Inferring influenza A infection attack rates from serologic data allowing for cross-reactive antibody responses

Benjamin J. Cowling[1], Vicky J. Fang[1], Alex R. Cook[2]

Affiliation(s): [1]School of Public Health, University of Hong Kong
[2]Department of Statistics, National University Singapore

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Context – household studies of flu in Hong Kong

- Randomized trial of hand hygiene and face masks to prevent flu transmission in households
  - 2007 – 128 households (Cowling et al. 2008 PLoS ONE).
  - 2008 – 322 households (Cowling et al. 2009 Ann Intern Med).
- Transmission study during and after the pandemic
  - 2009 – 99 households (Cowling et al. 2010 New Engl J Med).
  - 2010-11 – 78+ households (unpublished).

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- Transmission study during and after the pandemic
  - 2009 – 99 households (Cowling et al. 2010 New Engl J Med).
  - 2010-11 – 78+ households (unpublished).
- Indirect benefits of influenza vaccination in households (cohort).
  - 2008-09 – 119 households (Cowling et al. 2010 Clin Infect Dis).
  - 2009-10 – 796 households (unpublished).
  - 2010-11 – 599 households continuing follow-up.

Vaccination study design

Does vaccination of school-age children against seasonal influenza confer any indirect benefit to their household contacts?

- Cluster-randomized (at household level), placebo-controlled, double-blind study.
- One child in each household received either
  1. One dose of trivalent inactivated vaccine (60%)
  2. 0.5ml saline (placebo control) (40%)
- Periodic serology, intense illness follow-up.

