Supplemental Information – Protection against Infection with 1918 H1N1 Pandemic Strain

The current global population would be partially protected against an outbreak caused by the 1918 H1N1 pandemic strain of influenza due to immunity arising from exposure to the 2009 H1N1 pandemic strain (H1N1pdm09), through natural infection or vaccination. A sizeable percentage of the population was infected during the initial outbreak in 2009 - 2010 and more people have been infected since, as the virus has continued to circulate in subsequent flu seasons. In addition, monovalent H1N1pdm09 vaccines were administered during the pandemic, and the strain has continued to be a recommended component of the seasonal flu vaccine through the current flu season. Animal studies have demonstrated that H1N1pdm09 vaccines are highly protective against subsequent challenge with the 1918 strain. Epidemiological and serological studies support long-term duration of immunity against antigenically related strains. Together, these data suggest that the population's exposure to H1N1pdm09 would confer a substantial degree of protection in the event of a 1918 pandemic strain outbreak. (Chapter 6 uses the data presented in this section to estimate the degree of population immunity to the 1918 H1N1 virus given prior exposure to the 2009 H1N1 pandemic virus.)

Estimated Percentage of Global Population Exposed to H1N1pdm09

Published estimates for the percentage of the current global population that has been naturally infected with H1N1pdm09 are lacking. Van Kerkhove et al[1] estimated the cumulative global incidence of H1N1pdm09 infection during the 2009-2010 pandemic period by aggregating data from seroprevalence studies conducted in eleven countries. These studies compared hemagglutination inhibition (HI) or microneutralization (MN) titers from pre- and post-pandemic sera to determine incidence of infection in these countries. The continents of Asia, Europe, North America, and Oceania were represented. The pooled cumulative incidence was estimated to be 24%. A second estimate of cumulative incidence, which excluded countries that may have included vaccinated individuals in their seroprevalence data (US and Norway), yielded a value of 21% [95% CI 18-25%]. The estimate of 21% reflects the incidence of natural infection alone.

The H1N1pdm09 strain has continued to circulate seasonally since the 2009 – 2010 pandemic season, so that more than 21% of the current population has now been naturally infected with this strain. The total percentage of today's global population that has been infected with H1N1pdm09 (including and subsequent to the initial pandemic season) was estimated using available data. First, the 21% global cumulative incidence estimate was applied to the average of the 2009 and 2010 global population, reported by the US Census Bureau,[2] to yield an estimate of the total number of global cases that season. Next, the number of reported H1N1pdm09 cases from the WHO FluNet[3] database, summed over all geographic regions and all weeks during the 2009-10 pandemic,[4] was determined. The ratio of the total number of (estimated) global cases to the number of cases reported to FluNet during the 2009 – 2010 season was then calculated. Next, for each subsequent flu season from 2010-11 to present, where a flu season is defined July-June, the number of FluNet reported cases was multiplied by this ratio to yield an estimate of the total number of global cases that season. Table S1 lists the estimated number of total global cases for each season as a number and as a percentage of the 2016 global population. Finally,

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1 Van Kerkhove MD et al. (2013) Estimating age-specific cumulative incidence for the 2009 influenza pandemic: a meta-analysis of A(H1N1)pdm09 serological studies from 19 countries. *Influenza and other respiratory viruses.* 7 (5)**:** 872-886.

2 (2015) International Programs - Total Midyear Population for the World: 1950-2050 - U.S. Census Bureau. United States Census Bureau.

3 (2016) FluNet. World Health Organization.

4 Case data from March 2009 to June 2010 were summed to find the number of reported cases during the 2009-10 pandemic.

estimated cases were summed over all seasons to calculate an estimated total number of global cases of H1N1pdm09 since the emergence of the virus in 2009. This number of cases represents 31% of the current global population, indicating that about one-third of the world's people have been exposed to H1N1pdm09 through natural infection. This method makes several simplifying assumptions: (1) the ratio of actual global cases to FluNet reported cases remains consistent from year to year, (2) all H1N1pdm09 cases are first-time infections, and (3) all people that have been infected with H1N1pdm09 since 2009 are still living.

