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Monitoring the fitness of antiviral-resistant influenza strains during an epidemic: A mathematical modeling study

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Summary

Background

Antivirals (e.g. oseltamivir) are important for mitigating influenza epidemics. In 2007, an oseltamivir-resistant seasonal A(H1N1) strain emerged and spread to global fixation within one year. This showed that antiviral-resistant (AVR) strains can be intrinsically more transmissible than their contemporaneous antiviral-sensitive (AVS) counterpart. Surveillance of AVR fitness is therefore essential.

Methods

We define the fitness of AVR strains as their reproductive number relative to their co-circulating AVS counterparts. We develop a simple method for real-time estimation of AVR fitness from surveillance data. This method requires only information on generation time without other specific details regarding transmission dynamics. We first use simulations to validate this method by showing that it yields unbiased and robust fitness estimates in most epidemic scenarios. We then apply this method to two retrospective case studies and one hypothetical case study.

Findings

We estimate that (i) the oseltamivir-resistant A(H1N1) strain that emerged in 2007 was 4% (3-5%) more transmissible than its oseltamivir-sensitive predecessor and (ii) the oseltamivir-resistant pandemic A(H1N1) strain that emerged and circulated in Japan during 2013-2014 was 24% (17-30%) less transmissible than its oseltamivir-sensitive counterpart. We show that in the event of large-scale antiviral interventions during a pandemic with co-circulation of AVS and AVR strains, our method can be used to inform optimal use of antivirals by monitoring intrinsic AVR fitness and drug pressure on the AVS strain.

Conclusions

We have developed a simple method that can be easily integrated into contemporary influenza surveillance systems to provide reliable estimates of AVR fitness in real time.

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Influenza antiviral drugs are important for mitigating influenza epidemics. The neuraminidase (NA) inhibitor oseltamivir is the most commonly used influenza antivirals (1) and has been extensively stockpiled by many countries for pandemic preparedness (2). The effectiveness of antivirals is threatened by emergence and spread of antiviral resistance (AVR) viruses. For oseltamivir, the most commonly detected resistance mutation in A(H1N1) viruses is the NA H275Y substitution. Before 2007, emergence of oseltamivir-resistant influenza viruses were sporadically reported, and the fitness of detected resistant viruses had always been substantially compromised (3). As such, there was a consensus that AVR influenza viruses would always be outcompeted by their antiviral-sensitive (AVS) counterparts, and hence posed only minimal threat to public health.

Such conventional wisdom was refuted by events in 2007-2008 – a new oseltamivir-resistant A(H1N1) virus emerged and displaced its contemporaneous oseltamivir-sensitive counterpart to become the dominant A(H1N1) strain globally within only 12 months (4). The emergence and rapid fixation of this oseltamivir-resistant virus was not driven by widespread use of oseltamivir (4, 5). This event thus proved that AVR viruses are not necessarily less transmissible than their AVS counterparts. Furthermore, in the context of large-scale antiviral intervention during a pandemic, AVR fitness may be enhanced by the drug pressure on the AVS strain such that an intrinsically less transmissible AVR strain may become more fit than the AVS strain. Timely and accurate assessment of AVR fitness is therefore essential for informing situational awareness and optimal use of antivirals during both inter-pandemic and pandemic periods (6).

The spread of AVR influenza viruses can increase morbidity and mortality. For example, case-fatality risk may increase because antivirals would be ineffective for treating AVR cases. Furthermore, if AVR viruses spread during the early stage of a pandemic, populations at the downstream of global spread will be subject to substantial importation and hence higher incidence of AVR cases (7). In view of such risks, national and supranational agencies, especially the WHO's Global Influenza Surveillance and Response System (GISRS), have emphasized the need for timely and accurate assessment of AVR fitness (8). However, few advances have been made in data analytics and performance evaluation for AVR surveillance systems. Our objective is to help fill this knowledge gap by developing a simple method for estimating AVR fitness from surveillance data.

Methods

The model

We assume that there is only one transmissible AVR strain over the course of a single epidemic wave constituted by the A subtype or B lineage to which the AVR strain and its antiviral-sensitive counterpart (the AVS strain) belong. We define the intrinsic AVR fitness as the ratio of the basic reproductive number of the AVR strain to that of the AVS strain ($\sigma_0 = R_0^R / R_0^S$). Similarly, we define AVR fitness as the ratio of their reproductive numbers ($\sigma = R^R / R^S$) which encapsulates the combined effect of intrinsic fitness and any reduction in AVS transmissibility due to antiviral interventions.

We formulate our model under the following base case assumptions:

1. The AVS and AVR strains co-circulate during the epidemic.
2. Without antiviral treatment, AVS and AVR infections have the same severity such that all infections are equally likely to be selected for AVR testing.
3. Recovery from infection with either strain provides complete cross-protection against both strains during the epidemic.
4. The effect of viral interference (if any) caused by all other circulating influenza viruses (i.e. those from other subtypes and lineages) and pathogens are the same for both strains.
5. AVR fitness does not depend on age.
6. Age-specific susceptibility to the AVR virus is the same as that to the AVS virus.