Pilot study – timing of serology and TIV administration

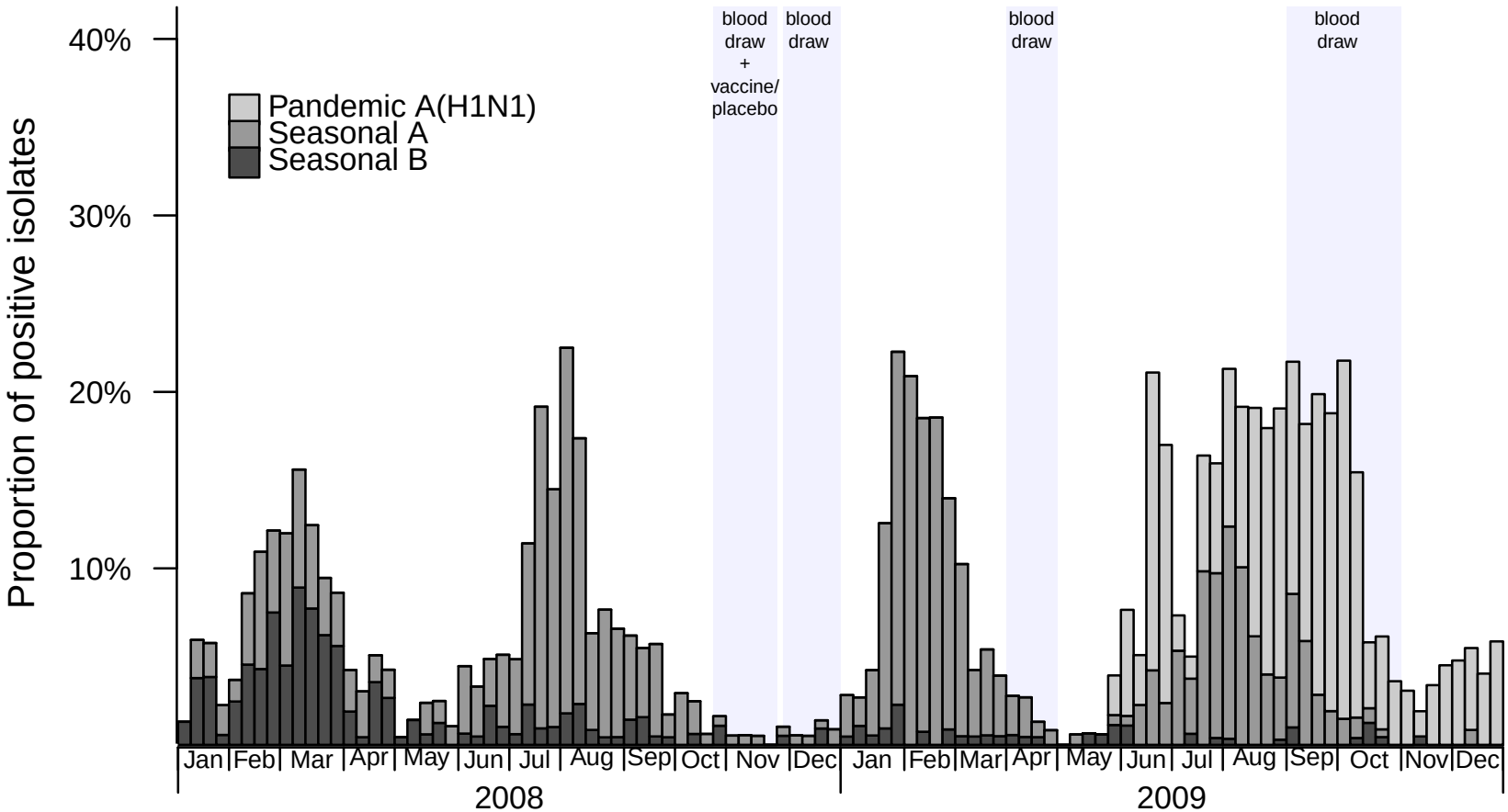

Figure: Study timeline versus virological surveillance from QMH

Cumulative incidence of infection in TIV/placebo recipients

	Vaccine		Placebo		p-value
	est.	(95% CI)	est.	(95% CI)	
Serologically-confirmed influenza*					
seasonal A/H1N1	0.08	(0.02, 0.15)	0.21	(0.09, 0.32)	0.10
seasonal A/H3N2	0.07	(0.01, 0.13)	0.12	(0.03, 0.22)	0.49
seasonal B	0.03	(0.00, 0.07)	0.08	(0.01, 0.16)	0.36
pandemic A/H1N1	0.32	(0.22, 0.43)	0.17	(0.06, 0.27)	0.09
PCR-confirmed influenza A	0.08	(0.03, 0.17)	0.08	(0.02, 0.20)	1.00
PCR-confirmed influenza B	0.00	(0.00, 0.05)	0.02	(0.00, 0.06)	0.84
Influenza-like illness (ILI)†	0.35	(0.24, 0.46)	0.38	(0.24, 0.51)	0.95
Acute respiratory infection (ARI)‡	0.66	(0.55, 0.77)	0.67	(0.53, 0.80)	0.89

* 4-fold rise in antibody titre by HAI (seasonal) or microneutralization (pandemic).

† ILI is fever$\geq$37.8°C plus cough or sore throat

‡ ARI is at least 2 of fever$\geq$37.8°C, cough, sore throat, phlegm, runny nose, muscle pain, myalgia.

Cumulative incidence of infection in household contacts

	Vaccine		Placebo		p-value
	est.	(95% CI)	est.	(95% CI)	
Serologically-confirmed influenza*					
seasonal A/H1N1	0.13	(0.08, 0.17)	0.14	(0.08, 0.20)	0.91
seasonal A/H3N2	0.21	(0.15, 0.26)	0.16	(0.10, 0.23)	0.41
seasonal B	0.06	(0.02, 0.09)	0.10	(0.05, 0.15)	0.28
pandemic A/H1N1	0.17	(0.12, 0.23)	0.14	(0.08, 0.20)	0.48
PCR-confirmed influenza A	0.07	(0.03, 0.12)	0.03	(0.01, 0.08)	0.29
PCR-confirmed influenza B	0.00	(0.00, 0.02)	0.00	(0.00, 0.03)	1.00
Influenza-like illness (ILI)†	0.16	(0.11, 0.22)	0.10	(0.06, 0.17)	0.29
Acute respiratory infection (ARI)‡	0.34	(0.28, 0.41)	0.28	(0.20, 0.36)	0.26

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Risk factors for pH1N1

Table: Factors affecting risk of pandemic H1N1 among all participants

	Lab-confirmed pH1N1*	
	AOR†	(95% CI)
Age (years)		
≤ 15	6.60	(2.17, 20.13)
$16 - 45$	2.53	(0.80, 7.99)
> 45	1.00	
Seasonal influenza during study‡	0.35	(0.14, 0.87)
Received 2008-09 seasonal TIV	1.11	(0.54, 2.26)

