Detection of influenza A(H3N2) virus in children with suspected mumps during winter 2014/15 in England

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Influenza A(H3N2) virus was detected in oral fluid from 16/107 children (aged 2 to 12 years) with a clinical diagnosis of mumps, who were sampled between December 2014 and February 2015 in England, during the peak of the 2014/15 influenza season. Sequence analysis of an A(H3N2) virus from a child with suspected mumps showed the virus was similar to other circulating A(H3N2) viruses detected in winter 2014/15, which were antigenically drifted from the A(H3N2) vaccine strain.

During winter 2014/15, clinical parotitis in children with confirmed influenza A(H3) infection was reported in North America [1]. In contrast, however, neither clinical nor virological surveillance for influenza-like illness (ILI) through sentinel general practitioners (GPs) in England detected an association between parotitis and influenza virus infection in the 2014/15 winter. In light of the observations from North America, gingival crevicular fluid (oral fluids) submitted for mumps surveillance in England were examined for influenza virus. Analysis of samples from 107 children (aged 2 to 12 years) with a clinical diagnosis of mumps but negative for mumps virus, sampled between December 2014 and February 2015, during the peak of the 2014/15 influenza season, showed that 16 (15%) were infected with influenza A(H3N2) virus.

Testing oral fluid samples from mumps surveillance for the presence of respiratory viruses

Of 116 residual oral fluids tested for mumps virus, a panel of 107 samples that were mumps virus negative was selected for testing for respiratory viruses based on age (2–12 years), sample taken between 1 and 5 days post onset of clinical parotitis between December 2014 and February 2015. Anonymised specimens were screened for respiratory viruses including influenza A(H1N1)pdm09 and A(H3N2), influenza B, respiratory syncytial virus A and B (RSV-A and RSV-B) and human metapneumovirus A and B (hMPV-A and hMPV-B) by real-time reverse transcription (RT) PCR using previously described assays [2-4] (Table 1). A total of 16 (15%) oral fluids tested were positive for influenza A(H3N2) virus in the mumps virus negative cohort (Table 2). One sample was found to contain a coinfection of influenza A(H3N2) virus and RSV-A.

As a control group, nine mumps virus positive oral fluids were screened using the same protocols and no respiratory viruses were detected. A further 63 control oral fluids were selected from the same age group (2–12 years) presenting with a different syndrome (suspected measles): only two (3%) of the measles virus positive samples were found to be positive for influenza A(H3N2) virus (Table 2). Two further separate coinfections of measles virus with RSV-A and hMPV-A were found.

Phylogenetic analysis of isolated influenza A(H3N2) virus haemagglutinin gene

Nucleotide sequencing of the haemagglutinin (HA) and neuraminidase (NA) genes of A(H3N2) viruses from influenza virus positive oral fluid samples by PCR (cycle threshold (Ct) values ‹ 33 (4/16) was undertaken (primer sequences available on request). Full-length HA and NA sequence was obtained from one oral fluid, Sample 69 (A/England/Sample69/2015), and an HA phylogenetic tree constructed as described previously [5] (Figure), using HA sequences from A(H3N2) viruses isolated in the 2014/15 influenza season in the United Kingdom, selected to be representative of the range of patient's age (2–12 years) and date of sample collection (between December 2014 and February 2015). Other reference virus sequences were obtained from the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database (Table 3).

Analysis of the HA gene from A/England/Sample69/2015 showed that it fell into the A(H3N2) HA 3C.2a clade, as did the majority of A(H3N2) influenza viruses genetically characterised in the United Kingdom (UK) in 2014/15 (Figure) [6,7]. Similarly, phylogenetic analysis of the NA gene from A/England/Sample69/2015

Table 1

Oral fluid samples from mumps and measles surveillance selected for respiratory virus study, England, December 2014–February 2015 (n = 179)

Characteristic	Mumps surveillance		Measles surveillance
	Mumps virus negative	Mumps virus positive	Measles virus positive
Number of samples	107	9	63
Date sample taken	2 Dec 2014 to 26 Feb 2015	13 Jan 2014 to 9 Feb 2015	5 Dec 2014 to 5 Mar 2015
Number of days symptomatic before sampling (median)	1–5 (4)	2–10 (4)[a]	1–5 (4)
Patient age in years (median)	2–12 (6)	2–12 (8)	2–12 (4)

[a] Number of days symptomatic before sampling unknown for one sample.

