Influenza A(H1N1)2009 antibody seroprevalence in Scotland following the 2010/11 influenza season

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Following the 2010/11 influenza season, we determined the age- and location-specific seroprevalence of antibodies against the influenza A(H1N1)2009 virus in Scotland. Samples were analysed by microneutralisation assay. Age/seropositivity profiles varied significantly between cities. The increases in seroprevalence relative to the previous influenza season (2009/10) were similar across age groups and geographic locations. However, the increased seropositivity in older adults appeared to be driven by exposure to vaccination, indicating significantly lower levels of infection than in younger age groups.

In 2010 we determined the age and location-specific seroprevalence of antibodies against the influenza A(H1N1)2009 virus in Scotland after the second wave of the pandemic [1]. Following the 2010/11 influenza season, we have carried out a similar study to identify the changes in seroprevalence in Scotland from the previous season. Although population demographics and contact patterns may vary between countries, this information can assist European public health policy makers in planning for the 2011/12 influenza season.

Methods

The collection of samples and the materials and methods utilised were identical to those described in our 2010 study [1]. Briefly, anonymised serum and plasma from leftover diagnostic samples taken in February 2011 (subsequently referred to as hospital/general practice (GP) samples) were obtained from biochemistry laboratories in four cities in Scotland: Aberdeen, Edinburgh, Glasgow and Inverness. For each site, samples were categorised by patients' age groups (20–29, 30–39, 40–49 and ≥50 years) and 100 samples of each age group at each site were analysed. In addition, 100 anonymised samples were collected from leftover diagnostic samples taken in February 2011 in genito-urinary medicine (GUM) clinics in each of the four cities. Antibody responses were detected by microneutralisation assays, according to standard methods [2] using the NYMC X-179A reassortant virus strain derived from A/California/7/2009 (supplied by the National Institute for Biological Standards and Control, Potters Bar). It has previously been demonstrated that serum and plasma samples are equally applicable to influenza A(H1N1)2009 microneutralisation assays [3]. Each sample was tested at a dilution of 1:40, since positivity at this dilution has previously been taken to indicate a significant antibody response [4]. Logistic regression analysis was used to estimate the effects of age group, location, sample type, and potential vaccine exposure on seroprevalence. We did not have information on the vaccination status of patients whose samples were tested in this study. However, data on vaccine uptake has been collected from a cohort of approximately 93,000 individuals from 17 general practices (GP) across Scotland [5]. The geographic spread of the cohort does not allow separate uptake calculations for each of the four locations; nevertheless, vaccine uptake can be derived for each age group.

Results

The table shows the percentage of samples that were found to be positive for antibodies against the influenza A(H1N1)2009 virus by age, location, and time point, and how these percentages have increased between March 2010 and February 2011.

The age/seropositivity profile is complex and varies with location (Figure 1A).

Positivity was found to vary significantly with age in Aberdeen (p=0.014), Edinburgh (p=0.003), and Inverness (p<0.001), but not in Glasgow (p=0.94). In Aberdeen, seropositivity in the 40–49 year-old age group was lower than in the 20–29 year-old age group (p=0.007). In Edinburgh, the three older age groups had significantly lower seropositivity than the 20–29

year-old age group (p=0.037, p<0.001, p=0.015 respectively). In Inverness the 20–29 year-old age group had higher seropositivity than all other age groups (p<0.001 in each case).

Location was found to have a significant effect in all age groups except the 40–49 year-old group (p=0.67). Among 20–29 year-olds, Glasgow showed a significantly lower seroprevalence than Aberdeen (p<0.001), while Edinburgh and Inverness did not. Among 30–39 year-olds, Edinburgh was similar to Aberdeen, with Glasgow (p=0.016) and Inverness (p=0.007) having significantly lower seroprevalence. In the ≥50 year-old age group, all locations had significantly lower seroprevalence than Aberdeen (Edinburgh: p=0.03; Glasgow: p<0.001; Inverness: p<0.001).

The samples obtained from GPs and hospital departments cannot be considered a random sample from the general population as they are likely to have an over-representation among patients in groups more likely to receive an influenza vaccination. It is not likely that patients attending GUM clinics are over-represented in such groups. Figure 1B shows the seropositivity among 20–29 year-old hospital/GP patients and 20–29 year-old GUM clinic attendees for each location. In Glasgow (p=0.013) and Inverness (p=0.014), seropositivity in hospital/GP samples was lower than in GUM samples.

No such differences were observed in Aberdeen and Edinburgh.