Table S1. Estimated Global Incidence of H1N1pdm09 Infection, 2009-Present

Flu Season	Estimated Global Cases of H1N1pdm09	Estimated Percentage of 2016 Population Infected with H1N1pdm09
2009-2010	1.43E9	20%
2010-2011	2.53E8	3%
2011-2012	5.52E7	1%
2012-2013	1.40E8	2%
2013-2014	2.60E8	4%
2014-2015	6.94E7	1%
2015-Present (2016 Week 5)	9.46E7	1%
Total	**2.31E9**	**31%**

Protection by H1N1 Vaccination against Morbidity, Mortality, and Transmissibility during Subsequent 1918 H1N1 Challenge

Vaccination using H1N1pdm09 provides a substantial degree of cross-protection against subsequent 1918 H1N1 infection in animal models, where protection is indicated by reduction of mortality, morbidity, or transmissibility following viral challenge. Vaccination with H1N1pdm09 and other strains antigenically similar to the 1918 pandemic H1N1 strain provides a high degree of protection against challenge with the 1918 strain, as or nearly as effective as vaccination with 1918 H1N1 itself. Seasonal H1N1 vaccination provides a moderate degree of protection, and seasonal H3N2 vaccination provides little to no protection against challenge with 1918 H1N1. Based on the results of animal studies, immunization with H1N1pdm09 is highly effective at protecting against infection with 1918 H1N1, providing nearly complete protection against mortality and reducing morbidity and transmissibility. Results of individual experiments are discussed below.

In a study by Medina et al,[5] mice were immunized intramuscularly using 1918 H1N1, H1N1pdm09, seasonal H1N1, or seasonal H3N2 vaccine, at days 28 and 14 pre-challenge, then challenged with 1918 pandemic H1N1. Mortality results were recorded for groups of n=5 mice and morbidity was measured by lung viral titer on day two or four post-challenge for groups of n=4-6 mice. For calculating the percentage of animals displaying morbidity, any lung viral titer above the baseline, 1E1 pfu/ml, was counted as an animal displaying morbidity regardless of severity. Experimental results are summarized in Table S2. H1N1pdm09 vaccination was as effective as 1918 H1N1 vaccination at reducing mortality and slightly more effective at reducing morbidity.[6] Seasonal H1N1 vaccination was nearly as effective as 1918 H1N1

[5] Medina RA et al. (2010) Pandemic 2009 H1N1 vaccine protects against 1918 Spanish influenza virus. *Nature communications.* 1**:** 28.

[6] Morbidity is recorded based on percentage of animals displaying any level of the morbidity symptom.

vaccination at reducing mortality but did not reduce morbidity. Seasonal H3N2 vaccination did not afford protection beyond that of the unvaccinated (PBS) control.

Table S2. Summary of Experimental Results from Medina et al, 2010, Part One

Vaccine strain	Challenge strain	Dose	Morbidity		Mortality	
			%[7]	Symptom	%	Day
1918 H1N1 VLP	1918 H1N1	300 LD_{50}	83% (5/6)	Lung viral titer	0% (0/5)	NA
H1N1pdm09 (Cal/04/09) inactivated virus	1918 H1N1	300 LD_{50}	67% (4/6)	Lung viral titer	0% (0/5)	NA
Seasonal H1N1 (Bris/59/07) inactivated virus	1918 H1N1	300 LD_{50}	100% (6/6)	Lung viral titer	20% (1/5)	7
Seasonal H3N2 (Bris/10/07) inactivated virus	1918 H1N1	300 LD_{50}	100% (4/4)	Lung viral titer	100% (5/5)	5-11
No vaccine control (PBS)	1918 H1N1	300 LD_{50}	100% (6/6)	Lung viral titer	100% (5/5)	5-8
NA	Mock-infected control (PBS)	NA	NA	NA	0% (0/5)	NA

In the same study by Medina et al, mice were passively immunized with human sera, intraperitoneally, 24 hours pre-challenge. Sera samples were drawn from three human volunteers pre-vaccination and post-vaccination with an H1N1pdm09 licensed inactivated vaccine. Morbidity and mortality results were recorded as described previously and are summarized in Table S3. Immunization with post-vaccination human sera reduced mortality completely in all groups but did not reduce the percentage of mice displaying morbidity symptoms.