Table 2

Detection of respiratory viruses in oral fluid samples from children with suspected mumps or measles, England, December 2014–February 2015 (n = 179)

Respiratory virus target	Mumps surveillance		Measles surveillance
	Mumps virus negative n = 107	Mumps virus positive n = 9	Measles positive n = 63
	Number of positive samples (%)	Number of positive samples	Number of positive samples (%)
Influenza A(H1N1)pdm09	0	0	0
Influenza A(H3N2)	16 (15%)	0	2 (3%)
Influenza B	0	0	0
Respiratory syncytial virus A	1 (1%)	0	1 (2%)
Respiratory syncytial virus B	0	0	0
Human metapneumovirus A	0	0	1 (2%)
Human metapneumovirus B	0	0	0

showed that it clustered with other 3C.2a clade viruses from 2014/15 (data not shown). Viruses from this clade are typically antigenically drifted from the 2014/15 northern hemisphere vaccine virus A/Texas/50/2102 and are more similar to A/Switzerland/9715293/2013, the A(H3N2) virus selected for the 2015/16 northern hemisphere influenza vaccine [8].

Discussion

Mumps is a notifiable disease in the UK, which is characterised by bilateral or unilateral inflammation of the parotid salivary glands. Mumps virus is the most common viral agent associated with parotitis, although other viral aetiologies have been investigated [9-11]. Surveillance for mumps virus infection is carried out widely in primary care through oral fluid testing of suspect cases of parotitis [12]. All oral fluid samples from throughout England are sent to the Public Health England (PHE) Virus Reference Department, Colindale, for testing. Recent infection is confirmed by detection of mumps virus-specific IgM antibodies and/or by detection of viral RNA by RT-PCR. Laboratory-confirmed positive cases are followed up to determine vaccination status.

An association between acute parotitis and influenza A virus has only rarely been made during previous epidemic periods in North America [13,14] and influenza is not often considered as part of differential diagnosis for this syndrome, and as a consequence oral fluid is not tested in England for the presence of influenza viruses [9,15].