Assumptions 5 and 6 are relatively less likely to hold, e.g. high-risk groups may be more likely to receive antiviral prophylaxis, susceptibility to the AVR virus may be different from that to the AVS virus (9). In the Appendix (see Appendix page 5), we extend our method to allow relaxation of these two assumptions.

Under the base case assumptions, the next generation matrix of AVR infections is simply σ times that of AVS infections. This remains true in the presence of seasonal forcing and interventions such as vaccination and school closure because transmission of the AVS and AVR strain are identically affected by these factors (see Appendix page 2). As the epidemic unfolds, the proportion of infections that are AVR, denoted by $\rho(t)$, will increase towards 1 if $\sigma > 1$, remain at the same level if $\sigma = 1$, and decline towards 0 if $\sigma < 1$. The key step of our method is to approximate $\rho(t)$ using the equation

$$\rho(t) = \frac{\int_0^t \sigma g^R(t-a)\rho(a)i(a)da}{\int_0^t \sigma g^R(t-a)\rho(a)i(a)da + \int_0^t g^S(t-a)(1-\rho(a))i(a)da} \quad (1)$$

where $i(t)$ is the total incidence rate of AVR and AVS infections, g^R and g^S are the generation time distributions for AVR and AVS infections, respectively. To verify the accuracy of this approximation, we randomly generate 100 epidemic scenarios driven by the UK contact matrix (10) with four age groups (0-5, 6-18, 18-65, and >65) using Latin-hypercube sampling from the following parameter space which covers a wide range of plausible epidemics:

- Initial susceptible proportion of each age group between 0.3 and 1;
- Initial reproductive number of the AVS strain ($R^S(0)$) between 1.2 and 3;
- Mean generation time (T_g) between 2 and 4 days;
- Intrinsic AVR fitness (σ_0) between 0.8 and 1.2;
- The proportion of seeding infections that are AVR between 0.1 and 0.9;

Figure A1 (see Appendix page 8) shows that the approximation in equation (1) is very accurate. As such, given $i(t)$ or a proxy of it (see below) and the generation time distribution for both strains, equation (1) allows us to accurately describe $\rho(t)$ without knowing other epidemiologic details such as basic reproductive number, contact matrix, symptomatic proportion, seasonality, etc.

Inference of AVR fitness

Our method requires the following two streams of data (for the subtype or lineage under investigation):

1. The incidence rate $i(t)$ or its proxy, e.g. based on the daily number of laboratory confirmed infections in the Hong Kong E-Flu system (11), Flu Near You (12), or other proxies used for calculating influenza excess mortality (13). We denote this data stream by $\tilde{i}(t)$. These data are typically confounded with temporal fluctuation in reporting rate and laboratory testing capacity. Our method, however, is robust against such fluctuation (see Results).
2. Data from AVR surveillance where Z_d^R and Z_d^S are the number of influenza positive isolates tested on day *d* that are found to be positive and negative for AVR, respectively. The subjects selected for AVR testing should (i) have not been treated with antivirals for their infection and (ii) have no recent travel history to avoid misclassifying imported cases as local cases.

We substitute $i(t)$ with its proxy $\tilde{i}(t)$ in equation (1) and denote the resulting approximation by $\tilde{\rho}(t)$. The approximate likelihood is

$$\prod_d \binom{Z_d^S + Z_d^R}{Z_d^R} p_d{}^{Z_d^R} (1-p_d)^{Z_d^S}$$

where $p_d = p_{sens}\int_d^{d+1} \tilde{\rho}(t)\,dt + (1-p_{spec})\left(1-\int_d^{d+1} \tilde{\rho}(t)\,dt\right)$, p_{sens} and p_{spec} are the sensitivity and specificity of AVR testing. With this likelihood and uniform priors, we estimate AVR fitness σ using Markov Chain Monte Carlo methods (see Appendix page 3).

Validation of the AVR fitness inference method

To validate our method, we simulate 100 stochastic realizations of the data streams for each of the 100 epidemic scenarios generated earlier assuming that (i) daily reporting proportions are uniform random variables ranging between 0.5% and 2%; and (ii) daily AVR testing capacity is 2, 5, 10, 20 or 80 isolates. AVR fitness is then inferred at the end of each epidemic.

Case Studies

After validating our method, we apply it to three case studies:

1. *A retrospective study of the oseltamivir-resistant influenza A(H1N1) virus in 2007 – 2008.* To estimate the (intrinsic) fitness of this oseltamivir-resistant strain in comparison to its oseltamivir-sensitive predecessor, we retrieve the data on influenza virus activity and AVR surveillance for 10 countries/regions from published literature and public online data (**Tables A1-A3 on page 13-17 of Appendix, Figure 3**). We assume that AVS and AVR infections had the same generation time distribution because there is no published evidence that indicates the contrary. Based on published serial interval estimates, we assume that the generation time distribution was lognormal with mean 2.8 days and coefficient of variation 0.54 (14). We first obtain a pooled estimate of AVR fitness by assuming that AVR fitness was the same in all populations. We then estimate AVR fitness in each population separately and compare them.
2. *A retrospective study of the oseltamivir-resistant influenza A(H1N1)pdm09 virus in Japan during 2013-2014.* Although 98% of the tested A(H1N1)pdm09 virus isolates were sensitive to oseltamivir by 2014 (8), large clusters of oseltamivir-resistant variants were detected in

Newcastle, Australia in 2011 (15) and Hokkaido, Japan in 2013-2014 (16). In the Japan cluster, the oseltamivir-resistant virus was causing community outbreaks until it was displaced by its oseltamivir-sensitive counterpart (**Figure 2**). We apply our method to estimate the fitness of this oseltamivir-resistant strain using published data (16) and the generation time distribution in case study 1.