Despite the differences in age/seropositivity profiles in each location, overall levels of seropositivity in each location increased by similar amounts (p=0.59) between 2010 and 2011 (Figure 2A).

The same is true for all age groups, with similar increases in seropositivity observed (p=0.65) (Figure 2B). An overall increase in seroprevalence was observed between 2010 and 2011 (p<0.001). These interactions indicate that between 2010 and 2011, there was no overall change in the relationship between seropositivity, age and location.

Figure 3 shows the relationship between seroprevalence and vaccine exposure in each age group for 2010 and 2011.

As expected, in all age groups, the proportion of individuals who have received the vaccine increases from 2010 to 2011. However, the increase in those aged ≥50 is much greater than in any other group (a consequence of people aged over 65 being routinely targeted for the seasonal vaccination in season 2010/11, but not for the influenza A(H1N1)2009 vaccination in season 2009/10).

Table

Increase in percentages of samples positive for antibodies against the influenza A(H1N1)2009 virus by age, location, and time point, Scotland, March 2010 and February 2011

Location	Age group [1,2] (years)	March 2010 [1] Percentage of positive samples (95% confidence interval)	February 2011 Percentage of positive samples (95% confidence interval)	Increase Percentage of positive samples (95% confidence interval)
Aberdeen	20–29	47 (39.8 to 53.6)	69 (62.6 to 75.4)	22 (9.0 to 35.6)
	30–39	51 (41.2 to 60.8)	63 (53.5 to 72.5)	12 (-7.3 to 11.7)
	40–49	39 (29.4 to 48.6)	53 (43.2 to 62.8)	14 (-5.4 to 33.4)
	≥50	39 (29.4 to 48.6)	73 (64.3 to 81.7)	24 (15.7 to 52.3)
Edinburgh	20–29	43 (36.1 to 50.1)	72 (65.8 to 78.2)	29 (15.7 to 42.1)
	30–39	35 (25.7 to 44.3)	60 (50.4 to 69.6)	25 (6.1 to 43.9)
	40–49	28 (19.2 to 36.8)	52 (42.2 to 61.8)	24 (5.4 to 42.6)
	≥50	45 (35.2 to 54.8)	58 (48.3 to 67.7)	13 (-6.5 to 32.5)
Glasgow	20–29	26 (20.0 to 32.2)	44 (36.6 to 50.4)	17 (4.4 to 30.4)
	30–39	18 (10.5 to 25.5)	46 (36.2 to 55.8)	28 (10.7 to 45.3)
	40–49	26 (17.4 to 34.6)	45 (35.2 to 54.8)	19 (0.6 to 37.4)
	≥50	33 (23.8 to 42.2)	42 (32.3 to 51.7)	9 (-9.9 to 27.9)
Inverness	20–29	50 (43.1 to 56.9)	71 (64.7 to 77.3)	21 (7.8 to 34.2)
	30–39	29 (20.1 to 37.9)	44 (34.3 to 53.7)	15 (-3.6 to 33.6)
	40–49	28 (19.2 to 36.8)	49 (39.2 to 58.8)	21 (2.4 to 39.6)
	≥50	19 (11.3 to 26.7)	30 (21.0 – 39.0)	11 (-5.7 to 27.7)

[1] In the 20–29 year-old groups, hospital/general practice and genito-urinary medicine clinic samples are combined (n=200 samples in each location for 2011, while in 2010 n=199 samples in Aberdeen, 195 in Edinburgh, 199 in Glasgow, 200 in Inverness).

[2] In the 30–39, 40–49, and ≥50 year-old groups, n=100 samples per age group in each location at each time point.

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Increases in vaccine exposure are strongly related to increased seroprevalence (p<0.001), but the increase in seropositivity among those aged ≥50 is significantly less than would have been expected relative to those aged less than 50 (p<0.001). This implies that in the ≥50 year-old age group a higher proportion of the increase in seropositivity is due to vaccination than in any other age group.

Discussion

Since the outbreak of influenza A(H1N1)2009, several studies have been undertaken to measure the frequency of antibodies against the virus [1, 6–10]. Taken together, these studies illustrate the spread of the virus at different time points and geographic locations since it began to spread in spring 2009. The work described here represents one of the earliest assessments of antibody seroprevalence following the

2010/11 influenza season in the northern hemisphere. In addition, due to the consistencies in sampling, materials, and methods with the study that we carried out following the 2009/10 influenza season [1], it has been possible to estimate increases in antibody seroprevalence in Scotland during the third wave of infection. While hospital/GP samples cannot be considered to be a random sample from the general population, such samples have previously been used to estimate seroprevalence [4].