* 4-fold rise in antibody titre to A/CA/2009 or infection confirmed by RT-PCR

† Adjusted Odds Ratio also adjusted for sex and date of completion of study

‡ Seasonal influenza infection indicated by 4-fold rise in antibody titer or confirmed by RT-PCR

Motivation

Table: Cumulative incidence of infection based on serology

	Children		Adults	
	Estimate	(95% CI)	Estimate	(95% CI)
pandemic A/H1N1	0.23	(0.17, 0.30)	0.08	(0.04, 0.12)
seasonal A/H1N1	0.06	(0.03, 0.11)	0.03	(0.01, 0.06)
seasonal A/H3N2	0.12	(0.07, 0.17)	0.06	(0.03, 0.10)
seasonal B	0.02	(0.00, 0.05)	0.01	(0.00, 0.05)

- Only have virologic confirmation of around 15% of these infections.
- Antibody titer rise could be associated with cross-reaction rather than infection with the same strain; imperfect sensitivity and specificity of 4-fold rise criteria.
- How to account for these when estimating influenza attack rates?

HI test

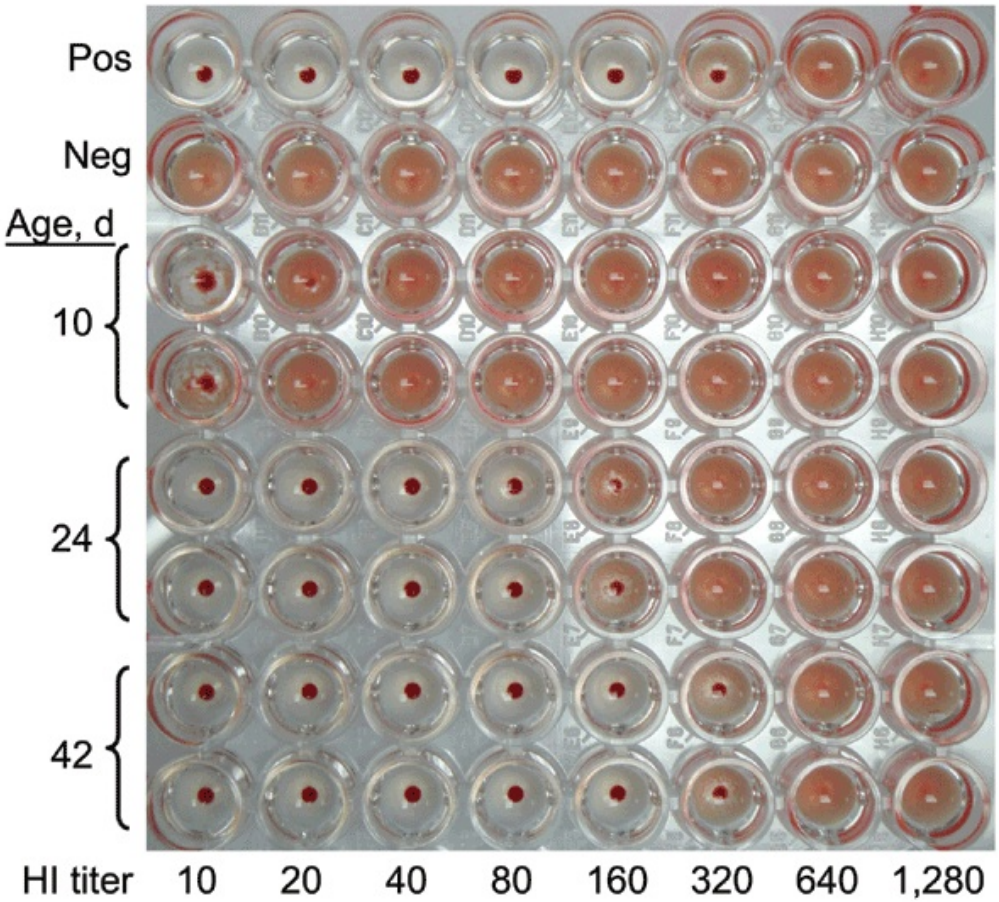

Figure: Example of an HI panel. The red button indicates antibody is present. Final sera (42d) has HI titer of 1:320.