3. *A hypothetical study of AVR fitness and drug pressure under large-scale antiviral interventions during a pandemic*. Oseltamivir resistance is not uncommon among influenza viruses with pandemic potential, e.g. avian influenza A(H5N1) (17) and A(H7N9) viruses (18). We consider a hypothetical but realistic situation in which large-scale antiviral interventions, comprising both prophylaxis and treatment, are implemented during a pandemic that comprises co-circulation of AVS and AVR viruses (7, 19-21). The epidemic parameters are the same as that in **Figure 2** with all individuals susceptible at time 0. We consider situations in which (i) the AVR strain is intrinsically less transmissible than the AVS strain with $\sigma_0 = 0.95$; and (ii) large-scale antiviral interventions reduce the AVS reproductive number by a proportion μ such that drug pressure renders the AVS strain less transmissible than the AVR strain, i.e. $\sigma = \sigma_0/(1-\mu) > 1$. We consider 10%, 15% and 20% coverage of antiviral prophylaxis that reduces susceptibility to the AVS virus by 81% (22); this corresponds to μ = 0.08, 0.12 and 0.16, respectively. We assume that σ_0, σ and μ are unknown a priori and demonstrate how our method can be used to estimate them in real-time to inform optimal use of antivirals. Specifically, if AVR fitness is consistently estimated to exceed 1 with high probability (say, above 0.9 for one week), then there is compelling evidence that an increasing proportion of severe cases would be AVR and hence not treatable with the antiviral. We assume that in response to this alert, antiviral use would be suspended except for treating high-risk and severe cases as policymakers deliberate (i) how to strategically adjust antiviral use to strike a balance between reducing transmission of AVS infections and increasing the number of severe AVR infections , and (ii) whether alternative treatment options such as convalescent plasma and antivirals with different resistance mechanisms should be considered (7, 20, 21, 23). The objective of this case study is to demonstrate how estimates of σ_0 and μ can be used to build an evidence base for this decision-making process.

Role of the funding sources

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Validating the method for estimating AVR fitness

Figure 1 summarizes the accuracy and precision of AVR fitness estimates across a wide range of plausible epidemic scenarios when AVR testing sensitivity and specificity are both 100% (a reasonable assumption for genotypic testing). The reliability of fitness estimates depends on epidemic characteristics mainly via the time span, expressed in terms of number of generation intervals, during which the AVS and AVR strains are both circulating in significant proportions. Fitness estimates are largely unbiased unless this time span is below 10 generation intervals (around 30 days) and AVR testing capacity is low (<5 samples per day). Increasing the daily testing capacity beyond 20 samples provides little improvement in the fitness estimates. The accuracy and precision of fitness estimates deteriorate significantly when testing sensitivity and specificity are both reduced to 90% which has a similar effect as halving the testing capacity (**Figure A2 on page 9 of Appendix**).

Timeliness of AVR fitness estimates

Figure 2 illustrates the timeliness of reliable AVR fitness estimates for one stochastic realization of an exemplary epidemic scenario. The AVS and AVR reproductive numbers differ by 5% which is sufficiently high to result in fixation within a single epidemic wave. The daily AVR testing capacity is 10 samples, a modest level for well-resourced populations like Hong Kong. Our method correctly predicts which virus would become dominant with posterior probability consistently above 0.9 as early as three weeks before the epidemic peak. However, stochasticity has a strong impact on the timeliness of reliable fitness estimates. **Figures A3** (see Appendix page 10) shows two alternative realizations of the same epidemic scenarios in which reliable fitness estimates are available a couple of weeks sooner or later than in **Figure 2**.

Case study 1: Oseltamivir-resistant influenza A(H1N1) virus, 2007 – 2008

The pooled (intrinsic) AVR fitness estimate is 1.04 (95% credible interval 1.03-1.05), i.e. the oseltamivir-resistant strain was 4% (3%-5%) more transmissible than its contemporaneous oseltamivir-sensitive counterpart (**Figure 3**). The fitness estimate increases (decreases) by 0.01

when we increase (decrease) T_g by one day. If the data were available in real-time, reliable fitness estimates would have been available by late February 2008, which was 15 weeks after the oseltamivir-resistant virus was first identified in Norway and months before it became dominant in populations outside Europe (24). If we estimate AVR fitness in each population separately, the results suggest that the oseltamivir-resistant strain was more transmissible than the oseltamivir-sensitive strain only in Canada, Luxembourg, the UK, Germany and France, but not in the other five populations (**Figure 3**). In particular, there is no strong evidence that the oseltamivir-resistant strain was more transmissible than its oseltamivir-sensitive counterpart in Japan (25). The intrinsic AVR fitness estimates remain unchanged when the effect of drug pressure in Japan is explicitly modelled (see Appendix page 4).

