to −73)**
All ‹16	Meningitis	0.4 (0.3 to 0.6)	0.2 (0.2 to 0.3)	**−0.2 (−0.4 to −0.1)**	**−49 (−69 to −20)**
	Non-meningitis	2.6 (1.9 to 3.3)	0.7 (0.4 to 0.9)	**−2.0 (−2.3 to −1.6)**	**−75 (−80 to −68)**

CI: confidence interval; ESPED: German paediatric surveillance unit; IRR: incidence rate ratio; PneumoWeb: web interface for reporting of cases through laboratory sentinel surveillance.

Two-digits after the decimal point were used when necessary to avoid figures being rounded to 0.

Figures in bold indicate a statistically significant decrease or increase, since the 95% CI does not include 0.

[a] Capture–recapture calculation was based on 231 vaccine-type invasive pneumococcal disease cases observed in two sources (without matches) – hospital (ESPED) and laboratory (PneumoWeb) surveillance – for 2009 and 2012 combined. For cases for whom serotype data were not available, the same serotype distribution as for the serotyped cases was assumed. The serotyping rate was 75%.

[b] Calculated as 1 − IRR, as a percentage.

Incidence and incidence changes of meningitis and non-meningitis invasive pneumococcal disease in children aged under 16 years caused by serotypes not included in the 13-valent pneumococcal conjugate vaccine, based on capture–recapture calculation[a], Germany, 2009 and 2012

| Age in years | Diagnosis | Non-vaccine type serotype incidence or change per 100,000 children | | | |
		Incidence (95% CI) 2009	Incidence (95% CI) 2012	Absolute incidence change (95% CI) 2009 vs 2012	Relative incidence change[b] % (95% CI) 2009 vs 2012
‹2	Meningitis	1.4 (0.9 to 2.0)	2.3 (1.5 to 3.0)	0.8 (−0.2 to 1.9)	59 (−10 to 82)
	Non-meningitis	1.6 (1.0 to 2.2)	5.2 (2.6 to 7.8)	**3.6 (2.2 to 5.1)**	**225 (101 to 428)**
2−4	Meningitis	0.7 (0.4 to 1.0)	0.4 (0.3 to 0.5)	−0.3 (−0.8 to 0.2)	−39 (−74 to 42)
	Non-meningitis	1.8 (1.1 to 2.5)	2.0 (0.6 to 3.3)	0.2 (−0.1 to 0.7)	11 (−73 to 29)
5−15	Meningitis	0.2 (0.1 to 0.3)	0.3 (0.2 to 0.4)	0.04 (−0.1 to 0.2)	17 (−18 to 37)
	Non-meningitis	0.4 (0.2 to 0.7)	0.09 (0.04 to 0.13)	**−0.4 (−0.5 to −0.2)**	**−79 (−91 to −54)**
All ‹16	Meningitis	0.5 (0.3 to 0.6)	0.5 (0.4 to 0.6)	0.1 (−0.1 to 0.3)	14 (−21 to 65)
	Non-meningitis	0.8 (0.6 to 1.0)	1.0 (0.6 to 1.5)	**0.3 (0.01 to 0.5)**	**33 (2 to 74)**

CI: confidence interval; ESPED: German paediatric surveillance unit; IRR: incidence rate ratio; PneumoWeb: web interface for reporting of cases through laboratory sentinel surveillance.

Two-digits after the decimal point were used when necessary to avoid figures being rounded to 0.

Figures in bold indicate a statistically significant decrease or increase, since the 95% CI does not include 0.

[a] Capture–recapture calculation was based on 181 non-vaccine-type invasive pneumococcal disease cases observed in two sources (without matches) – hospital (ESPED) and laboratory (PneumoWeb) surveillance – for 2009 and 2012 combined. For cases for whom serotype data were not available, the same serotype distribution as for the serotyped cases was assumed. The serotyping rate was 75%.

[b] Calculated as 1 − IRR, as a percentage.