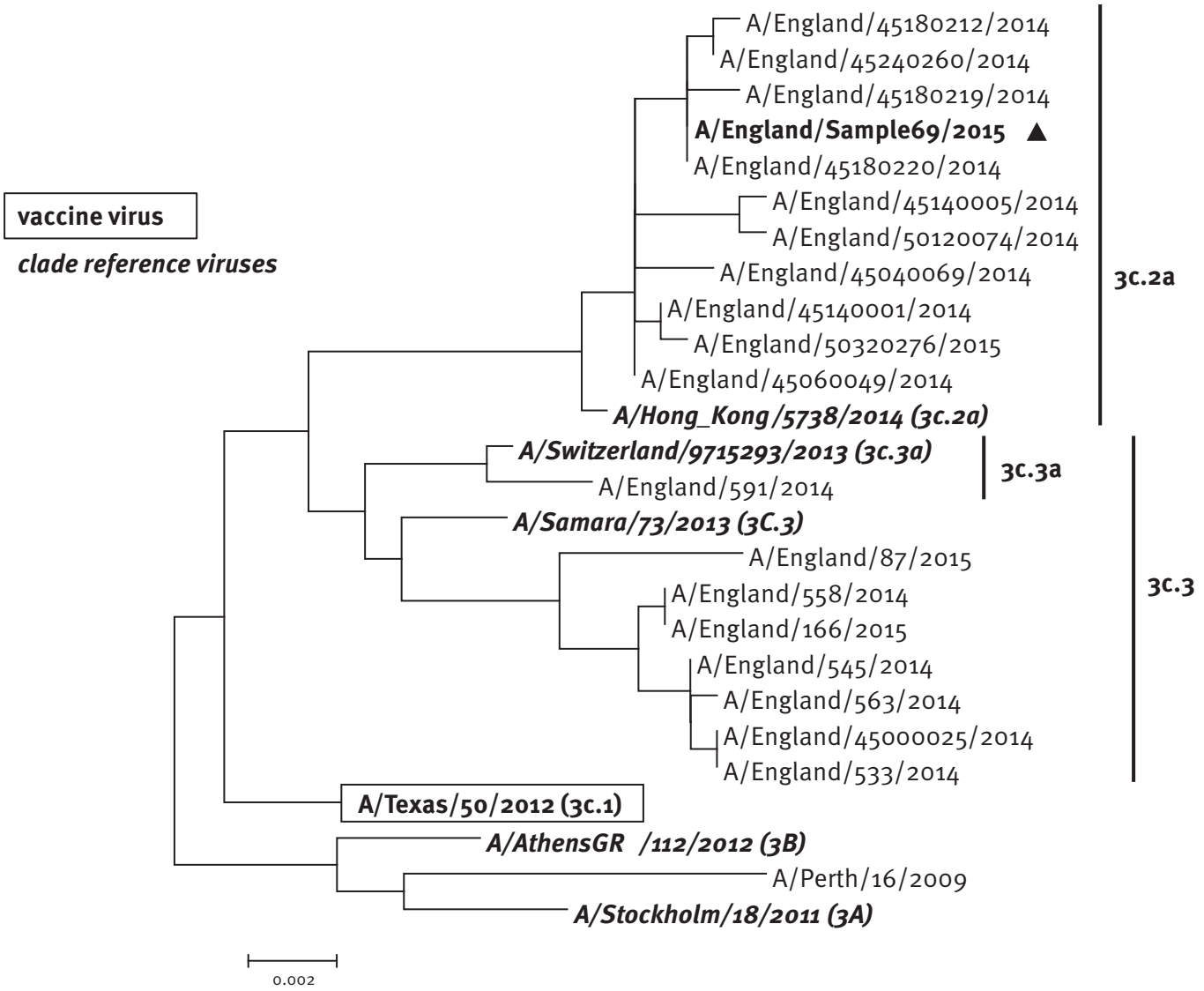
In the presence of a well-performing measles-mumps-rubella vaccination and surveillance programme in England, mumps infection is rarely confirmed in children aged under 12 years, with less than 10% of samples submitted from this age group being positive. During the last quarter of 2014, only 2% of samples submitted to the PHE Virus Reference Department were positive.

Systematic surveillance for influenza-associated parotitis using optimised specimen types, sensitive RT-PCR technology and appropriately timed studies during periods of heightened influenza virus circulation has, to the best of our knowledge, not hitherto been performed. This study, using oral fluid, provides evidence that a proportion of children presenting with suspected mumps during winter 2014/15 had influenza A(H3N2) virus infections. Mumps virus negative oral fluids were tested as a proxy for sampling for parotitis due to non-mumps aetiology. Although the oral fluids were not specifically collected for respiratory virus detection, the study selection criteria included only those that were taken within five days of symptom onset, to optimise detection of respiratory viruses during the period of greatest virus shedding [16]. Furthermore, analysis of oral fluid samples that were from patients without parotitis (i.e. measles virus positive) also found evidence of coinfections with respiratory viruses including influenza A(H3N2), RSV-A and hMPV-A.