Identifying infections from serology

- In analysis of paired sera collected before and after a period of influenza activity, a rise in antibody response to a particular strain of influenza could due to:
  - infection with that particular strain, or
  - infection with another strain (i.e. a cross-reactive response), or
  - vaccination, or
  - no infection – false positive.

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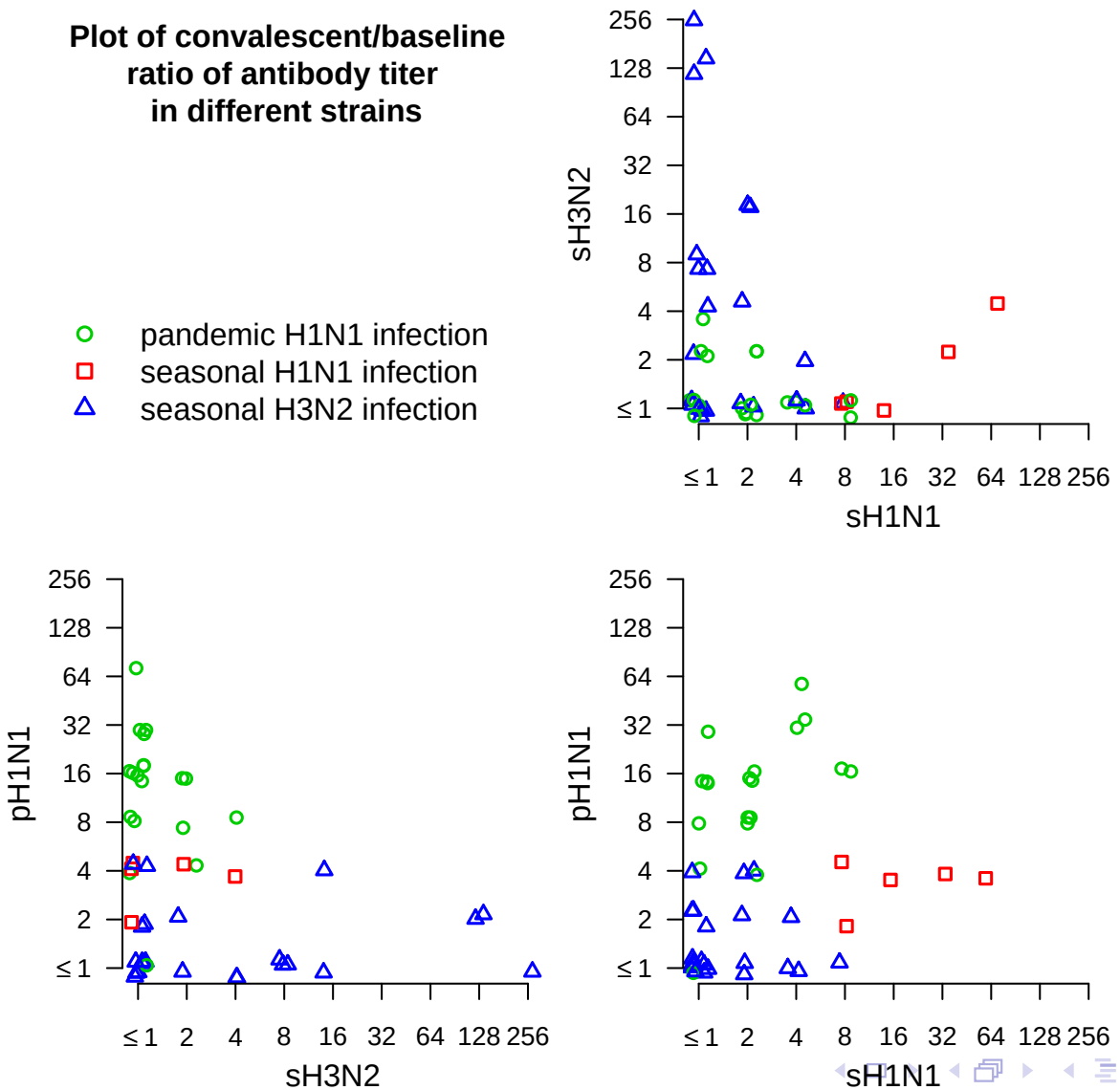
  - vaccination, or
  - no infection – false positive.
- Some infected individuals may not have a substantial rise in antibody.
- How is the risk of antibody titer rise associated with infection with homologous or heterologous influenza strains?