TABLE 3

Meningitis invasive pneumococcal disease in children aged under 16 years: reported cases, incidence estimates and rate ratios, Germany, 2009–12

Value	Age group in years	Last year of PCV7 vaccination period	PCV10 and PCV13 vaccination period		
		2009	2010	2011	2012
Number of reported cases in the two surveillance sources: ESPED; Pneumoweb	‹2	24; 15	30; 21	21; 11	29; 16
	2–4	15; 7	16; 10	8; 7	8; 5
	5–15	21; 9	9; 4	12; 10	14; 12
	All‹16	60; 31	55; 35	41; 28	51; 33
Estimated number of cases by CRC (95% CI)	‹2	49.0 (30.0 to 68.0)	55.8 (39.5 to 72.1)	51.8 (23.4 to 80.2)	50.0 (34.2 to 65.8)
	2–4	24.6 (14.2 to 35.0)	30.2 (17.6 to 42.7)	23.0 (7.8 to 38.2)	9.8 (7.4 to 12.2)
	5–15	30.4 (20.6 to 40.3)	24.0 (4.4 to 43.6)	22.8 (14.1 to 31.6)	26.9 (17.3 to 36.4)
	All‹16	104.0 (80.2 to 127.9)	110.0 (81.6 to 138.4)	97.6 (64.3 to 131.0)	86.7 (68.0 to 105.3)
Estimated incidence by CRC per 100,000 children (95% CI)	‹2	3.6 (2.2 to 5.0)	4.1 (2.9 to 5.3)	3.8 (1.7 to 5.9)	3.7 (2.4 to 4.5)
	2–4	1.2 (0.7 to 1.7)	1.5 (0.9 to 2.1)	1.1 (0.4 to 1.9)	0.5 (0.4 to 0.6)
	5–15	0.4 (0.2 to 0.5)	0.3 (0.1 to 0.5)	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)
	All‹16	0.9 (0.7 to 1.1)	0.9 (0.7 to 1.2)	0.8 (0.6 to 1.1)	0.7 (0.6 to 0.9)
IRR[a] (95% CI)	‹2	NA Year of reference	1.14 (0.78 to 1.68)	1.06 (0.72 to 1.57)	1.03 (0.69 to 1.52)
	2–4		1.22 (0.72 to 2.09)	0.93 (0.53 to 1.65)	**0.40 (0.19 to 0.83)**
	5–15		0.80 (0.47 to 1.36)	0.77 (0.44 to 1.32)	0.91 (0.54 to 1.53)
	All‹16		1.06 (0.82 to 1.39)	0.95 (0.72 to 1.26)	0.85 (0.64 to 1.13)

CI: confidence interval; CRC: capture–recapture calculation; ESPED: German paediatric surveillance unit; IRR: incidence rate ratio; NA: not applicable; PCV: pneumococcal conjugate vaccine; PneumoWeb: web interface for reporting of cases through laboratory sentinel surveillance.

[a] With reference to the incidence rate in 2009. IRRs with 95% CIs excluding 1 are shown in bold.

TABLE 4

Non-meningitis invasive pneumococcal disease in children aged under 16 years: reported cases, incidence estimates and rate ratios, Germany, 2009–12