Case study 2: Oseltamivir-resistant influenza A(H1N1)pdm09 virus in Japan, 2013-2014

We estimate that this oseltamivir-resistant A(H1N1)pdm09 virus was 24% (17%-30%) less transmissible than the oseltamivir-sensitive strain that displaced it (**Figure 4**). Such differential transmissibility was not detected by *in vitro* competitive growth and *in vivo* ferret transmission experiments (16). In retrospect, our method could have correctly predicted that the AVR virus was less transmissible that its AVS counterpart (with posterior probability > 0.95) after both viruses had co-circulated for two weeks, which corresponds to four weeks before the AVR virus was displaced.

Case study 3: Estimating AVR fitness and drug pressure on the AVS strain under large-scale antiviral interventions during a pandemic

Figure 5 shows that reliable estimates of σ_0 and μ are typically available within one to two weeks after antiviral interventions are suspended. These estimates can be used to inform the optimal use of antivirals. For example, if policymakers resume large-scale antiviral prophylaxis with coverage equal to γ times the baseline level, then the resulting AVR fitness would be $\sigma_0/(1-\gamma\mu)$ which can be used to assess the downstream effect of increased AVR incidence, e.g. increase in case-fatality risk due to more cases not treatable with antivirals.

Discussion

We have developed a simple method for estimating AVR fitness from influenza AVR surveillance data. Characterization of the nonlinear epidemic dynamics underlying surveillance data typically

requires inference of multiple parameters in transmission models (e.g. basic reproductive number, reporting rate, etc.) (26). Our method bypasses such complexity and is therefore easy to implement.

Conventionally, AVR fitness is assessed based on *in vitro* experiments examining kinetics of neuraminidases and virus replications in cell cultures, or *in vivo* experiments examining viral load and virus transmission in animal models (27). As illustrated in our second case study, fitness estimates from such laboratory settings do not necessarily conform with that observed in actual community transmission settings (16). Moreover, as the 2007 experience showed, experiments performed using different genetic background may give different results (28). Nonetheless, these experiments are indispensable for early detection of transmissible AVR viruses. Our method complements these experiments by providing population-level fitness estimates when both AVS and AVR viruses co-circulate.

Timeliness of AVR surveillance depends on the capacity and turnaround time of AVR testing. Current influenza AVR surveillance mainly relies on the WHO Collaborating Centers (WHO CCs) in GISRS with antiviral susceptibility testing capacity available mainly in five WHO CCs, namely Atlanta, Beijing, London, Melbourne and Tokyo (8). National influenza centers collect clinical specimens and send representative virus isolates to one of the WHO CCs for more advanced analyses. However, patient-specific clinical and epidemiological data for these isolates, such as gender, age, geographic location, healthcare setting, antiviral treatment history and vaccination status, are often incomplete or missing, especially when these samples are not collected by the sentinel surveillance systems. Routine collection of these data (e.g. antiviral treatment history) can enhance the performance of AVR surveillance.

The turnaround time of AVR testing depends on our knowledge regarding the genetic mechanisms that confer AVR. If the genetic markers associated with AVR are known a priori (e.g. the NA H275Y mutation (27)), the turnaround time for genotypic tests are usually 1-2 days. In contrast, phenotypic tests for antiviral susceptibility (e.g. neuraminidase inhibition assay (8)) are necessary for monitoring emergence of AVR strains with previously unknown AVR mechanisms (27). Phenotypic tests are much more labor intensive than genotypic tests with a turnaround time of 1-2 weeks. Following the discovery of a new strain with unknown AVR mechanism, further investigations would be needed to characterize the associated genetic markers. As such, real-time surveillance for novel AVR strains will likely incur a lead time of at least several weeks.

In our first case study, we estimate that the oseltamivir-resistant influenza A(H1N1) virus that emerged and became globally dominant in 2007-2008 was 4% more transmissible than its oseltamivir-sensitive predecessor. This is consistent with the findings in Chao et al (29) in which the fitness advantage of the oseltamivir-resistant strain was estimated to be 1.7% to 2.4% based on the rate at which it spread around the globe. Both studies indicate that an AVR strain with a fitness advantage of as little as 2% to 4% would spread to fixation both locally and globally within months. If large-scale antiviral intervention is implemented during a pandemic, the resulting drug pressure on the AVS strain might confer such magnitude of fitness advantage to an intrinsically less transmissible AVR strain. In such context, timely and robust surveillance of AVR fitness is essential for informing optimal use of antivirals. For example, given that antiviral therapy will likely be the first-line treatment for severe cases during a pandemic, an increase in AVR/AVS incidence ratio and growing ineffectiveness of antivirals in treating AVR cases might increase the overall pandemic mortality. Estimates of intrinsic AVR fitness and drug pressure on the AVS strain provided by our method would thus be useful for assessing the risk of such outcome, though a comprehensive evaluation of optimal antiviral use would require knowledge of additional parameters (e.g. reproductive number, antiviral efficacy in reducing mortality, etc.) (30).

