

# Burden of influenza B virus infections in Scotland in 2012/13 and epidemiological investigations between 2000 and 2012

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We describe the burden of influenza B infections in Scotland during a 13-year study period. Influenza A and B viruses cocirculated throughout the period, with numbers of influenza B cases approaching or exceeding those of influenza A during six influenza seasons. Influenza B viruses of both Victoria and Yamagata lineage were detected in two of six seasons investigated. For the 2012/13 season, influenza B accounted for 44.4% of all influenzas, with the highest incidence in those under the age of five years. Influenza B virus infections led to fewer admissions to an intensive care unit (ICU) and a lower mortality rate than influenza A (37 vs 81 ICU admissions and three vs 29 deaths) during the 2012/13 season. However, a quarter of those admitted to ICU with influenza B had not been immunised and 60% had not received specific influenza antiviral therapy. This highlights the need for consistent influenza vaccination and prompt usage of antiviral treatment for identified risk groups. Combining the newly introduced vaccination programme for children with the use of a tetravalent vaccine may provide the opportunity to improve the control of influenza B in those with the highest influenza B burden, children and young adolescents.

## Introduction

Both influenza A and B viruses show antigenic variation with sequence variability in the haemagglutinin (H) and neuraminidase (N) genes, altering neutralising properties and protection from reinfection [1]. However, they differ in the dynamics of turnover of antigenic variants and in their propensity for reassortment [2]. Variants of influenza A viruses are classified into subtypes based on the H and N genes; two subtypes (H1N1 and H3N2) are currently found in humans in Europe. Although influenza B viruses are not formally classified into subtypes, two antigenically distinct lineages defined

by the reference strains B/Victoria/2/87 (Victoria lineage) and B/Yamagata/16/88 (Yamagata lineage) have been circulating globally since 1983 [3]. Influenza B viruses of the Victoria and Yamagata lineages have been shown to circulate simultaneously or individually in the past and switching between lineages occurs almost every other year [2]. In contrast, antigenic shift of influenza A, characterised by changes in the circulating H and N types occurs less frequently. Because several influenza variants are present in the same season, influenza vaccines currently in use in the United Kingdom (UK) and elsewhere in Europe are trivalent, containing two influenza A subtypes (H1N1 and H3N2) and one influenza B lineage. However, it provides only limited immunity against the influenza B strains of the other lineage [4].

In order to improve our understanding of the burden and epidemiology of influenza B, we have reviewed the influenza B viruses circulating in Scotland over the period 2000 to 2013 and estimated the clinical burden of influenza B virus infections in more detail for the season 2012/13.