Value	Age group in years	Last year of PCV7 vaccination period	PCV10 and PCV13 vaccination period		
		2009	2010	2011	2012
Number of reported cases in the two surveillance sources: ESPED; Pneumoweb	‹2	42; 33	35; 31	21; 16	24; 23
	2–4	47; 30	22; 19	27; 16	21; 14
	5–15	32; 25	23; 28	21; 29	10; 8
	All‹16	121; 88	80; 78	69; 61	55; 45
Estimated number of cases by CRC (95% CI)	‹2	120.8 (75.6 to 166.1)	127.0 (68.8 to 185.2)	61.3 (29.7 to 93.0)	84.7 (42.3 to 127.1)
	2–4	134.3 (80.3 to 188.2)	75.7 (34.8 to 116.5)	67.0 (35.7 to 98.3)	81.5 (25.5 to 137.5)
	5–15	142.0 (58.0 to 226.0)	115.0 (48.7 to 181.3)	109.0 (46.8 to 171.2)	23.8 (10.9 to 36.6)
	All‹16	397.1 (287.5 to 506.8)	317.7 (220.4 to 414.9)	237.3 (160.9 to 313.8)	190.0 (118.5 to 261.4)
Estimated incidence by CRC per 100,000 children (95% CI)	‹2	8.9 (5.6 to 12.3)	9.4 (5.1 to 13.7)	4.6 (2.2 to 6.9)	6.3 (3.1 to 9.4)
	2–4	6.5 (3.9 to 9.2)	3.7 (1.7 to 5.7)	3.3 (1.7 to 4.8)	4.0 (1.2 to 6.4)
	5–15	1.7 (0.7 to 2.7)	1.4 (0.6 to 2.2)	1.3 (0.6 to 2.1)	0.3 (0.1 to 0.4)
	All‹16	3.4 (2.4 to 4.3)	2.7 (1.9 to 3.5)	2.0 (1.4 to 2.7)	1.6 (1.0 to 2.3)
IRR[a] (95% CI)	‹2	NA Year of reference	1.05 (0.82 to 1.35)	**0.51 (0.37 to 0.69)**	**0.70 (0.53 to 0.93)**
	2–4		**0.56 (0.42 to 0.75)**	**0.50 (0.37 to 0.67)**	**0.61 (0.46 to 0.80)**
	5–15		0.82 (0.64 to 1.05)	0.78 (0.61 to 1.01)	**0.17 (0.11 to 0.27)**
	All‹16		**0.81 (0.70 to 0.93)**	**0.61 (0.52 to 0.71)**	**0.50 (0.42 to 0.60)**

CI: confidence interval; CRC: capture–recapture calculation; ESPED: German paediatric surveillance unit; IRR: incidence rate ratio; NA: not applicable; PCV: pneumococcal conjugate vaccine; PneumoWeb: web interface for reporting of cases through laboratory sentinel surveillance.

[a] With reference to the incidence rate in 2009. IRRs with 95% CIs excluding 1 are shown in bold.

Sensitivity analysis: incidence rate ratios of meningitis and non-meningitis invasive pneumococcal disease in children aged under 16 years (mean of 2011–12 vs mean of 2008–09), based on capture–recapture calculation[a], Germany

Age group in years	IRR: average of 2011–12 vs average of 2008–09 (95% CI)	
	Meningitis IPD	Non-meningitis IPD
< 2	1.06 (0.80 to 1.40)	**0.58 (0.47 to 0.71)**
2–4	**0.56 (0.36 to 0.87)**	**0.76 (0.61 to 0.94)**
5–15	1.16 (0.77 to 1.72)	**0.53 (0.43 to 0.65)**
All < 16	0.95 (0.78 to 1.16)	**0.65 (0.58 to 0.73)**

CI: confidence interval; ESPED: German paediatric surveillance unit; IPD: invasive pneumococcal disease; IRR: incidence rate ratio; PneumoWeb: web interface for reporting of cases through laboratory sentinel surveillance.

95% CIs not including 1 are shown in bold.

[a] Capture–recapture calculation was based on 798 IPD cases observed in two sources (without matches) – hospital (ESPED) and laboratory (PneumoWeb) surveillance – for 2008, 2009, 2011 and 2012 combined.

presentation. The relative incidence decrease was lowest (and not statistically significant) for meningitis IPD in the youngest age group, i.e. under two years (Table 1).