In our method, AVR fitness corresponds to the combined effect of intrinsic AVR fitness and the drug pressure posed on the AVS strain by population-wide antiviral interventions. AVR fitness will vary across populations if the drug pressure in each localities are different. Therefore, comparison of AVR fitness estimates from different populations should account for heterogeneities in drug pressure. We have demonstrated how to do this in our case study 1 in which we jointly estimate intrinsic AVR fitness and drug pressure in Japan using data from 10 populations (see Appendix page 4).

Our study has several important limitations. First, our method is applicable only when AVS and AVR strains co-circulate and hence cannot be used to estimate the fitness of a newly emerged AVR strain that has not yet spread in the community. Second, our method requires accurate specification of the generation time distribution. If data on exposure or onset times of infector-infectee pairs are available, our method can be extended to jointly infer the generation time distribution (see Appendix page 4). The resulting fitness estimate remains largely unbiased, but its precision would be lower due to uncertainty in the generation time distribution. Third, our method has not

accounted for importation of AVS and AVR viruses. In the presence of such importation, our method would still be valid if (i) cases with recent travel history are excluded from AVR surveillance and (ii) the number of imported cases is small compared to incidence from local transmission (which is generally the case after the local epidemic has undergone exponential growth for 1-2 weeks).

Timely and accurate estimates of AVR fitness is important during both inter-pandemic and pandemic periods because the spread of AVR viruses can substantially attenuate the effectiveness of antivirals. Robust real-time interpretation of AVR surveillance data for estimating AVR fitness is thus an essential but currently missing function of AVR surveillance. Our method has the potential to fill this knowledge gap and can be easily integrated into contemporary surveillance systems.

Contributors

J.T.W., M.L. and K.L. designed the experiments; K.L. and J.T.W. performed the data collection and analysis; K.L., M.L., K.Y.Y. and J.T.W. interpreted the results and wrote the manuscript.

Conflicts of interest

We declare that we have no conflicts of interest.

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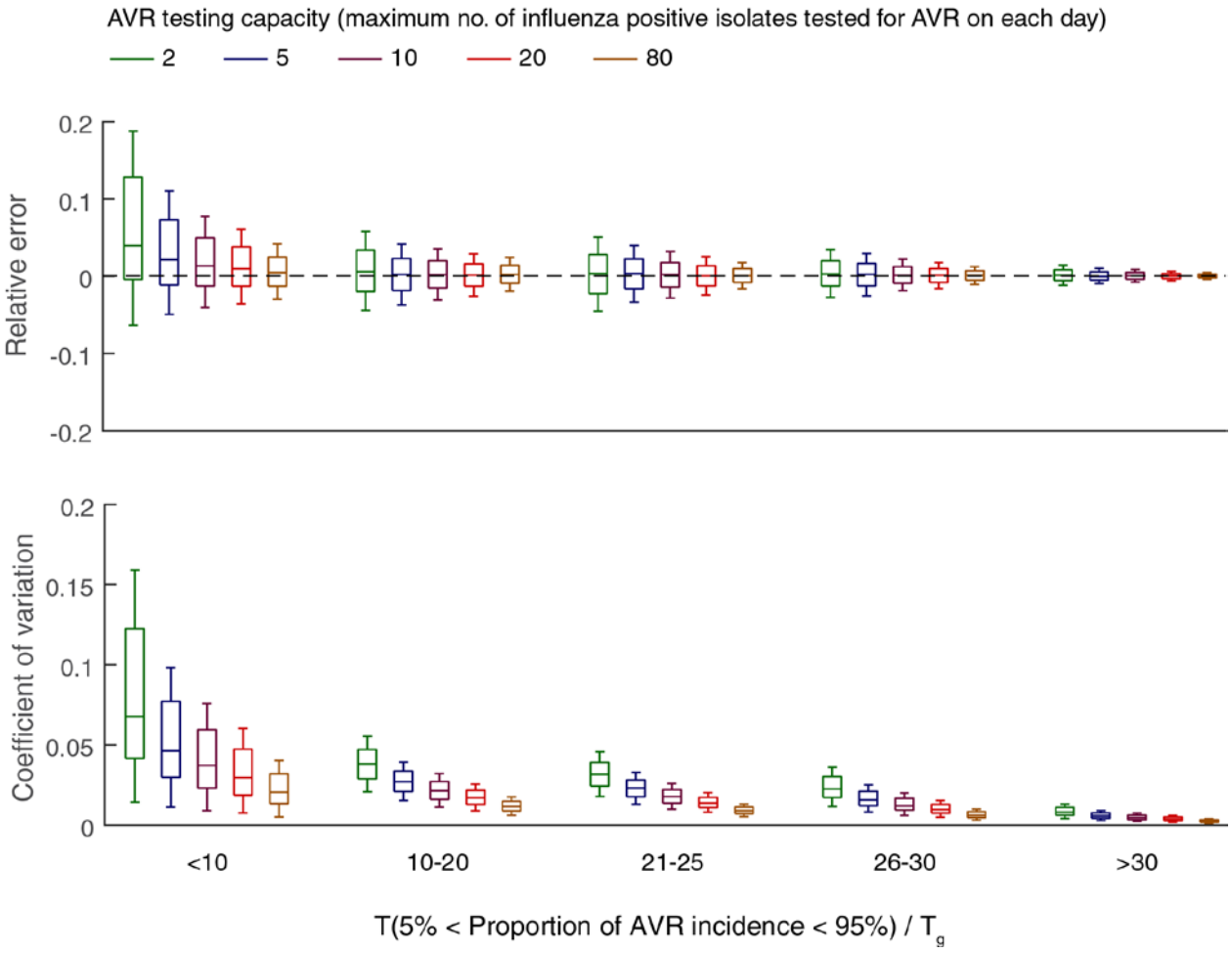