Influenza A(H3N2) was the predominant virus circulating during the 2014/15 influenza season in the UK [6]. Genetic characterisation of an A(H3N2) virus

Figure

Phylogenetic analysis of influenza A(H3N2) virus haemagglutinin gene sequence from an oral fluid sample from a child with suspected mumps, England, December 2014–February 2015



The haemagglutinin gene sequence from the child's sample (A/England/Sample69/2015, marked with ▲) was analysed with HA sequences from A(H3N2) viruses isolated in the 2014/15 influenza season in the United Kingdom, selected to be representative of the range of patient's age (2–12 years) and date of sample collection (between December 2014 and February 2015). These representative gene sequences were deposited in the EpiFlu sequence database of the Global Initiative on Sharing All Influenza Data (GISAID) (accession numbers provided in Table 3). Other reference virus sequences were obtained from the GISAID EpiFlu database (Table 3). The phylogenetic tree was constructed with a neighbour-joining algorithm available in the Mega 6 software [5,24]. Branch lengths are drawn to scale.

from a child with parotitis found the viral HA and NA sequences were from the 3C.2a genetic subgroup, which is antigenically distinguishable from the vaccine strain, and similar to other A(H3N2) viruses detected through ILI surveillance [7]. Previous studies have found very limited sporadic evidence of seasonal influenza A(H3N2) or zoonotic virus infection with swine influenza A(H3N2) in children with parotitis [14,17] or occasionally in adults [18]. Former seasonal influenza A(H1N1) or A(H1N1)pdm09 viruses in patients with parotitis has not been described, to the best of our knowledge, although influenza A subtyping has not always been performed [9,15].

Information on influenza vaccination status in our study population was not collected so it is not possible to determine whether this could have affected the positivity rate for detection of influenza virus. The recent introduction of routine live-attenuated influenza vaccine (LAIV) for children aged 2–4 years in the UK may change the influenza positivity rate compared with that in an unvaccinated population [19] and further work to determine LAIV vaccine effectiveness against parotitis could be considered in future seasons.

Influenza virus as an atypical cause of acute viral parotitis should be considered especially during epidemic

Table 3

Origin of influenza A(H3N2) virus haemagglutinin sequences used in the phylogenetic analysis

Segment ID	Segment	Country	Collection date	Virus isolate name	Originating laboratory	Submitting laboratory
EPI539806	HA	China	2014-Apr-30	A/Hong Kong/5738/2014	Government Virus Unit, Hong Kong (SAR)	National Institute for Medical Research, London, UK
EPI530687	HA	Switzerland	2013-Dec-06	A/Switzerland/9715293/2013	Hopital Cantonal Universitaire de Geneves, Switzerland	National Institute for Medical Research, London, UK
EPI460558	HA	Russian Federation	2013-Mar-12	A/Samara/73/2013	WHO National Influenza Centre, Saint Petersburg, Russian Federation	National Institute for Medical Research, London, UK
EPI391247	HA	United States	2012-Apr-15	A/Texas/50/2012	Texas Department of State Health Services, Austin, US	Centers for Disease Control and Prevention, Atlanta, US
EPI358885	HA	Greece	2012-Feb-01	A/AthensGR/112/2012	Hellenic Pasteur Institute, Athens, Greece	National Institute for Medical Research, London, UK
EPI326139	HA	Sweden	2011-Mar-28	A/Stockholm/18/2011	Swedish Institute for Infectious Disease Control, Solna, Sweden	National Institute for Medical Research, London, UK
EPI211334	HA	Australia/ Western Australia	2009-unknown	A/Perth/16/2009	WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia	Centers for Disease Control and Prevention, Atlanta, US
EPI611914	HA	England, UK	2014-Dec-23	A/England/50120074/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611874	HA	England, UK	2014-Dec-24	A/England/563/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611826	HA	England, UK	2014-Dec-22	A/England/45240260/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611810	HA	England, UK	2014-Dec-23	A/England/558/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611714	HA	England, UK	2014-Dec-16	A/England/545/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611698	HA	England, UK	2014-Dec-17	A/England/45180220/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611690	HA	England, UK	2014-Dec-17	A/England/45180219/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611674	HA	England, UK	2014-Dec-16	A/England/45180212/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611626	HA	England, UK	2014-Dec-15	A/England/45140005/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611618	HA	England, UK	2014-Dec-16	A/England/45140001/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611528	HA	England, UK	2014-Dec-09	A/England/45060049/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611520	HA	England, UK	2014-Dec-09	A/England/45040069/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611496	HA	England, UK	2014-Dec-01	A/England/533/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI611488	HA	England, UK	2014-Dec-01	A/England/45000025/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI608041	HA	England, UK	2015-Feb-12	A/England/166/2015	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI607881	HA	England, UK	2015-Jan-22	A/England/87/2015	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI607785	HA	England, UK	2015-Jan-12	A/England/50320276/2015	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI607601	HA	England, UK	2014-Dec-23	A/England/591/2014	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale
EPI612846	HA	England, UK	NA	A/England/Sample69/2015	Public Health England, Reference Microbiology - Colindale	Public Health England, Reference Microbiology - Colindale