In the under two years age group, a distinction needs to be made between children too young to be vaccinated (≤ 2 months) and those for whom the universal vaccination recommendation applies (3–23 months). In 2009, VT meningitis IPD cases aged ≤ 2 months accounted for 5 of 19 VT cases in children aged under two years compared with 9 of 14 cases in 2012. Among the nine 2012 cases, five were due to serotype 7F. The high proportion of 7F among these cases contrasts with the vaccine serotype distribution in all older age groups (> 2 months), among whom serotype 7F had almost vanished (one case in the age group 3–23 months, one among children aged 2–15 years). The age group ≤ 2 months was the only group in which VT meningitis IPD increased. In children aged 3–23 months, the number of meningitis cases with PCV13 serotypes decreased from 14 cases in 2009 to five in 2012.

Regarding non-meningitis IPD cases, in children aged ≤ 2 months, the number of VT IPD cases decreased from six (2009) to three (2012); in children aged 3–23 months, the number of VT IPD cases fell from 47 to five.

Impact on non-vaccine-type invasive pneumococcal disease incidence

For non-PCV13 vaccine serotypes, an inconsistent pattern was observed. There was an increase in the incidence of NVT serotypes in children aged under two years (for children ≤ 2 months as well as for those aged 3–23 months), which was statistically significant for non-meningitis IPD but not for meningitis IPD. In older

age groups, the incidence of NVT IPD increased in some age groups while decreasing in others. There was a statistically significant decrease of non-meningitis IPD in 5–15 year-old children (Table 2).

Impact on overall (vaccine type and non-vaccine type) incidence of meningitis and non-meningitis invasive pneumococcal disease

The impact of PCV13 on the overall number and incidence of meningitis IPD cases is shown in Table 3. While barely no change or even a slight rise in the number of meningitis IPD cases is shown in the laboratory source PneumoWeb, the hospital data source showed a decrease in the number of these cases among children aged two years or older, but also no decrease in those aged under two years. This was confirmed in the capture–recapture analysis: some decrease in older age groups (two years and above) and virtually no incidence reduction in children aged under two years. The IRRs suggest an incidence reduction, which was significant for the 2–4 years age group only: −60% (95% CI: −81 to −17). For children aged 5–15 years, CRC incidence changed by −9% (95% CI: −46 to 53). For children aged under two years, the change was 3% (95% CI: −31 to 52).

In order to disentangle the changes in children too young to be vaccinated (≤ 2 months) and those for whom the universal vaccination recommendation applies (3–23 months), we report data for each subgroup: for children ≤ 2 months, the incidence of meningitis IPD

Absolute number and percentage of vaccine type serotypes[a] in paediatric (< 16 years) invasive pneumococcal disease cases by diagnosis (meningitis and non-meningitis), Germany, 2009

Age group in years	VT meningitis IPD		VT non-meningitis IPD	
	Number of cases[b]	Percentage of meningitis IPD cases by any serotype (95% CI)	Number of cases[b]	Percentage of non-meningitis IPD cases by any serotype (95% CI)
< 2	30	61 (48 to 75)	99	82 (74 to 90)
2–4	11	43 (28 to 64)	98	73 (64 to 82)
5–15	12	44 (26 to 58)	107	75 (65 to 85)
All < 16	53	49 (40 to 59)	304	77 (72 to 82)

CI: confidence interval; ESPED: German paediatric surveillance unit; IPD: invasive pneumococcal disease; PneumoWeb: web interface for reporting of cases through laboratory sentinel surveillance; VT: vaccine type.

[a] VT serotypes were all serotypes included in the 13-valent pneumococcal vaccine (PCV13), i.e. seven-valent pneumococcal vaccine (PCV7) serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) plus an additional six serotypes (1, 3, 5, 6A, 7F, 19A).

[b] Numbers according to capture–recapture calculation based on 257 invasive pneumococcal disease cases observed in two sources (without matches) – hospital (ESPED) and laboratory (PneumoWeb) surveillance – for 2009. For cases for whom serotype data were not available, the same serotype distribution as for the serotyped cases was assumed. The serotyping rate was 75%.

increased from 1.1 per 100,000 children in 2009 to 1.5 per 100,000 in 2012. In children aged 3–23 months, it was 2.1/100,000 in 2009 and 2.2/100,000 in 2012.