In our previous study, we speculated that Glasgow and Inverness might experience higher levels of influenza activity than Aberdeen and Edinburgh during the 2010/11 influenza season [1]. However the results described here indicate that similar levels of influenza activity occurred in each of the four locations (although geographical variations in vaccine uptake are not known). Overall, age/seroprevalence graphs for each city have essentially shifted upwards in relation to 2010: Aberdeen and Edinburgh still show higher levels of seropositivity than Glasgow, with seropositivity in Inverness still decreasing with increased patient age.
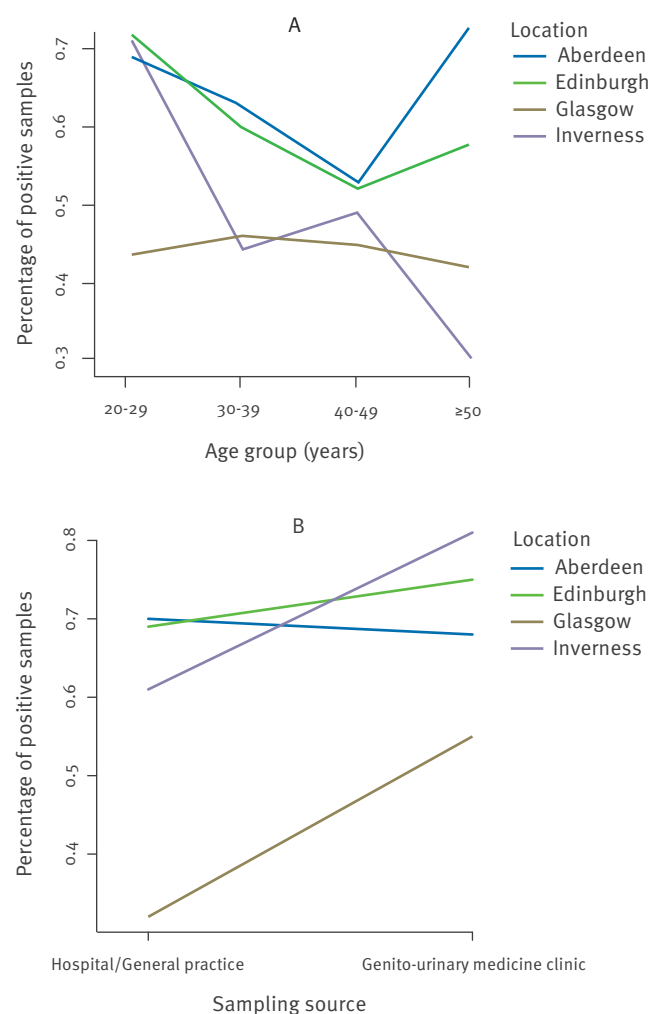
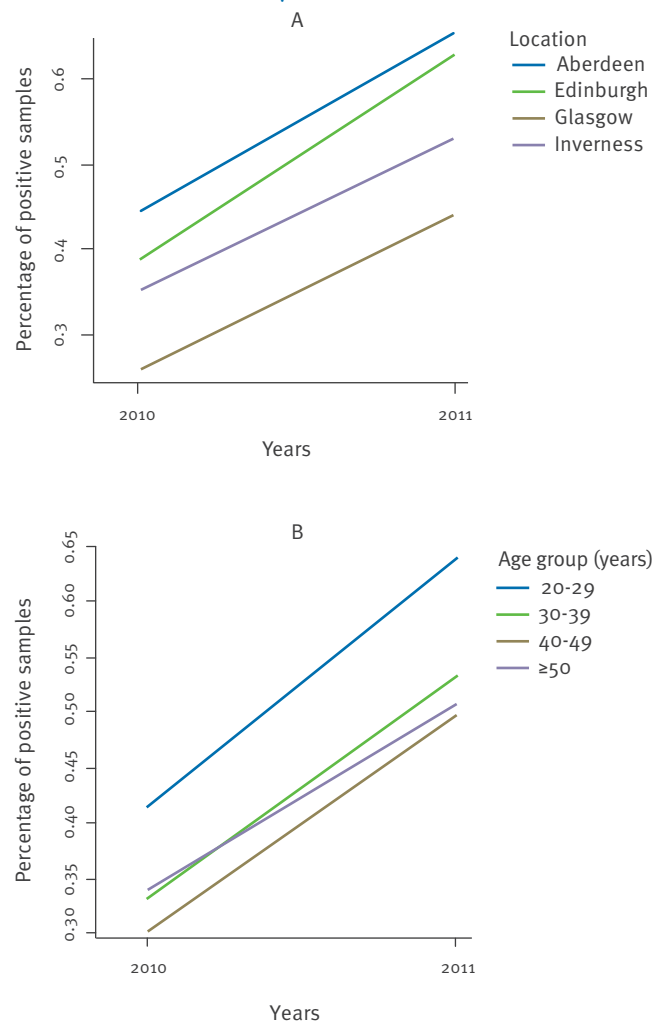A: Variations in age/seropositivity profile by location.
B: Seropositivity among 20–29 year-olds attending hospital/ general practice and genito-urinary medicine clinics for each location.