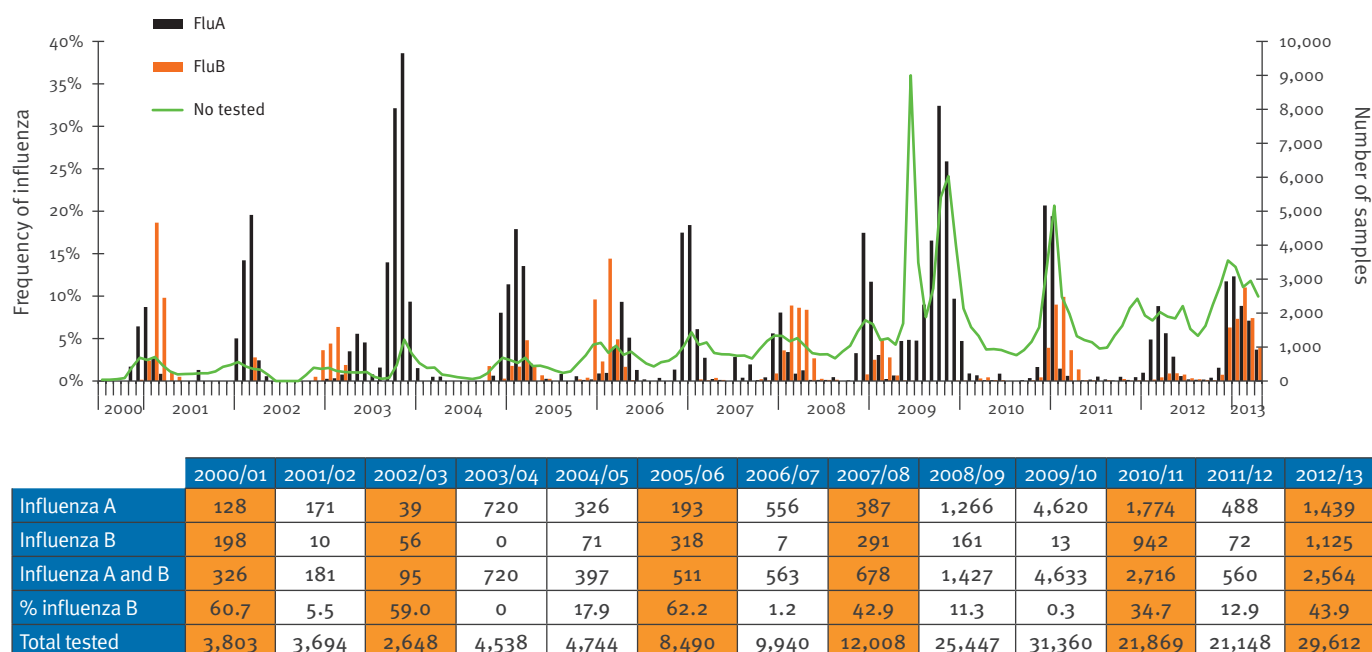
## Methods

### Routine laboratory testing

In Scotland, diagnostic testing by reverse transcription-polymerase chain reaction (RT-PCR) for influenza B virus forms part of multiplex real-time screening for a range of respiratory viruses. PCR-based influenza B virus screening has been carried out in National Health Service (NHS) laboratories in Glasgow since early 2000 [5], in Edinburgh since 2005 [6], in Dundee since 2007 and in Aberdeen since 2008. Thus Figure 1 from 2000 to 2005 is based on Glasgow results only. In addition to individuals presenting to hospital with presumed

**FIGURE 1**

Frequency of influenza A and B virus infections compared with total number of samples tested, Scotland, November 2012–April 2013



Years when more than 30% of all influenza infections were caused by influenza B virus are shown in orange.

respiratory virus infection, a sample of those presenting in the community is tested for respiratory viruses including influenza B virus via the Scottish sentinel surveillance scheme [7].

### Prospective data collection

All reports of influenza B virus detection from NHS laboratories in Scotland are collated centrally by the national public health body Health Protection Scotland (HPS) via the Electronic Communication of Surveillance in Scotland (ECOSS) non-mandatory reporting system. The population of Scotland was estimated to be 5,295,400 in 2011 [8].

General practitioners (GP) participating in the Scottish sentinel surveillance scheme are requested to systematically submit nose and throat swabs from the first five patients per week consulting with influenza-like illness or acute respiratory tract infection (based on clinical judgement) [7]. Supplementary data on demography, clinical signs and risk group as well as vaccination status are collected for each patient. Since season 2000/01 between 18 and 89 GPs per season have participated in the scheme, submitting between 600 and 5,000 samples per season.

Furthermore, the Scottish Severe Acute Respiratory Illness (SARI) surveillance system collects individual-level data on confirmed influenza cases admitted to an intensive care unit (ICU) during the influenza season

from all 30 hospitals with intensive care provision across the 14 NHS boards.