Table S3. Summary of Experimental Results from Medina et al, 2010, Part Two

Vaccine strain	Challenge strain	Dose	Morbidity		Mortality	
			%	Symptom	%	Day
Human sera (pre- vaccination) #2	1918 H1N1	50 LD_{50}	100% (6/6)	Lung viral titer	100% (5/5)	7-10
Human sera (pre- vaccination) #6	1918 H1N1	50 LD_{50}	100% (6/6)	Lung viral titer	100% (5/5)	10-11
Human sera (pre- vaccination) #7	1918 H1N1	50 LD_{50}	100% (6/6)	Lung viral titer	100% (5/5)	9-10
Human sera (2009 H1N1 post-vaccination) #2	1918 H1N1	50 LD_{50}	100% (6/6)	Lung viral titer	0% (0/5)	NA
Human sera (2009 H1N1 post-vaccination) #6	1918 H1N1	50 LD_{50}	100% (6/6)	Lung viral titer	0% (0/5)	NA
Human sera (2009 H1N1 post-vaccination) #7	1918 H1N1	50 LD_{50}	100% (6/6)	Lung viral titer	0% (0/5)	NA
No sera control (PBS)	1918 H1N1	50 LD_{50}	100% (5/5)	Lung viral titer	100% (5/5)	7-10

A second study conducted by Easterbrook et al[8] involved immunization of mice with 1918 H1N1, H1N1pdm09, seasonal trivalent, or 1976 H1N1 vaccine, at four and two weeks pre-challenge, followed by challenge with 1918 pandemic H1N1 virus. Mortality results were recorded for groups of n=5 mice and morbidity was measured by weight loss for groups of n=5 mice. Experimental results are summarized

[7] Morbidity percentages were not reported directly in this study, but judged based on the number of lung viral titer data points visually above the baseline level.

[8] Easterbrook JD et al. (2011) Immunization with 1976 swine H1N1- or 2009 pandemic H1N1-inactivated vaccines protects mice from a lethal 1918 influenza infection. *Influenza and other respiratory viruses.* 5 (3)**:** 198-205.

in Table S4. Vaccination H1N1pdm09 and with 1976 H1N1, both antigenically similar to 1918 H1N1, reduced morbidity and mortality percentages completely. The seasonal trivalent vaccine, containing a pre-2009 H1N1, H3N2, and influenza B strains, reduced mortality but did not reduce the percentage of animals experiencing morbidity.

Table S4. Summary of Experimental Results from Easterbrook et al, 2011

Vaccine strain	Challenge strain	Dose	Morbidity		Mortality	
			%[9]	Symptom	%	Day
1918 H1N1 (South Carolina/1/18) whole virus vaccine	1918 H1N1	10 LD_{50}	0% (0/5)	Weight loss	0% (0/5)	NA
2009 H1N1pdm (A/Cal/7/09) subunit vaccine	1918 H1N1	10 LD_{50}	0% (0/5)	Weight loss	0% (0/5)	NA
2009 seasonal trivalent subunit vaccine (A/Brisbane/59/07 H1N1 + A/Uruguay/716/07 H3N2 + B/Brisbane/60/08)	1918 H1N1	10 LD_{50}	100% (5/5)	Weight loss	20% (1/5)	8
1976 swH1N1 whole virus vaccine (A/New Jersey/11/76)	1918 H1N1	10 LD_{50}	0% (0/5)	Weight loss	0% (0/5)	NA
No vaccine control (PBS)	1918 H1N1	10 LD_{50}	100% (5/5)	Weight loss	100% (5/5)	7-8

In a third study, Pearce at al[10] studied protection in a ferret model. Ferrets were immunized with the 2010-11 seasonal trivalent vaccine containing an H1N1pdm09 pandemic strain isolate as well as H3N2 and influenza B strains. Vaccination was performed three times intramuscularly, four to five weeks apart, with the final injection five to eight weeks pre-challenge. Morbidity was indicated by weight loss and fever. Transmissibility was measured by peak nasal viral titer during the eight days post-challenge, measured every other day. Experimental results are summarized in Table S5. Although vaccination did not lower morbidity percentage, vaccination did lead to a reduction in nasal wash viral titers, indicating decreased transmissibility compared to the unvaccinated control group.