A different pattern was observed for non-meningitis IPD cases (Table 4). The numbers in each data source showed a decrease from 2009 to 2012 (with some fluctuations in 2010 and 2011). This decrease was confirmed in the capture–recapture analysis: a statistically significant reduction in incidence of non-meningitis IPD was observed in all age groups, except for children ≤ 2 months, in whom incidence was 1.4/100,000 in both 2009 and 2012. For children aged under 2 years, the decrease was −30% (95% CI: −46 to −7). For older children aged 2–4 and 5–15 years, the decrease was −39% (95% CI: −54 to −20) and −83% (95% CI: −89 to −73), respectively.

In order to confirm that the observed results did not reflect an artefact resulting from the use of CRC, we also assessed the changes in case numbers and incidence rates between 2009 and 2012 solely based on the observed number of cases reported in both surveillance sources (counting cases observed in both sources only once): the changes (IRRs) related to the introduction of PCV10 and PCV13 were almost identical to those based on CRCs.

We also calculated CRC-based IRRs for the two-year periods 2008–09 and 2011–12 (Table 5), which yielded very similar results to our comparison of 2012 with 2009. Only the decrease of non-meningitis IPD in 5–15 year-old children (1 − IRR = −46% (95% CI: −57 to −35) was outside the 95% CIs of the corresponding result in Table 4 (1 − IRR: −83% (95% CI: −89 to −73) and thus significantly and substantially smaller in the analysis which compared 2008-09 to 2011-12, reflecting the sharp decrease in incidence in this age group in 2012.

The percentages of VT serotypes in IPD cases among children aged under 16 years in 2009 is shown in Table 6. This percentage was smaller for meningitis IPD than for non-meningitis IPD in all age groups. The VT serotypes in 2009 consisted mainly (84%, 300/357) of the six serotypes (1, 3, 5, 6A, 7F, 19A) that were not contained in PCV7.

Discussion

For non-meningitis IPD, the introduction of PCV10 and PCV13 prompted a marked decrease in the incidence of IPD cases caused by PCV13 serotypes. This was not confined to the PCV13-vaccinated cohorts aged under two years but was almost as pronounced in those older than two years. An increasing incidence of IPD cases caused by NVT serotypes was solely observed in children under two years-old. This NVT increase, however, was smaller than the VT decrease, so that an overall decrease of non-meningitis IPD (regardless of serotype) was observed in all age groups.

In contrast, for meningitis IPD, we did not observe a decrease in the VT incidence in children aged under two years, nor in the oldest age group (5–15 years). Therefore, the overall incidence decrease (regardless of serotype) was limited to 2–4 year-old children.

The clear decrease of PCV13 serotypes in non-meningitis IPD in children under two years-old reflects the high effectiveness of PCV13 against the six additional serotypes (1, 3, 5, 6A, 7F and 19A) not contained in PCV7, as demonstrated by Miller et al. [4]. The additional effect in older age groups not vaccinated with PCV10 or PCV13 indicates herd protection.

In accordance with the previously reported increase in non-PCV7 serotypes after the introduction of PCV7 [3], we observed an increase of non-PCV13 serotypes three years after the introduction of PCV13. In our data, this early increase was confined to the vaccinated cohorts aged under two years, where it was substantial in non-meningitis IPD, but did not reach statistical significance in meningitis IPD. From a clinical and public health perspective, the overall net impact on IPD incidence (regardless of VT or NVT serotype) is most relevant. While there was an overall decrease of non-meningitis IPD incidence in all age groups, the decrease of meningitis IPD incidence was confined to children in the age group 2–4 years. At first glance, this differential impact is surprising. A likely explanation is the lower proportion of PCV13 serotypes among meningitis IPD cases (49%; 95% CI: 40 to 59) than among non-meningitis IPD cases (77%; 95% CI: 72 to 82) in 2009 when PCV13 was introduced in Germany, meaning that a larger proportion of non-meningitis IPD was preventable by PCV13. The clear decrease in the non-meningitis IPD incidence (regardless of serotype) might theoretically be due to less frequent blood culturing. However, previous findings rather suggested increasing blood culturing rates since the introduction of infant pneumococcal vaccination in Germany [2].