### Retrospective data collection

All reports of influenza B virus detections submitted to HPS via ECOSS between 1 November 2012 and 31 April 2013 were obtained electronically and analysed in this study. Where multiple samples collected from the same patient tested positive for influenza B within an eight week period, only the first influenza B detection was included. Data were anonymised by HPS before extraction for analysis by month and year of reporting, age group and source of referral (GP/hospital). Since the ECOSS records do not include a denominator of total number of tests performed, additional data were collected on the numbers of respiratory samples referred for influenza B testing in Glasgow (January 2000–May 2013), Edinburgh (October 2005–May 2013), Aberdeen (November 2012–May 2013) and Dundee (November 2012–May 2013). Furthermore, all cases of severe influenza B admitted to ICU in Scotland between November 2012 and April 2013 were identified from the SARI surveillance system. For each case, anonymised data on age group, sex, date of onset, outcome, vaccination status, antiviral treatment and specific clinical risk group including underlying illnesses were obtained.

Estimates of the incidence of infection in different age groups were calculated for central and southern Scotland based on the most recent mid-year population

estimate of 4,252,000 including 235,200 children under the age of five years [8].

## Sequencing

Nucleotide sequences of the influenza B haemagglutinin gene were obtained for each influenza season from a representative subset of archived nucleic acid samples derived from anonymised respiratory samples from patients with confirmed influenza B (collected since 2007). The haemagglutinin gene was amplified using hemi-nested primers (sense: TACTACTCATGGTAGTAACATCC; outer antisense: TTTCGTTTGSAGTTCATCCATSGC; inner antisense: AATCATK CCTTCCCAKCKCCT). Nucleotide sequences were aligned with reference sequences using SSE v1.1 [9] and phylogenetic analysis was performed using Mega 5.1 [10]. In addition, influenza B virus-positive samples tested in Glasgow in 2012/13 were typed using lineage-specific real-time PCR [11], and sequences from 39 previously characterised influenza B virus strains from England were obtained from the database of the Global Initiative on Sharing All Influenza Data (GISAID; <http://platform.gisaid.org/epi3/frontend#48fe7c>). The authors gratefully acknowledge the originating and submitting laboratories who contributed these sequences to GISAID, in particular Public Health England (formerly Health Protection Agency) and the National Institute for Medical Research in the UK (n=37 sequences) and the National Institute for Medical Research and the Centers for Disease Control and Prevention in the United States (US) (n=2 sequences).

## Results

### Epidemiology of influenza B virus during 2000 to 2013

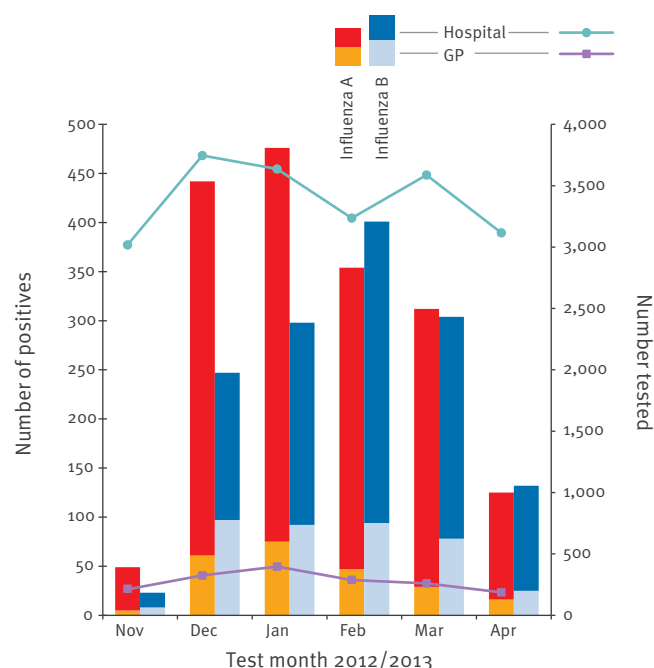
During the period January 2000 to April 2013, several distinct waves of influenza A and B viruses infections were observed in Scotland (Figure 1). Influenza A and B viruses cocirculated during most influenza seasons, with numbers of influenza B infections approaching or exceeding those of influenza A virus during six seasons (2000/01, 2002/03, 2005/06, 2007/08, 2010/11 and 2012/13). The number of samples tested for influenza viruses by PCR in Scotland increased by a factor of 7 over this period from an average of 3,381 per season in 2000 to 2003 (all Scottish samples were tested in Glasgow) to an average of 24,209 per season in 2010 to 2013 (testing done in all four centres).