Table S5. Summary of Experimental Results from Pearce at al, 2012

Vaccine strain	Challenge strain	Dose[11]	Morbidity[12]		Mortality		Transmissibility	
			%	Symptom	%	Day	Titers	Day
2010-2011 seasonal trivalent vaccine (A/Mexico/4482/09 H1N1 + A/Perth/16/09 H3N2 + B/Brisbane/60/08)	1918 H1N1	1 LD_{50}	100% (5/5)	Weight loss, fever	0% (0/5)	NA	1E3.7 pfu/ml (5/5)	2
No vaccine control (PBS)	1918 H1N1	1 LD_{50}	100% (5/5)	Weight loss, fever	0% (0/5)	NA	1E5.6 pfu/ml (5/5)	4

[9] Morbidity is recorded based on percentage of animals displaying any level of the morbidity symptom.

[10] Pearce MB et al. (2012) Seasonal trivalent inactivated influenza vaccine protects against 1918 Spanish influenza virus infection in ferrets. *Journal of virology*. 86 (13)**:** 7118-7125.

[11] Dosage was 1E6 pfu which roughly translates to 1 LD_{50}.

[12] Morbidity is recorded based on percentage of animals displaying any level of the morbidity symptom.

Finally, Tumpey et al[13] examined the protection conferred by H1N1 strains other than the H1N1pdm09 strain. In brief, mice were immunized against 1918 H1N1, one of several H1N1 strains, or H3N2, intramuscularly three weeks pre-challenge, then challenged with one of two recombinant strains. Challenge strains contained key 1918 H1N1 genes in an A/WSN/33 backbone, at either a 2:6R or 5:3R ratio. Morbidity was measured by observations of weight loss and listlessness. In addition, the investigators measured viral titers in the nose from post-mortem tissue cultures, removed five days post-challenge, an indicator of transmissibility. Results are summarized in Table S6. Vaccination with the 1930 H1N1 strain, which is antigenically similar to the 1918 strain, was as effective as the 1918 H1N1 vaccine at reducing morbidity, mortality, and transmissibility. Vaccination with 1934 H1N1, another antigenically similar strain, effectively reduced mortality and lowered transmissibility to an extent. However, more recent H1N1 strains, such as a 1999 isolate, were much less effective than vaccines based on the 1918 and similar strains, though slightly reduced mortality and transmissibility compared to unvaccinated control animals. H3N2 vaccination did not confer protection against 1918 H1N1.

Table S6. Summary of Experimental Results from Tumpey et al, 2004

Vaccine strain	Challenge strain	Dose	Morbidity		Mortality		Transmissibility	
			%	Symptom	%	Day	Titers	Day
1918 recombinant (HA/NA) H1N1 vaccine	2:6R strain: 1918 HA/NA:WSN	100 LD_{50}	0%	Weight loss, listlessness, lung viral titer	0%	NA	<1E1 EID_{50}/ml	5
1934 H1N1 (A/PR/8/34) vaccine	2:6R strain: 1918 HA/NA:WSN	100 LD_{50}	100%	Weight loss, listlessness, lung viral titer	0%	NA	~1E3 EID_{50}/ml	5
1999 H1N1 (A/New Caledonia/20/99) vaccine	2:6R strain: 1918 HA/NA:WSN	100 LD_{50}	100%	Weight loss, listlessness, lung viral titer	~25%	12	~1E4 EID_{50}/ml	5
H3N2 (X-31) vaccine	2:6R strain: 1918 HA/NA:WSN	100 LD_{50}	100%	Weight loss, listlessness, lung viral titer	~80%	6-11	~1E5 EID_{50}/ml	5
No vaccine control (PBS)	2:6R strain: 1918 HA/NA:WSN	100 LD_{50}	100%	Weight loss, listlessness, lung viral titer	100%	9-11	~1E5 EID_{50}/ml	5

[13] Tumpey TM et al. (2004) Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. *Proceedings of the National Academy of Sciences of the United States of America.* 101 (9)**:** 3166-3171.