Although chance might be another explanation, given the wide 95% CIs of the IRRs, biological explanations related to the tropism of different pneumococcal serotypes appear more likely: German data collected in 1997−98 (during the pre-vaccination period) showed an about equal incidence of meningitis and non-meningitis IPD for serotypes 6A, 7F and 19A, whereas the incidence of serotypes 1, 3 and 5 was significantly higher in non-meningitis than in meningitis IPD [6]. A similar pattern was observed after the introduction of PCV in 2007 (data not shown). A literature review published in 2013, with observations from other countries, addressed the issue of tropism of different pneumococcal serotypes: serotypes 1, 19A and 3 were identified as predominant for pneumococcal pneumonia but not for meningitis IPD during the post-PCV7 period [14]. A recent paper from India, not included in the review, reported that serotypes 1, 5 and 7F (three of the six additional serotypes in PCV13) caused more pneumonia than meningitis [15].

For children aged under two years, the decreased incidence of meningitis IPD was not only less than that of non-meningitis IPD in this age group, but no decrease was observed at all. One explanation might be an increase in the number of cases caused by NVT serotypes. However, this increase was not statistically significant. Notable was an increase in the number of cases caused by VT serotypes in children ≤ 2 months-old, in particular serotype 7F. Children ≤ 2 months of age are too young to be vaccinated, according to the German vaccination schedule, and can thus only indirectly benefit from vaccination through herd protection. The reason for the absence of herd protection in this age group in 2012 is unclear.

There are limited data on the impact of PCV13 on the incidence of IPD in previously PCV7-vaccinated populations and distinctions between non-meningitis and meningitis IPD are only made in one study: for England and Wales, where PCV13 replaced PCV7 in April 2010, Miller et al. reported a 50% reduction in the number of cases caused by PCV13 serotypes (for meningitis and non-meningitis IPD combined) in children aged under two years after one year of PCV13 use [4]. In a multicentre study from eight hospitals in the United States (where PCV13 was used as of March 2010), IPD cases per total admissions were analysed, comparing the mean of 2007–09 with the number of cases in 2011. A 57% decrease in PCV13 serotypes and a 42% reduction in IPD cases per total admissions for children aged under five years was reported. Meningitis cases were reduced the least [16]. Data from Denmark, where PCV13 was introduced in April 2010, show a decrease of IPD incidence (regardless of clinical entity) by about a third in children under two years-old [17].

A strength of our study is the separate analysis of meningitis and non-meningitis IPD cases. Differences in incidence of meningitis and non-meningitis IPD show the importance of separate data analysis according to clinical entity. The high proportion of meningitis among incident IPD in children aged under 16 years in Germany (26% (628/2,394) for 2007–12) was similar to the proportion in Denmark (20%) [18], but much higher than the proportion in the United Kingdom (6%) [19]. For countries with high blood culturing rates, and therefore a high proportion of non-meningitis IPD, aggregate estimates of PCV impact on IPD may mask a differential effect on meningitis IPD. Meningitis IPD, however, has a higher clinical relevance and public health impact because case fatality and sequelae rates are about fourfold higher than for non-meningitis IPD [6].

Separate analysis (and publication) of meningitis and non-meningitis IPD cases is also useful for comparison of international data, since meningitis IPD incidence is less likely to be affected by ascertainment bias. This is demonstrated by similar incidence rates in different countries [20], while non-meningitis IPD incidence is dependent on blood culturing practices for children

with fever – practices that differ between countries and can change over time [21].

Another strength of our data is the use of two independent data sources, which allows us to correct for underreporting by CRC.

