**Epidemiology and Infection**

**Title: Immunity against influenza A(H1N1) infections is determined by age at the time of initial strain circulation**

**Authors:**

R. M. Delabre, N. Salez, N. Lapidus, M. Lemaitre, M. Leruez-Ville, X. de Lamballerie, F. Carrat

**Supplementary Material**

**1. Models of HI titre as a function of age**

Models used by Lessler *et al.* [[1](#_ENREF_1)]were adapted to our longitudinal study data; a random subject and strain specific intercept was added for all tested models.

Starting from the most complex models (Models A1-4), strain-specific HI titre was modeled as a function of age at inclusion and age at the time of initial strain circulation, then simplified to either age at inclusion (Models B1-2) or age at the time of strain circulation (Models C1-2). All models were tested with and without strain dependence on the age variables. Splines were used to smooth the relationship between the HI titre and covariates.

HI titre of subject to strain

subject and strain specific intercept

and regression coefficient

and strain-specific regression coefficients

subject age at inclusion

years since strain first circulated

smooth spline at age *a*

Models A1-4: HI titre was modeled as a function of age at inclusion and age at the time of initial strain circulation

Model A1: both age variables were strain-dependent

Model A2: only age at inclusion was strain-dependent

Model A3: only age at the time of initial strain circulation was strain-dependent

Model A4: age was independent of viral strain

Models B1-2: HI titre was modeled as a function of age at inclusion

Model B1: age at inclusion was strain-dependent

Model B2: age at inclusion was independent of viral strain

Models C1-2: HI titre was modeled as a function of age at the time of initial strain circulation

Model C1: age at the time of initial strain circulation was strain-dependent

Model C2: age at the time of initial strain circulation was independent of strain

**2. Sensitivity analysis**

This cohort study was conducted over three influenza seasons (2007-2008, 2008-2009 and 2009-2010) in which subjects were recruited to the study cohort during the first two years. During this period, the primary viruses circulating in France were as follows: influenza A /Brisbane/59/2007 (H1N1) during the 2007-2008 season [[2](#_ENREF_2)], influenza A/Brisbane/10/2007 (H3N2) [[3](#_ENREF_3), [4](#_ENREF_4)] during the 2008-2009 influenza season, and influenza A/California/7/2009 (H1N1pdm09) during the 2009-2010 season [[5](#_ENREF_5)].

*Laboratory procedures*

Influenza infection among ILI subjects was diagnosed from nasopharyngeal swab samples (VIROCULT®, KITVIA, Labarthe Inard, France) collected at inclusion and/or ILI visits using one-step real time RT-PCR. More details regarding procedures have been described elsewhere [[6](#_ENREF_6)].

*Statistical methods*

Partial correlation tests and models of HI titre and age were conducted as described in the main analysis with the exclusion of all subjects identified with influenza A(H1N1) infection (2007 A(H1N1) or A(H1N1)pdm09) by RT-PCR.

**Bibliography**

(1) Lessler J, et al. Evidence for antigenic seniority in influenza A (H3N2) antibody responses in southern China. *PLoS pathogens* 2012; 8(7): e1002802.

(2) Vaux SV, M.; Enouf, V.; Bensoussan, J.; Turbelin, C.; Blanchon, T. Surveillance épidémiologique et virologique de la grippe en France : saison 2007-2008. *Bull Epidemiol Hebd* 2008; 34: 301-301.

(3) Centre National de Reference des Virus Influenzae. Rapport d'Activité Annuel Saison 2008-2009. n.d.

(4) European Centre for Disease Prevention and Control. Influenza surveillance in Europe 2008/09: ECDC; 2010.

(5) Centre National de Reference des Virus Influenzae. Rapport D'Activité Annuel Saison 2009-2010; n.d.

(6) Lemaitre M, et al. Seasonal H1N1 2007 influenza virus infection is associated with elevated pre-exposure antibody titers to the 2009 pandemic influenza A (H1N1) virus. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2011; 17(5): 732-737.

**Figure Captions (Supplementary Material):**

**Supplementary Table S1:** Strain-specific HI titre was modeled as a function of age at inclusion and age at the time of initial strain circulation (Models A1-4), or just age at study inclusion (Models B1-2) or just age at the time of initial strain circulation (Models C1-2). All models included a random subject and strain-specific intercept. Strain dependency was tested for all age variables in the three types of models. Models were compared using BIC; main model was selected based on lowest BIC (\*). †Difference in BIC with main model (Model C1) is shown.

**Supplementary Figure S1:** Sensitivity analysis without H1N1 infected subjects (n=46); partial rank correlations (Spearman’s rho) between A(H1N1) viral strains by birth cohort group: 1977-1991 (n=88), 1956-1976 (n=159), 1930-1955 (n=105). Asterisks denote p-significance (\* significant at p < 0.05; \*\* significant at p < 0.01, and \*\*\* significant at p < 0.001).

**Supplementary Figure S2:** Sensitivity analysis without H1N1 infected subjects (n=46); strain-specific GMT as a function of age at initial strain circulation (lines) and observed GMT per subject (points) where A(H1N1)pdm09 is represented in red, seasonal 2007 A(H1N1) is in green, 1977 A(H1N1) in orange, and 1956 A(H1N1) in dark blue.