1918 recombinant (HA/NA) H1N1 vaccine	5:3R strain: 1918 HA/NA/M/NP /NS:WSN	100 LD_{50}	0%	Weight loss, listlessness, lung viral titer	0%	NA	<1E1 EID_{50}/ml	5
1930 H1N1 (A/Swine/Iowa/30) vaccine	5:3R strain: 1918 HA/NA/M/NP /NS:WSN	100 LD_{50}	0%	Weight loss, listlessness, lung viral titer	0%	NA	<1E1 EID_{50}/ml	5
1934 H1N1 (A/PR/8/34) vaccine	5:3R strain: 1918 HA/NA/M/NP /NS:WSN	100 LD_{50}	100%	Lung viral titer	~10%	8	~1E3 EID_{50}/ml	5
1991 H1N1 (A/Texas/36/91) vaccine	5:3R strain: 1918 HA/NA/M/NP /NS:WSN	100 LD_{50}	100%	Lung viral titer	~35%	6	~1E3.5 EID_{50}/ml	5
1999 H1N1 (A/New Caledonia/20/99) vaccine	5:3R strain: 1918 HA/NA/M/NP /NS:WSN	100 LD_{50}	100%	Lung viral titer	~45%	9-10	~1E3 EID_{50}/ml	5
H3N2 (X-31) vaccine	5:3R strain: 1918 HA/NA/M/NP /NS:WSN	100 LD_{50}	100%	Lung viral titer	100%	6-12	~1E4.5 EID_{50}/ml	5
No vaccine control (PBS)	5:3R strain: 1918 HA/NA/M/NP /NS:WSN	100 LD_{50}	100%	Lung viral titer	100%	7-9	~1E4.5 EID_{50}/ml	5

In sum, these studies show that vaccination with H1N1pdm09 and other strains that are antigenically similar to 1918 H1N1 provide excellent protection against the 1918 H1N1 virus in short-term challenge studies using mouse and ferret animal models for influenza infection. The most effective vaccines reduce mortality to 0% and reduce morbidity by varying amounts. Even when the fraction of animals displaying morbidity is not reduced following 2009H1N1pdm09 vaccination, vaccinated animals display less severe symptoms (weight loss, lung viral titers, etc.). Measures of transmission, *i.e.* nasal viral titers, are reduced following vaccination as well. Interestingly, vaccination using pre-2009 H1N1 seasonal strains, which are less antigenically similar to 1918 H1N1 than the H1N1pdm09 virus, also partially protects against mortality following 1918 H1N1 challenge. In contrast, vaccination against the human seasonal H3N2 virus has no protective effects against 1918 H1N1. Collectively, these results indicate that H1N1pdm09 immunization provides robust cross-protection against 1918 H1N1 infection.

No animal studies have directly examined whether natural infection with H1N1pdm09 confers protection against subsequent infection with 1918 H1N1. However, given that natural infection induces a more robust immune response than vaccination, the above results strongly suggest that natural infection with H1N1pdm09 would confer a similar degree and type of protection against infection with 1918 H1N1 (if not higher).