### Burden of influenza B infections in the 2012/13 season

More detailed information on influenza virus incidence and clinical outcomes was available for the season 2012/13. A total of 22,015 respiratory samples were tested in Scotland for influenza A and B viruses between November 2012 and April 2013. The overall detection frequency of influenza viruses was 14.4%, varying from 2.3% in November to 20.8% in February. Influenza B virus infections accounted for 44.4% of all

**FIGURE 2**

Detection frequency of influenza A and B virus infections and total number of samples submitted for testing by general practitioners and hospitals, Scotland, November 2012–April 2013 (n=22,015)



Virology testing in Scotland is performed in four NHS laboratories, located in Dundee, Aberdeen, Edinburgh and Glasgow.

influenza detections (1,405/3,163), exceeding the number of influenza A infections in February (Figure 2).

### Community versus hospital

Compared with 1,405 laboratory detections of influenza B virus in the 2012/13 season, 1,279 episodes of influenza B virus infection were reported to HPS during the same time period, showing the robustness of automated reporting (data not shown). The discrepancy probably reflects individuals who tested positive for influenza B more than once. Of the 1,279 reported infections, 394 related to samples collected by GPs and represented influenza B infections in the community, whereas the remaining 885 influenza B-positive samples were obtained during hospital visits (Figure 2).

The community-based influenza B infections will not be discussed further; the remaining analysis focuses on hospital-based influenza B infections only.

### Age distribution

In the 2012/13 season, hospital-based influenza A and B infections were widely distributed between age groups (Table 1). The highest frequency of influenza A virus detection (over 80% typed as H3N2) was seen in individuals over 65 years of age (23.5%; 451/1,916), whereas influenza B detections were significantly underrepresented in this age group in Scotland (7.0%, 134/1,916;  $p < 0.0001$  by chi-square test of association). When comparing the age-specific incidence of

**TABLE 1**

Age distribution of influenza A and B virus infections in individuals tested in Edinburgh and Glasgow, November 2012–April 2013 (n=16,146)

Age group	Samples	Influenza A virus		Influenza B virus	
		Positive	Rate	Positive	Rate
<5 years	5,555	399	7.2%	258	4.6%
5–10 years	1,637	129	7.9%	194	11.9%
11–15 years	719	53	7.4%	78	10.8%
16–20 years	551	40	7.3%	41	7.4%
21–36 years	1,494	63	4.2%	184	12.3%
37–65 years	4,274	485	11.3%	397	9.3%
>65 years	1,916	451	23.5%	134	7.0%

laboratory detections of hospital-based influenza in the Edinburgh and Glasgow area, based on the total population size in each age group, the highest incidence of influenza A(H3N2) and B infections were seen in children under the age of five years with, respectively, 170 and 110 infections per 100,000 (Figure 3). This age group was also the most frequently tested and accounted for 34% of samples tested for influenza (5,555/16,146), although this group represented only 5.6% of population (235,200/4,237,200). A similar age distribution of hospital-based influenza A(H3N2) and B infections was also observed in the previous seasons 2000 to 2012, whereas a higher incidence of influenza A(H1N1) infections was noted in adults between 21 and 65 years (data not shown).

### Severity of influenza B

In 2012–13, a total of 37 influenza B virus-infected individuals admitted to ICU were reported through the Scottish SARI surveillance system; three of them died. In comparison, 21 H1N1 and 60 H3N2 influenza A virus-infected individuals were admitted to ICU, of whom nine and 22 died, respectively. Most patients admitted to ICU with influenza B were older than 37 years (n=20), whereas a small number (n=9) of ICU admissions were observed in those younger than 20 years (Figure 4). The three mortalities occurred in the age groups 15–19, 20–24 and >65 years.