A potential limitation is the comparison of two one-year periods (2009 and 2012, three years after the introduction of PCV7 and PCV13, respectively). We chose these time periods to include two cohorts of children under two years-old, who could have been fully vaccinated with either PCV7 or PCV13. To reduce random variability due to small numbers when comparing two one-year periods only, we also compared 2008–09 with 2011–12. This yielded almost identical results, rendering chance a less likely explanation of our findings.

A limitiation of our data is the confinement of our analysis to children aged under 16 years.

It might also be argued that the strength of the effect of higher-valent pneumococcal vaccines might be underestimated as PCV10 was already introduced in Germany in April 2009 and PCV13 in December 2009. Both higher-valent vaccines were used, when PCV7 was still on the market. The market shares of the higher-valent vaccines in 2009, however, were small (PCV10: 21%; PCV13: 7%) and PCV7 was the predominantly used vaccine during 2009.

An overestimation of the impact of PCV13 is possible as well because of a potential increase in IPD incidence due to the influenza A(H1N1)pdm09 pandemic in 2009, which had reached its peak during calendar weeks 45–48 of 2009 [2,22,23]. Again, our two-year period comparison (2008–09 vs 2011–12) did not suggest bias due to picking 2009 as the base year for comparisons.

In a commentary, Katharine O'Brian described pneumococcal disease impact evaluations as a 'messy affair' and called for 'epidemiologic rigor' [24]. The heterogeneous findings in our study, once again, point to the importance of sustained surveillance of IPD and the need for thorough and detailed analysis disentangling the vaccine impact on different clinical entities.

Conflict of interest

Authors' contributions

Data administration (ESPED), data analysis, interpretation of data, drafting of the manuscript: Susanne Weiss.
Data administration (PneumoWeb), interpretation of data, drafting of the manuscript: Gerhard Falkenhorst.
Interpretation of data, drafting manuscript: Rüdiger von Kries.
Drafting manuscript and serotyping: Mark van der Linden; Matthias Imöhl.

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WHO publishes first guidelines for hepatitis B treatment

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The World Health Organization (WHO) issued today its first hepatitis B treatment guidelines: 'Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection', laying out a simplified approach to the care and treatment of persons living with chronic hepatitis B infection [1].

These guidelines are intended for country programme managers to help plan the development and scale up of hepatitis B prevention, care and treatment and also for healthcare providers who care for persons infected with hepatitis B virus (HBV).

Key recommendations include:
- the use of simple non-invasive tests to assess the stage of liver disease;
- prioritising treatment for those with most advanced stage of liver disease;
- the use of safe and effective medicines for treatment;
- regular monitoring using simple tests for early detection of liver cancer, to assess whether treatment is working, and if treatment can be stopped.

The special needs of specific populations, such as people co-infected with HIV, children and adolescents, and pregnant women are also considered.

Existing recommendations for the prevention of HBV transmission from relevant WHO guidelines are also summarised in the newly published guidelines and include prevention of perinatal and early childhood HBV infection through infant hepatitis B vaccination; catch-up vaccination and other prevention strategies in key affected populations (people who inject drugs, men who have sex with men, sex workers) as well as prevention of transmission in healthcare settings.

Hepatitis B infection is caused by the HBV, an enveloped DNA virus that infects the liver, causing hepatocellular necrosis and inflammation. Chronic hepatitis B – defined as persistence of hepatitis B surface antigen (HBsAg) for six months or more – is a major public health problem.

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ESCAIDE 2015 – save the date

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The European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) will take place in Stockholm, Sweden, between 11 and 13 November 2015.

The Early-Bird discounted registration will be possible between 30 March and 16 August and the abstracts can be submitted between 30 March and 11 May.

The call for 'late breaker' abstracts will be open from 1 to 30 September and the online registration will close on 1 November.

As in previous years, it is anticipated that the conference will be accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide CME credits for participants. In 2014, ESCAIDE was designated for a maximum of 18 hours of European external CME credits.

For further information about the conference, visit **www.escaide.eu** or contact **escaide.conference@ecdc.europa.eu**.