Household transmission study (2009)

- Recruited index cases from outpatient clinics during summer 2009 in Hong Kong.
- Home visit arranged within 36 hours (usually within 12h).
- Nose and throat swabs from all household members at initial visit and after 3 and 6 days regardless of illness.
- Blood draws from a subset on days 0 (baseline), and 21-30 (convalescent) for serologic testing.
- Define antibody titer rise as 4-fold or greater rise in antibody titers and the convalescent antibody titer at least 1:40.

Antibody titer rises after confirmed infections



A model for infection vs antibody titer rise

- $k = 1, 2, 3$ indexes influenza virus strains (pH1N1/sH1N1/sH3N2).
- $X_{ki} = 1$ if individual i had confirmed infection with the respective strain k, set $X_{0i} = 1$ if no infection. (observed data)
- $Y_{ki} = 1$ if individual i had a 4-fold or greater rise in antibody titer against strain k and the convalescent titer $\geq 1 : 40$. (also observed data)

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- $Y_{ki} = 1$ if individual i had a 4-fold or greater rise in antibody titer against strain k and the convalescent titer $\geq 1 : 40$. (also observed data)
- p_k are the probabilities of developing a 4-fold or greater rise in antibody titer against the infecting (homologous) strain. After preliminary investigation set $p_1 = p_2$.
- $q_{jk,\ j \neq k}$ are the probabilities of developing 4-fold or greater rise in antibody titer against different strains. Assume symmetry i.e. $q_{12} = q_{21}, q_{13} = q_{31}, q_{23} = q_{32}$.

A model for infection vs antibody titer rise

$$\begin{cases} \delta_{1i} = p_1 X_{1i} + q_{12} X_{2i} + q_{13} X_{3i} \\ \delta_{2i} = q_{12} X_{1i} + p_1 X_{2i} + q_{23} X_{3i} \\ \delta_{3i} = q_{13} X_{1i} + q_{22} X_{2i} + p_3 X_{3i} \end{cases}$$

$$Y_{ki} \sim Bernoulli(b + (c - b)\delta_{ki}), k = 1, 2, 3.$$

$$(X_{0i}, X_{1i}, X_{2i}, X_{3i}) \sim Multinom(1, (\alpha_0, \alpha_1, \alpha_2, \alpha_3))$$

- Note that ≥ 1 infections are not permitted; $X_{0i} + X_{1i} + X_{2i} + X_{3i} = 1$.
- δ_{ki} represents i's probability of a 4-fold or greater rise against strain k.
- Set $(\alpha_0, \alpha_1, \alpha_2, \alpha_3) \sim Dirichlet(1, 1, 1, 1)$.
- Specify non-informative priors for each parameter.
- Implement the model using MCMC.