Only 16 of 27 influenza B-infected individuals were recorded to have been treated with antiviral drugs in the ICU (data not available for the remaining 10 individuals). Of the 37 influenza B patients treated in ICU, 24 belonged to groups recommended for vaccination (65 years and older or a clinical risk group), but only six had actually received the seasonal influenza vaccination.

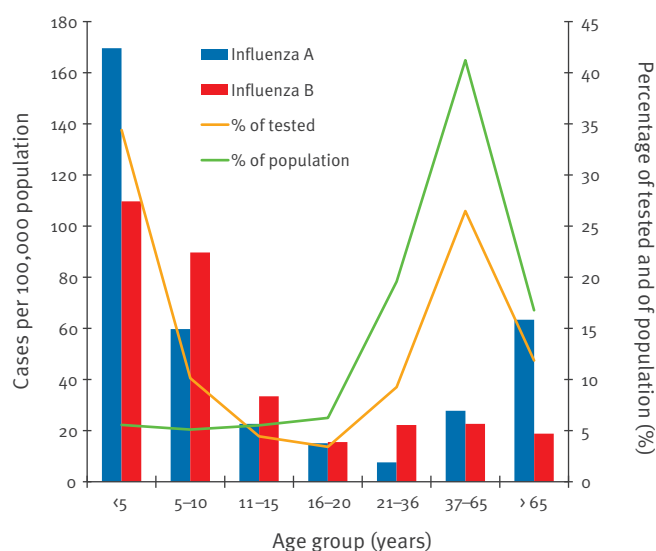
### Influenza B virus genetic lineages

Nucleotide sequence analysis of the haemagglutinin gene from viruses sampled monthly in Edinburgh (n=61) and Glasgow (n=86) between 2007/08 and 2012/13, and real-time PCR typing data in Glasgow in

2012/13 (n=488) revealed complex changes in the circulating lineages of influenza B virus (Table 2). The distribution of influenza B virus genetic lineages did not differ between Edinburgh and Glasgow. Before 2010/11, only one lineage was found per season, either B/Brisbane/60 (belonging to the Victoria lineage) or B/Florida/4 (Yamagata lineage). However, since only a limited number of influenza B virus strains were available for this study, it cannot be excluded that both genetic lineages may have been circulating simultaneously. Furthermore, in 2010/11 and 2012/13, both lineages were represented among sequences from both Edinburgh and Glasgow. Sequences collected in

**FIGURE 3**

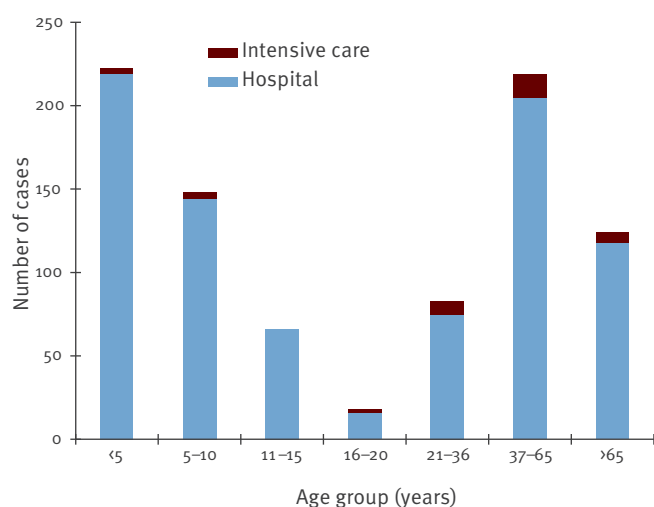
Estimated incidence of influenza A and B virus infections in southern and central Scotland by age group, based on laboratory testing data from Edinburgh and Glasgow, November 2012–April 2013 (n=16,146)



Incidences are calculated based on and census data from 2011 [8]. Line graphs refer to the right y-axis and show the percentage of samples tested per age group vs the percentage of each age group in the population of southern and central Scotland.