Duration of Immunity

Epidemiological Studies

Epidemiological studies demonstrate that natural infection with H1N1 confers a degree of protection against infection in subsequent years. A study by Davies et al[14] found evidence of enduring immunity against H1N1 in a boarding school in England, based on outcomes during outbreaks in 1978, 1979, and 1983. The 1978 and 1979 H1N1 strains were antigenically similar, with infection in 1978 conferring 92% protection during the 1978 outbreak; protection is defined as the percentage reduction in the attack rate in the previously infected (immunized) population relative to the susceptible population.[15] Infection in 1978 or 1979 conferred 55% protection against the 1983 H1N1 strain. Because the 1983 strain diverged antigenically from previous years' strains due to antigenic drift, the reduction in protection likely reflected both waning immunity and less effective protection due to antigenic mismatch. These data suggest that protection against H1N1 infection is long lasting, with substantial effects five years out. This study also found that in 1983, previously infected subjects with antibodies reacting with the 1983 strain (A/Eng/83) experienced protection of 73%, whereas subjects without these antibodies experienced protection of just 45%. This result indicates that, as in the animal studies, antigenic similarity is an important component of the protective effect.

A study by Fox et al[16] found that the odds of infection with various flu strains were decreased if infected with a related subtype in the previous season; this study was performed for an unvaccinated population in Vietnam. The odds ratio associating 2009-2010 pandemic H1N1 infection and 2008-2009 seasonal H1N1 infection (the odds of contracting H1N1 during the 2009 pandemic given natural infection with the seasonal H1N1 strain in the previous flu season compared to the odds of contracting H1N1 during the 2009 pandemic in the absence of natural infection with seasonal H1N1 in the previous season) was 0.23 with p=0.004. This result suggests that natural infection with a pre-2009 seasonal H1N1 strain confers some level of protection against the 2009 H1N1 pandemic strain one year following infection.

Giles et al[17] demonstrated the long-lasting effects of pandemic H1N1 vaccination in a mouse study. Mice were vaccinated with 1918 H1N1 virus-like particles then challenged with an H1N1pdm09 isolate known to cause morbidity but not lethality in adult mice. Viral challenge took place either two weeks or 16 months post-vaccination. Vaccination fully protected mice from morbidity, as measured by weight loss and observable clinical sickness, in both groups. Lung viral titers were significantly reduced, compared to unvaccinated mice of the same age, in the mice challenged two weeks post-vaccination but not in the mice challenged 16 months post-vaccination. Thus, vaccination maintained efficacy in preventing observable clinical illness but not in reducing viral load following long-term challenge in mice.

Serological Studies

Enduring immunity following natural infection or vaccination is also evident in human serological data. HI titer is well established as a correlate of immune protection against flu, with titers of 32-40 (1:32 to

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[14] Davies JR, Grilli EA, Smith AJ. (1986) Infection with influenza A H1N1. 2. The effect of past experience on natural challenge. *The Journal of hygiene.* 96 (2)**:** 345-352.

[15] Several values of protection percentage were reported directly by the study; the remainder were calculated from the provided raw data.

[16] Fox JP et al. (1982) Influenzavirus infections in Seattle families, 1975-1979. I. Study design, methods and the occurrence of infections by time and age. *American journal of epidemiology.* 116 (2)**:** 212-227.

[17] Giles BM et al. (2012) Elicitation of anti-1918 influenza virus immunity early in life prevents morbidity and lower levels of lung infection by 2009 pandemic H1N1 influenza virus in aged mice. *Journal of virology.* 86 (3)**:** 1500-1513.

1:40 dilution of serum required to prevent agglutination of red blood cells) generally associated with 50% protection against infection in a susceptible population.[18] FDA guidelines for vaccine licensure suggest using the percentage of the vaccinated population with HI titer > 40 (1:40) as an immunogenicity endpoint.[19]

The relationship between HI titer and protection from H1N1 has been elucidated in papers by Ng et al[20] and Tsang et al.[21] Based on curves modeled in these papers, relative risk reduction (*i.e.* protection) is a quadratic function of the logarithm of the HI titer. Protection rises quickly as HI titers increase from undetectable levels to the 50% protective level, beyond which protection rises more slowly with increasing titer. The study by Ng et al found that a titer of 40 (1:40) against H1N1pdm09 following seasonal trivalent flu vaccination in children corresponded to 48% protection, which stands in agreement with the conventional rule that a titer of 40 is the 50% protective dose. Tsang et al found that an HI titer of 40 corresponded to just 31% protection [95% CI 13% - 46%] against seasonal H1N1 infection following previous natural infection. Though there is a disparity in measures of the 50% protective dose between the two papers, both agree on the general shape of the curve relating protection to HI titer.