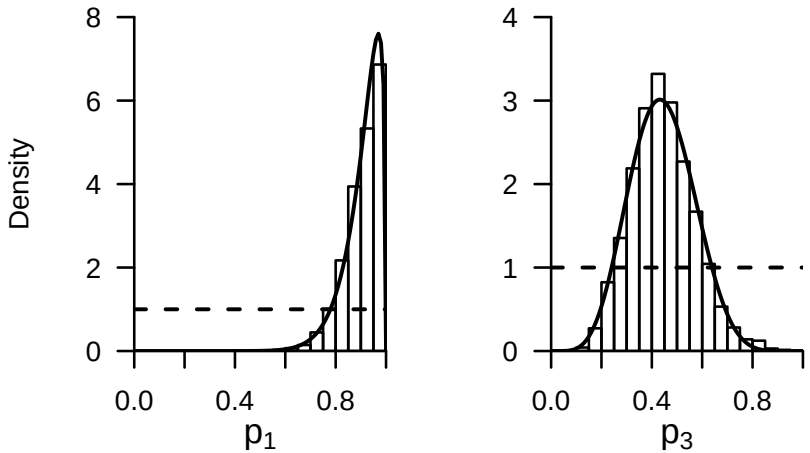
Data

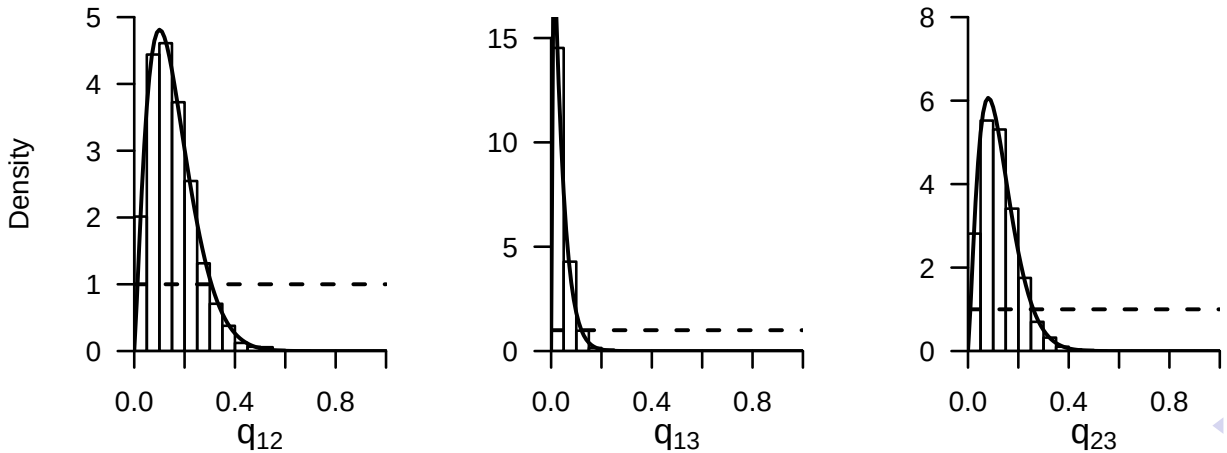
- Obtained complete swab and sera samples from 138 participants.
- The proportion of RT-PCR-confirmed infections were 14%, 4%, and 17% for pH1N1, sH1N1 and sH3N2, while the corresponding proportions of individuals with 4-fold or greater rises in antibody titer were 13%, 9%, and 10%.

	Antibody titer rise		
	pH1N1	sH1N1	sH3N2
RT-PCR-confirmed influenza			
pandemic A/H1N1 ($n = 19$)	84%	11%	5%
seasonal A/H1N1 ($n = 5$)	20%	100%	20%
seasonal A/H3N2 ($n = 23$)	0%	9%	39%
No infection ($n = 91$)	1%	4%	3%

Fitted model

Prior distributions (dashed lines), posterior samples (histograms) and smoothed posterior distributions (solid line) for risk of developing 4-fold or greater rise in homologous (first row) or heterologous (second row) antibody titers.





	Risk of antibody titer rise		
	pH1N1	sH1N1	sH3N2
Infection			
pH1N1	91%	15%	4%
sH1N1		91%	12%
sH3N2			44%