Figure 1. Validating the accuracy and precision of AVR fitness estimates when the sensitivity and specificity of AVR testing are both 100%. One hundred epidemic scenarios are randomly generated and 100 stochastic realizations of the data streams are simulated for each scenario (see Methods). AVR fitness is inferred at the end of each simulated epidemic. **A** Frequency distribution of the relative error in the fitness estimates $\hat{\sigma}$ (i.e. $1-E[\hat{\sigma}]/\sigma$) across all scenarios and realizations when the daily AVR testing capacity is 2, 5, 10, 20 and 80 samples. The smaller the relative error, the more accurate the estimates. **B** Frequency distribution of the coefficient of variation of $\hat{\sigma}$. The smaller the coefficient of variation, the more precise the estimates.

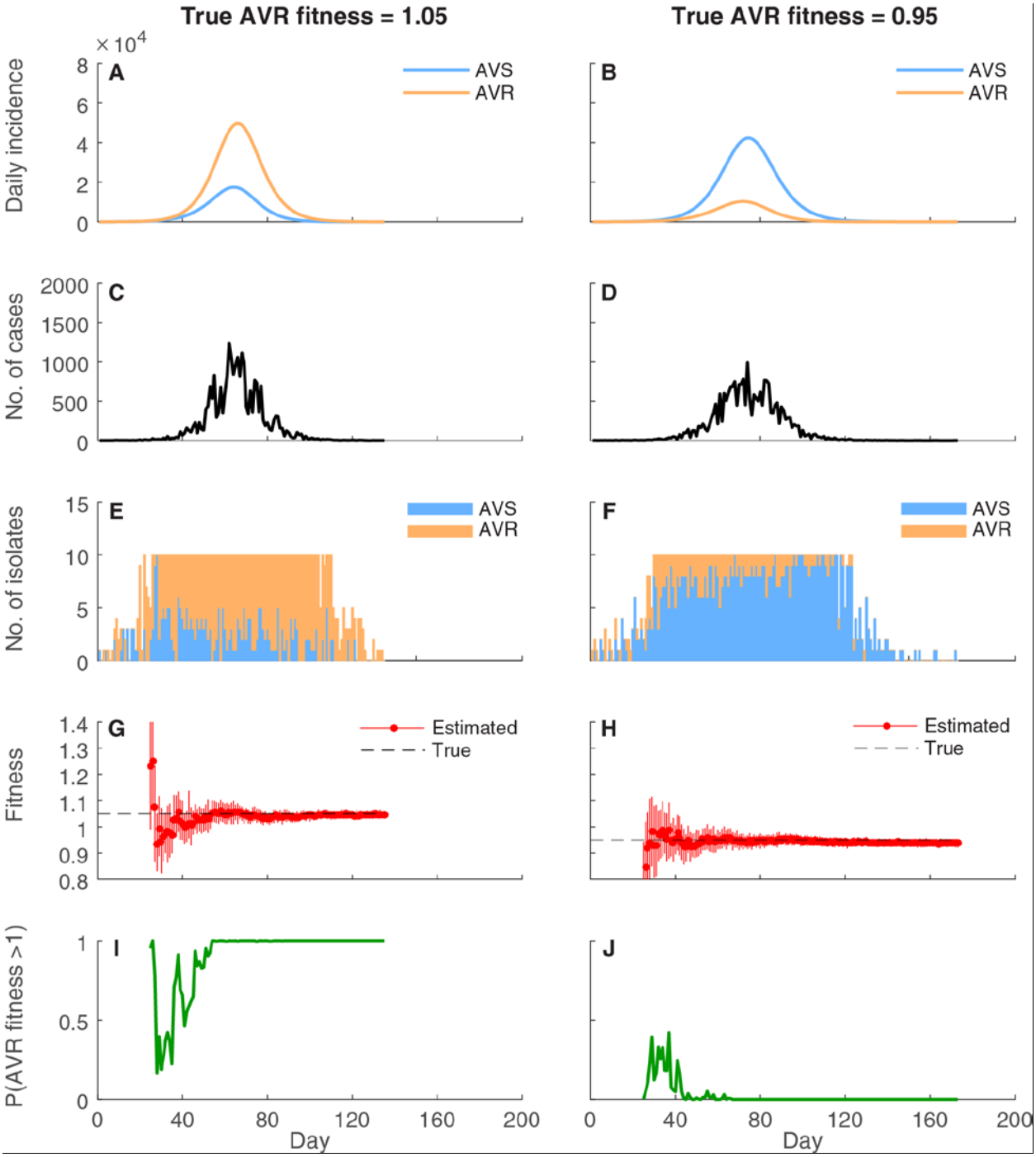

Figure 2. A simulated example to illustrate the timeliness of reliable AVR fitness estimates. The epidemic parameters are $R^S(0) = 1.4$ and $T_g = 2.8$ days. At time 0, 50% of each age group are susceptible and the epidemic is seeded with 10 AVS and 10 AVR infections. **A-B** Incidence of AVS and AVR infections in two fitness scenarios: σ = 1.05 or 0.95. **C-D** The daily number of