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In contrast to 2010, we observed higher levels of seropositivity in GUM samples than in hospital/GP samples in Glasgow and Inverness. The reason for this is unclear; however, a possible explanation might involve differences in social interactions between the two patient groups, with GUM patients mixing with other individuals more than those in the hospital/GP group. In Aberdeen and Edinburgh, seropositivity levels were higher, with less opportunity for the virus to be transmitted to susceptible individuals regardless of social mixing.

A weakness of this study is that we do not have any information on the risk group and vaccination status of the patients as only aggregate data could be used, which could not be linked to any patient characteristics. This means that we are unable to separate the effect of vaccination from infection, or to adjust seroprevalence among hospital samples for possible selection bias associated with risk groups.

The observation that increased seropositivity in the ≥50 age group between 2010 and 2011 is strongly correlated with vaccination may suggest that compared to younger individuals that the force of infection is weaker in the older age group. This hypothesis assumes that the cohort of 93,000 individuals is representative of the influenza vaccine profile in samples taken from hospital/GP and GUM sites. This might be due to older individuals being protected from influenza A(H1N1)2009 as a result of previous exposure. If this is the case then it indicates that testing samples in the microneutralisation assay at a dilution of 1:40 might represent too conservative an estimate of levels of protection against influenza A(H1N1)2009. To examine this in more detail, we have tested the samples described in this study at lower dilution levels. Initial findings indicate that low levels of antibodies that are reactive against influenza A(H1N1)2009 can be detected in a significant proportion of patients who are seronegative at 1:40, and that

this observation is particularly true for patients in the ≥50 age group. These data are currently being collated for publication.

There remains significant variation in antibodies by age and location to influenza A(H1N1)2009 virus among the Scottish population with between 27% and 70% of any age group or location being susceptible to infection. These observations support the World Health Organization recommendation of the inclusion influenza A(H1N1)2009 in the trivalent seasonal influenza vaccine for the northern hemisphere this coming season [11]. However, these overall figures may be revised following the analysis of samples at other dilutions in the microneutralisation assay.

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FIGURE 3

The relationship between seroprevalence, vaccine coverage, age, and year, Scotland, March 2010 and February 2011
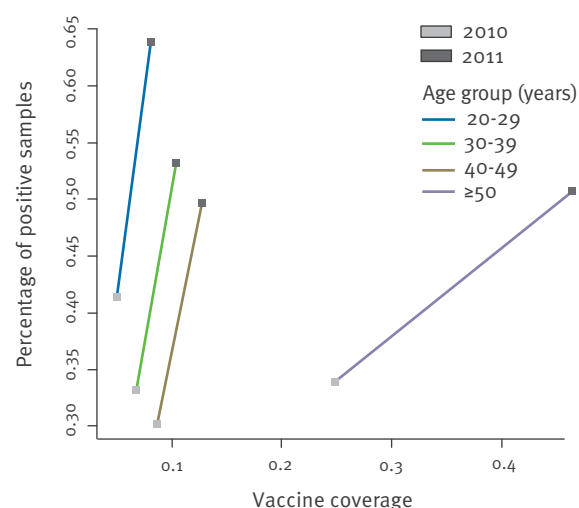
Age group (years)	Number vaccinated against influenza A(H1N1)-2009 in 2009/10	Number vaccinated against seasonal influenza in 2010/11	Number receiving at least one vaccine against influenza A(H1N1)-2009 in 2009/10 or 2010/11	Denominator	Cumulative vaccine coverage in February 2011 (%)
20−29	696	654	1,119	13,722	8.1
30−39	884	823	1,353	12,931	10.5
40−49	1,271	1,373	1,866	14,562	12.8
≥50	8,267	14,117	15,432	33,214	46.5
All age groups	**11,118**	**16,967**	**19,770**	**74,429**	**26.6**

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