Infer infection rates in the cohort study

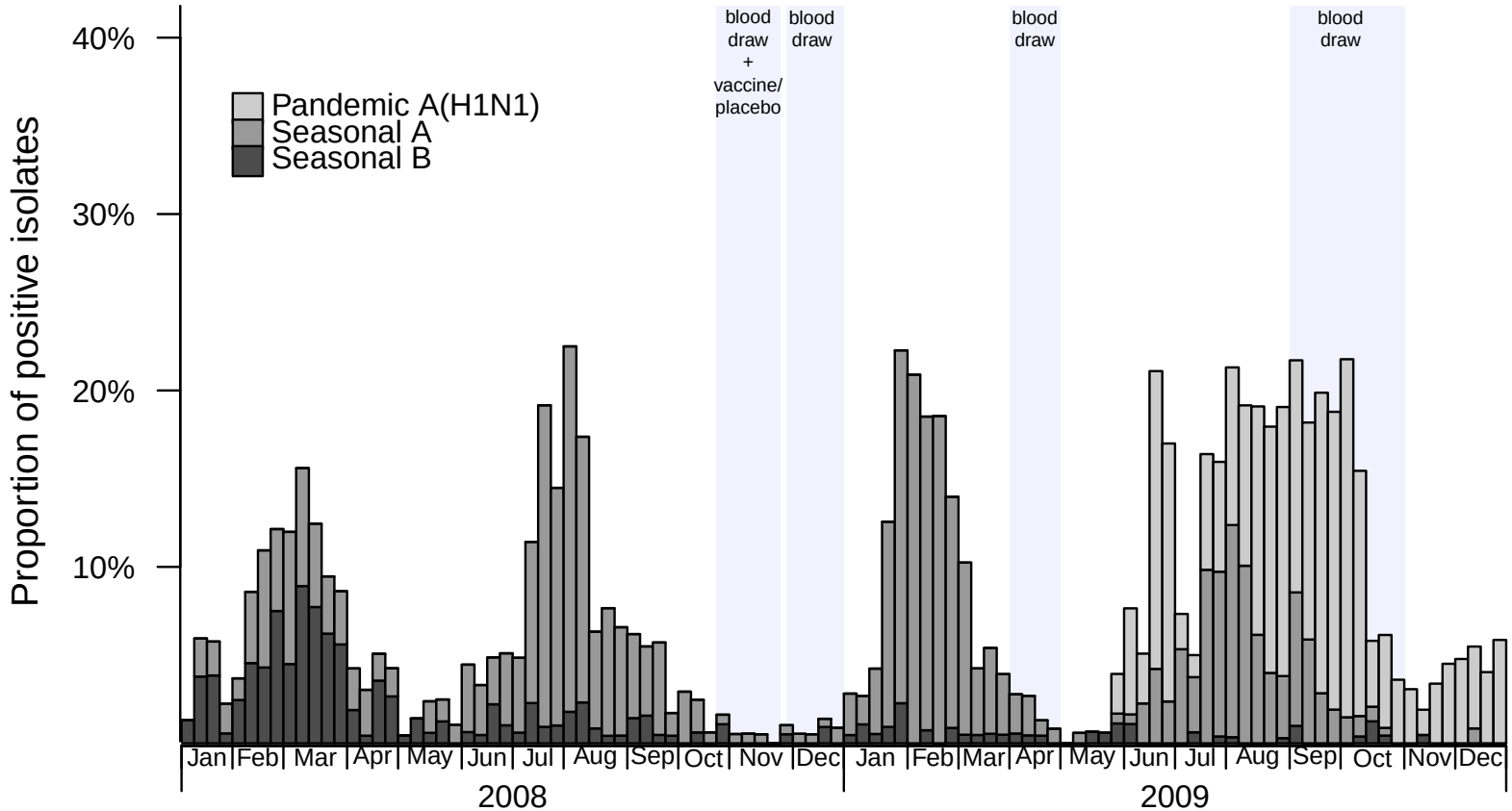


Figure: Study timeline versus local inpatient virological surveillance.

Current analyses focus on the period Apr 2009 through Sep-Oct 2009.

Data analysed

- Paired sera were collected from 376 individuals over the period April to September-October 2009.
- 28% of individuals had a 4-fold or greater antibody titer rise against at least one strain.
- Limited swab samples due to infrequent home visits – considered the infection data ($X_{\cdot i}$) as missing.
- Idea – infer the 'true' infection rates for each individual through the model constructed from the household transmission study.
- Incorporated the posteriors from the household transmission study as priors for this analysis.

Estimated infection rates

Table: Observed antibody titer rises and estimated infections for influenza A by subtype, in the cohort study.

Flu type	Observed 4-fold rise		Estimated infection	
	Estimate	(95% CI)	Estimate	(95% CI)
Pandemic H1N1	0.19	(0.15, 0.23)	0.17	(0.14, 0.20)
Seasonal H1N1	0.05	(0.03, 0.08)	0.02	(0.02, 0.03)
Seasonal H3N2	0.10	(0.07, 0.14)	0.16	(0.15, 0.18)

Illness episodes

- Daily symptom diaries kept by each participant.
- Acute respiratory illness (ARI) – 2+ signs or symptoms: measured temperature $\geq 37.8°C$, headache, cough, sore throat, aches or pains in muscles, runny nose, and phlegm.
- Influenza-like illness (ILI) – temperature $\geq 37.8°C$ plus cough or sore throat.
- Count number of ILI and ARI episodes for each participant.
- Aim – to estimate the risk of illness conditional on infection.