**FIGURE 4**

Hospital vs intensive care admissions due to influenza B infection, by age, Scotland, November 2012–April 2013 (n=880)



the same period in England (n=39), followed a similar trend. Which strains were circulating did not appear to be correlated with the vaccine strain used in a given season (Brisbane apart from 2008/09 and 2012/13) or with the intensity of influenza B infection (low in 2006/07, 2009/10 and 2011/12). In seasons with two circulating lineages there was no relationship between the distribution of virus lineages and the patients' age (data not shown).

Most influenza B viruses typed from patients treated in ICU were identified as Yamagata lineage viruses (16/19, two of which were vaccinated and thus vaccine failure was suspected); the remaining three were two Victoria lineage viruses and one untyped. This is in

line with the finding that 78.5% (400/509) of influenza B viruses were identified as Yamagata lineage viruses in Scotland during the last season 2012/13.

## Discussion

This epidemiological study of influenza B virus infections in Scotland is timely in view of the recent changes in the influenza vaccination programme in the UK and the possible introduction of a tetravalent influenza vaccine containing influenza B viruses of both the Victoria and Yamagata lineage. Our study reveals that influenza B virus was the predominant overall cause of influenza in four of the 13 influenza seasons from 2000/01 through 2012/13, and cocirculation of the Yamagata and Victoria lineages was observed in two of the six seasons investigated. Similar patterns of influenza B virus circulation have been described in the US, Europe and Hong Kong [12–13].

## Laboratory testing

With influenza diagnostic screening now improved through faster turnaround times and the capacity for screening larger numbers of samples in all four centres (a sevenfold increase during the past 10 years), virological data currently obtained through routine testing of samples from patients presenting to hospitals can be used effectively and directly to support influenza surveillance. Interestingly, 45% of samples submitted in 2012/13 for virological investigations in Edinburgh (NHS Lothian Health Board) were obtained from children under the age of five years. The high rate of testing in this age group results from a policy that all children presenting with respiratory symptoms to the accident and emergency department at the Royal Hospital for Sick Children in Edinburgh will be sampled and tested for respiratory viruses irrespective of whether they require hospital admission or not. Almost 10% of the population under five years of age in Lothian (4,141/48,600) were tested for influenza between November 2012 and

**TABLE 2**

Number of influenza B isolates grouping with the Victoria lineage (Brisbane/06) or Yamagata lineage (Florida/04 or Wisconsin/10), by season and location, Scotland, November 2007–April 2013 (n=186)

Season	Edinburgh		Glasgow		United Kingdom		Vaccine
	Victoria	Yamagata	Victoria	Yamagata	Victoria	Yamagata	
2007/08	0	10	No data	No data	0	3	Brisbane
2008/09	9	0	24	0	1	0	Florida
2009/10	2	0	1	0	3	0	Brisbane
2010/11	16	3	33	9	8	3	Brisbane
2011/12	0	0	3	0	4	7	Brisbane
2012/13	2	19	8 (107) <sup>a</sup>	8 (381) <sup>a</sup>	4	6	Wisconsin

<sup>a</sup> Additional typing results based on lineage-specific real-time PCR.

The vaccine strain used in each season is indicated.

The authors acknowledge the laboratories that submitted 39 UK sequences used in this Table to GISAID: Public Health England (formerly Health Protection Agency) and the National Institute for Medical Research in the UK (n=37 sequences) and the National Institute for Medical Research and the Centers for Disease Control and Prevention in the United States (n=2 sequences).



April 2013. These screening results can therefore be an indicator for influenza A and B virus incidence and circulation in that age group irrespective of whether infections lead to hospital admission. However, this approach is not routinely used elsewhere in Scotland: Only 20% of samples submitted for virological screening in Glasgow were obtained from children under the age of five years. This is likely to reflect different screening policies adopted in hospitals (i.e. screening only those admitted vs everybody screened).