HA: haemagglutinin; NA: not available; SAR: Special Administrative Region; UK: United Kingdom; US: United States.

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seasons with drifted A(H3N2) strains, such as in 2014/15, or when zoonotic exposure has occurred.

Influenza A(H3N2) viruses from the 3C.2a clade that have predominantly circulated in the UK during winter 2014/15 show reduced agglutination of red blood cells used in laboratory tests suggestive of a change in binding specificity or avidity for sialic acid receptors [20]. Both mumps and influenza viruses bind sialic acid receptors on cells in the upper respiratory tract [21]. The unusual clinical presentation of parotitis during the 2014/15 season in conjunction with a change in virus receptor binding properties warrants further investigation.

In England, oral fluid sampling is routine for suspected mumps. Young adults (between 15 and 30 years) are the expected demographic for true mumps infection [22,23]. The target population for our study was younger children, where non-mumps parotitis is likely to be more common than true mumps infection.

Oral fluid sampling for detection of influenza virus should be further explored with appropriately timed studies in different age groups. Systematic collection of oral fluids alongside conventional nose/throat swabs during sentinel ILI surveillance would be a useful source of oral fluids for validation of detection of respiratory viruses. This would be a mechanism both for exploring less invasive sampling for influenza virus infection and also investigation of the incidence of influenza virus infection in uncommon clinical presentations such as parotitis. Oral fluids are not typically used as clinical samples for the detection of influenza and their utility for conducting influenza virological surveillance remains uncertain. The specific detection of influenza A(H3N2), and not influenza B or A(H1N1) pdm09 in this sample set, in combination with the clinical syndrome reporting from North America, suggests that influenza should be considered as part of the differential diagnosis for parotitis at the time when influenza virus is circulating, but this conclusion requires evaluation with different circulating influenza virus strains. The question of whether 3C.2a A(H3N2) influenza virus strains have an unusual tissue distribution compared with other A(H3N2) viruses or whether a subset of children infected with any influenza strain experience parotitis remains to be determined. Our conclusions would be strengthened by evaluation of further studies during future influenza seasons where oral fluids are taken in parallel with nasal swabs from children with influenza, with and without parotitis. Nevertheless, greater awareness of influenza virus as a potential cause of parotitis especially during epidemic periods associated with a drifted A(H3N2) strain is an important clinical and public health message.

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Conflict of interest

None declared.

Authors' contributions

CT was involved in acquisition and analysis of laboratory data, and wrote the manuscript. JE was involved in acquisition and analysis of laboratory data, and reviewed the manuscript. MG provided phylogenetic analysis, and reviewed the manuscript. MR reviewed the manuscript. KB was involved in study design, analysis of surveillance data, and reviewed the manuscript. MZ initiated the study, was involved in study design, and revision of the manuscript. All authors read and approved the final manuscript.

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