reported cases. **E-F** The daily number of influenza-positive isolates that are AVS and AVR with a testing capacity of 10 samples per day. **G-H** Posterior distribution of the fitness estimate $\hat{\sigma}$ on each day. Circles and error bars indicate the posterior medians and the 95% credible intervals, respectively. **I-J** The posterior probability that AVR fitness is above 1.

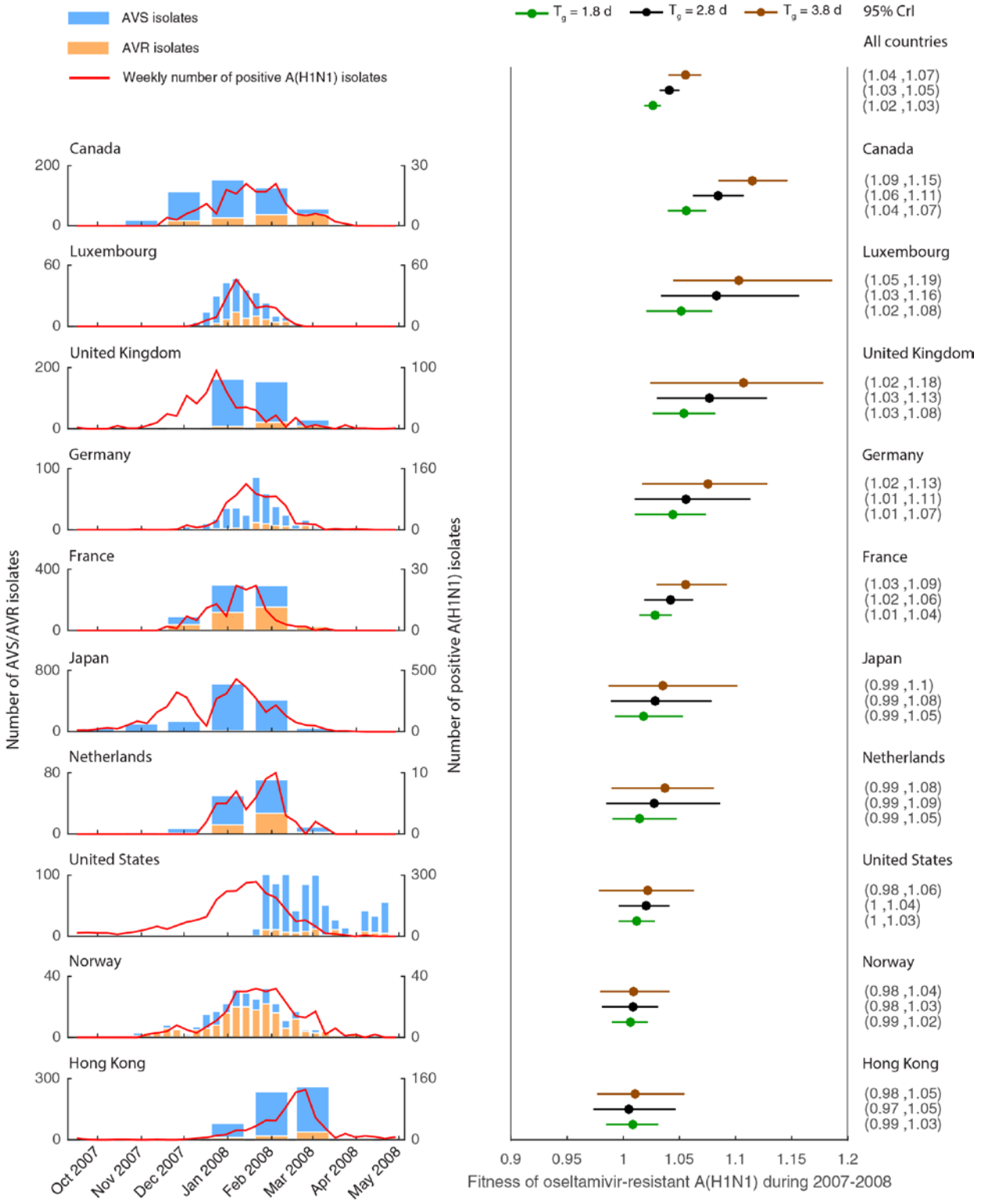
AVS isolates
AVR isolates
Weekly number of positive A(H1N1) isolates
Canada
Luxembourg
United Kingdom
Germany
France
Japan
Netherlands
United States
Norway
Hong Kong
Number of AVS/AVR isolates
Oct 2007
Nov 2007
Dec 2007
Jan 2008
Feb 2008
Mar 2008
Apr 2008
May 2008
T_g = 1.8 d
T_g = 2.8 d
T_g = 3.8 d
95% CrI
All countries
(1.04 ,1.07)
(1.03 ,1.05)
(1.02 ,1.03)
Canada
(1.09 ,1.15)
(1.06 ,1.11)
(1.04 ,1.07)
Luxembourg
(1.05 ,1.19)
(1.03 ,1.16)
(1.02 ,1.08)
United Kingdom
(1.02 ,1.18)
(1.03 ,1.13)
(1.03 ,1.08)
Germany
(1.02 ,1.13)
(1.01 ,1.11)
(1.01 ,1.07)
France
(1.03 ,1.09)
(1.02 ,1.06)
(1.01 ,1.04)
Japan
(0.99 ,1.1)
(0.99 ,1.08)
(0.99 ,1.05)
Netherlands
(0.99 ,1.08)
(0.99 ,1.09)
(0.99 ,1.05)
United States
(0.98 ,1.06)
(1 ,1.04)
(1 ,1.03)
Norway
(0.98 ,1.04)
(0.98 ,1.03)
(0.99 ,1.02)
Hong Kong
(0.98 ,1.05)
(0.97 ,1.05)
(0.99 ,1.03)
Number of positive A(H1N1) isolates
Fitness of oseltamivir-resistant A(H1N1) during 2007-2008