### Influenza B, a paediatric infection?

Influenza is generally recognised as an important disease with high mortality rate among the elderly, although children have been shown to have an important role in the dissemination of influenza [14-15]. In contrast to influenza A(H3N2) virus, influenza B virus predominantly infects children and young adults [16-17]. In the current study, influenza B contributed to 45% of influenza-related hospital visits overall during the 2012/13 season, as much as 60% for children aged six to 15 years and 75% for young adults aged 21 to 36 years. The detection rate for influenza A dropped remarkably from those younger than five years to those aged six to 10 years, whereas only a slight drop was seen for influenza B infections (Figure 3) This is consistent with previous studies [12,18] and may be due to children accumulating natural immunity to influenza B more slowly than to influenza A [19].

### Severity of influenza B infections

Data on severe influenza B virus infections and mortality are limited. A large study from the US covering the period from 1976 to 1999 reported that 25% of all influenza-related mortality could be attributed to influenza B virus. This is substantially higher than seen in this study where influenza B infections accounted for ca 9% of all influenza virus-related deaths (3/34) during the 2012/13 season. Among those admitted to ICU with influenza B infections, we saw two deaths in young adults (15 to 24 years; one without underlying health problems). Whether they and others who were admitted to ICU with influenza B virus infection were genetically predisposed for severe infection remains to be investigated further [20].

### Prevention and treatment

Influenza immunisation has been recommended in the UK since the late 1960s and has been targeted to those predisposed towards greater morbidity following influenza. Although influenza vaccine uptake in Scotland during the 2012/13 season was 77% for those older than 65 years and 56% for those 65 years and younger in a risk group [21], only a quarter of those treated in ICU who should have been vaccinated actually had received the seasonal influenza vaccine. Most individuals (16/18) in ICU who had missed their vaccination were younger than 65 years but should have been vaccinated due to an underlying chronic medical condition. Whether the mechanism to target these individuals for

influenza vaccinations could be improved remains to be studied.

The neuraminidase inhibitors zanamivir and oseltamivir have been shown to be effective in the treatment of influenza [22-23], and are recommended for those hospitalised with influenza. Only 60% of individuals admitted to ICU with influenza B were treated with antiviral drugs; none of those who died were reported to have received antiviral treatment. However, the effectiveness of neuraminidase inhibitors against influenza B virus has been questioned and needs to be investigated further because inhibitory concentrations in vivo differ by up to 10-fold [24].

As the disease burden of influenza B is higher in children and young adolescents than in older age groups and because of recent cocirculation of influenza B viruses of both lineages, tetravalent vaccines may need to be combined with the newly introduced vaccination programme for children to improve the control of influenza B. The national programme aims to offer annual influenza vaccination to all school children across Scotland by autumn 2015, but there is no defined time frame for the introduction of the tetravalent vaccine in this group. Our study demonstrates that influenza B virus infections are associated with substantial morbidity and that influenza surveillance and interventions including vaccination and treatment are still suboptimal.

### Acknowledgements

We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database from which 39 UK influenza B virus sequences were obtained for comparison.

### Conflict of interest

None declared.

### Authors' contributions

Heli Harvala analysed the data and wrote the manuscript. Donald Smith, Peter Simmonds and Beatrix von Wissman also wrote part of the manuscript. The study was planned together with Heli Harvala, Rory Gunson, Kate Templeton, Arlene Reynolds, Beatrix von Wissmann and Jim McMenamin. Peter Simmonds designed the influenza B virus sequencing primers, whereas Donald Smith and Karina Salvatierra did the virus sequencing, and Alastair MacLean virus typing based on real-time PCR. Catherine Frew, Alison Hunt and David Yirrell provided the laboratory data, whereas Beatrix von Wissmann and Arlene Reynolds provided the data from Health Protection Scotland.

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