Expanded model

- Denote the number of ARI (ILI) episodes for each individual as $nARI_i (nILI_i)$, caused by influenza virus $(nARI_i^{flu}, nILI_i^{flu})$ or other non-flu-A virus $(nARI_i^{other}, nILI_i^{other})$.
- Let θ^p_{ARI} (θ^p_{ILI}) be the probability of developing ARI (ILI) episode if infected with pH1N1, and θ^s_{ARI} (θ^s_{ILI}) if infected with sH1N1/sH3N2.
- Let λ_{ARI} (λ_{ILI}) be the rate of ARI (ILI) episodes not associated with influenza A over the follow-up period.

Fitting expanded model

- Use posterior distributions from Model 1 (transmission study) as priors here, specify non-informative priors for θ, λ.
- Analyzed children and adults separately.
- Problems with data quality ... subjects who did not return any diary, returned a blank symptom diary or never record any symptom throughout the study period were treated as having missing data on ARI/ILI episodes.

Risk of illness – children

Table: ARI/ILI episodes among 164 children.

	ARI	(95% CI)	ILI	(95% CI)
Probability of develop illness episode if infected with				
Pandemic H1N1	0.83	(0.43, 1.00)	0.73	(0.42, 0.97)
Seasonal H1N1/H3N2	0.72	(0.13, 1.00)	0.53	(0.06, 0.93)
Average number of illness episodes not associated with influenza A	0.55	(0.40, 0.75)	0.09	(0.03, 0.16)

Risk of illness – adults

Table: ARI/ILI episodes among 212 adults.

	ARI	(95% CI)	ILI	(95% CI)
Probability of develop illness episode if infected with				
Pandemic H1N1	0.83	(0.42, 1.00)	0.76	(0.39, 0.98)
Seasonal H1N1/H3N2	0.15	(0.00, 0.48)	0.07	(0.00, 0.25)
Average number of illness episodes not associated with influenza A	0.34	(0.26, 0.47)	0.04	(0.02, 0.06)

Conclusions

- Most 4-fold or greater antibody titer rise to pH1N1/sH3N2 corresponded to infection with that strain, but sH1N1 rises tended to be cross-reactions.
- Prevalent H3N2 virus in summer 2009 was a drift variant and the transmission study suggested that the corresponding HI test was not sensitive to infection, allowing correction of cumulative infection estimates in the cohort study.
- Adults have lower risk of illness if infected with seasonal influenza than children? Or just less likely to report?

Limitations

- Did not incorporate household structure (should provide additional information on risk of infection with specific strains).
- Did not condition infection status on illness data (yet).
- Very mild infections may not be confirmed, perhaps biasing antibody dynamics in model 1?

Future plans

- Incorporating household structure.
- HI tests for other strains, incorporate virus sequencing.
- Modeling exact antibody titers using mixture models, rather than 4-fold rises
  - cross-reactions may tend to be smaller rises.
  - can allow for falls in titers over time (particularly after vaccination).
  - could facilitate analysis of paired sera without parallel testing.

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  - can allow for falls in titers over time (particularly after vaccination).
  - could facilitate analysis of paired sera without parallel testing.
- Apply methods to larger datasets / longer follow-up.
  - Including effects of covariates (risk factors) on the latent infection status.
  - Duration/strength of immunity against reinfection.

Reproducibility

- Concerns about reproducibility of epidemiologic research,
- e.g. Peng 2006 AJE – "The replication of important findings by multiple independent investigators is fundamental to the accumulation of scientific evidence. . . . However, because of the time, expense, and opportunism of many current epidemiologic studies, it is often impossible to fully replicate their findings. An attainable minimum standard is reproducibility, which calls for data sets and software to be made available for verifying published findings and conducting alternative analyses."

Acknowledgments

- Household studies run in collaboration with Gabriel Leung, Malik Peiris.
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THE END