HI titers against H1N1 gradually decrease following their high point after natural infection or vaccination, though the slope of the decrease varies between studies. Delabre et al[22] studied patients infected with seasonal H1N1. Pre-infection titers of approximately 35 (1:35) rose to 54 following infection and slowly declined to 41.5 two years later, remaining significantly higher than pre-infection levels. A study at Sridhar et al[23] found no significant decline in HI titers against H1N1pdm09 following the initial increase after infection over a period of 480 days. The ending geometric mean titer was 47.66 [95% CI 24.45 – 92.89], remaining above the conventionally accepted protective threshold of 40. A study by Song et al[24] studied vaccination using a monovalent unadjuvanted H1N1pdm09 vaccine and found that titers of 146.1 one month post-vaccination sharply decreased to 47.4 six months post-vaccination, then steadied and were found to be 40.9 ten months post-vaccination. Taken together, these data suggest that if the vaccinated population renews immunizations annually, serum antibodies could remain at the 50% protective level throughout the year.

Interestingly, several studies have documented enduring immunity persisting decades following initial exposure to a virus through natural infection or vaccination. Yu et al[25] found evidence of long-lasting immunity approximately 90 years after the 1918 influenza pandemic. In subjects born in 1915 or earlier, who presumably would have been exposed to 1918 H1N1, 100% of the group exhibited serum neutralizing titers of at least 40 against 1918 H1N1 and 94% exhibited HI titers of at least 40. In contrast, just 20-30% of subjects born between 1926 and 1955 exhibited serum neutralizing titers and HI titers at

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18 Hannoun C, Megas F, Piercy J. (2004) Immunogenicity and protective efficacy of influenza vaccination. *Virus research.* 103 (1-2)**:** 133-138.

19 Center for Biologics Evaluation and Research - Food and Drug Administration. (2007) Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines.

20 Ng S et al. (2013) Estimation of the association between antibody titers and protection against confirmed influenza virus infection in children. *The Journal of infectious diseases.* 208 (8)**:** 1320-1324.

21 Tsang TK et al. (2014) Association between antibody titers and protection against influenza virus infection within households. Ibid.210 (5)**:** 684-692.

22 Delabre RM et al. (2015) Antibody persistence and serological protection among seasonal 2007 influenza A(H1N1) infected subjects: Results from the FLUREC cohort study. *Vaccine.* 33 (49)**:** 7015-7021.

23 Sridhar S et al. (2015) Longevity and determinants of protective humoral immunity after pandemic influenza infection. *American journal of respiratory and critical care medicine.* 191 (3)**:** 325-332.

24 Song JY et al. (2012) Comparison of the long-term immunogenicity of two pandemic influenza A/H1N1 2009 vaccines, the MF59-adjuvanted and unadjuvanted vaccines, in adults. *Clinical and vaccine immunology : CVI.* 19 (5)**:** 638-641.

25 Yu X et al. (2008) Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature.* 455 (7212)**:** 532-536.

these levels. Another study by McCullers et al[26] found enduring immunity following vaccination against 1976 H1N1, which is antigenically similar to the H1N1pdm09. Comparison of two groups of hospital workers, one group vaccinated against 1976 H1N1 in 1976 and one group not vaccinated, revealed a significant difference in the percentage of subjects with MN titers of at least 160 against H1N1pdm09. This study was based on samples gathered in 2009 prior to widespread circulation of the pandemic strain. These studies demonstrate that infection or vaccination with 1918-like H1N1 strains (i.e. 1918, 1976, or 2009 H1N1) triggers long-lasting immune memory, including for antigenically similar strains, despite the gradual decline of protective antibodies over time.

[26] McCullers JA et al. (2010) Recipients of vaccine against the 1976 "swine flu" have enhanced neutralization responses to the 2009 novel H1N1 influenza virus. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 50 (11)**:** 1487-1492.