Figure 3. Surveillance data for seasonal influenza A(H1N1) and fitness estimates for the oseltamivir-resistant strain during 2007-2008 in Canada, Luxembourg, United Kingdom, Germany, France, Japan, Netherlands, United States, Norway and Hong Kong. A The number of positive A(H1N1) virus isolates and the number of oseltamivir-sensitive and resistant A(H1N1) isolates over time in each population. **B** Fitness estimates for the oseltamivir-resistant A(H1N1) virus under three assumed generation time distributions. The pooled AVR fitness estimate (at the top) is obtained by assuming that AVR fitness was the same in all populations.

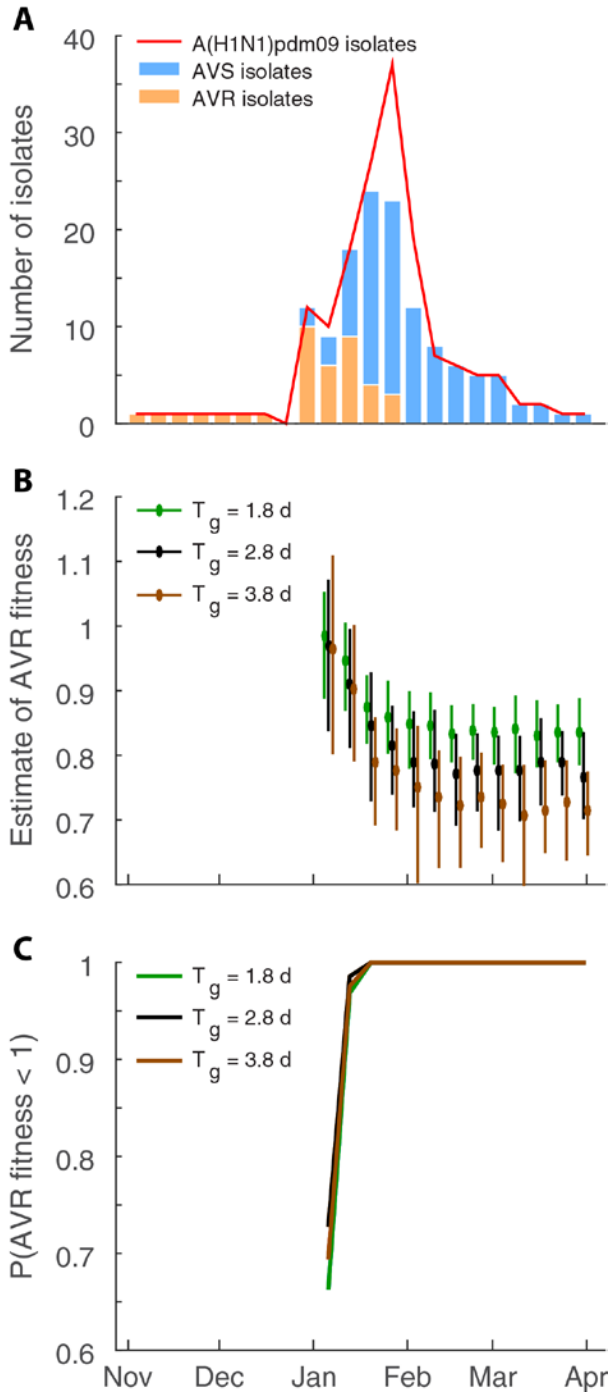

Figure 4. Retrospective real-time fitness estimate for the oseltamivir-resistant A(H1N1)pdm09 virus that circulated in Hokkaido, Japan during the 2013-2014 influenza season. A Data on influenza A(H1N1) activity and AVR surveillance. **B** Weekly fitness estimate using the same generation time distributions considered in Figure 3. **C** The posterior probability that AVR fitness was above 1.

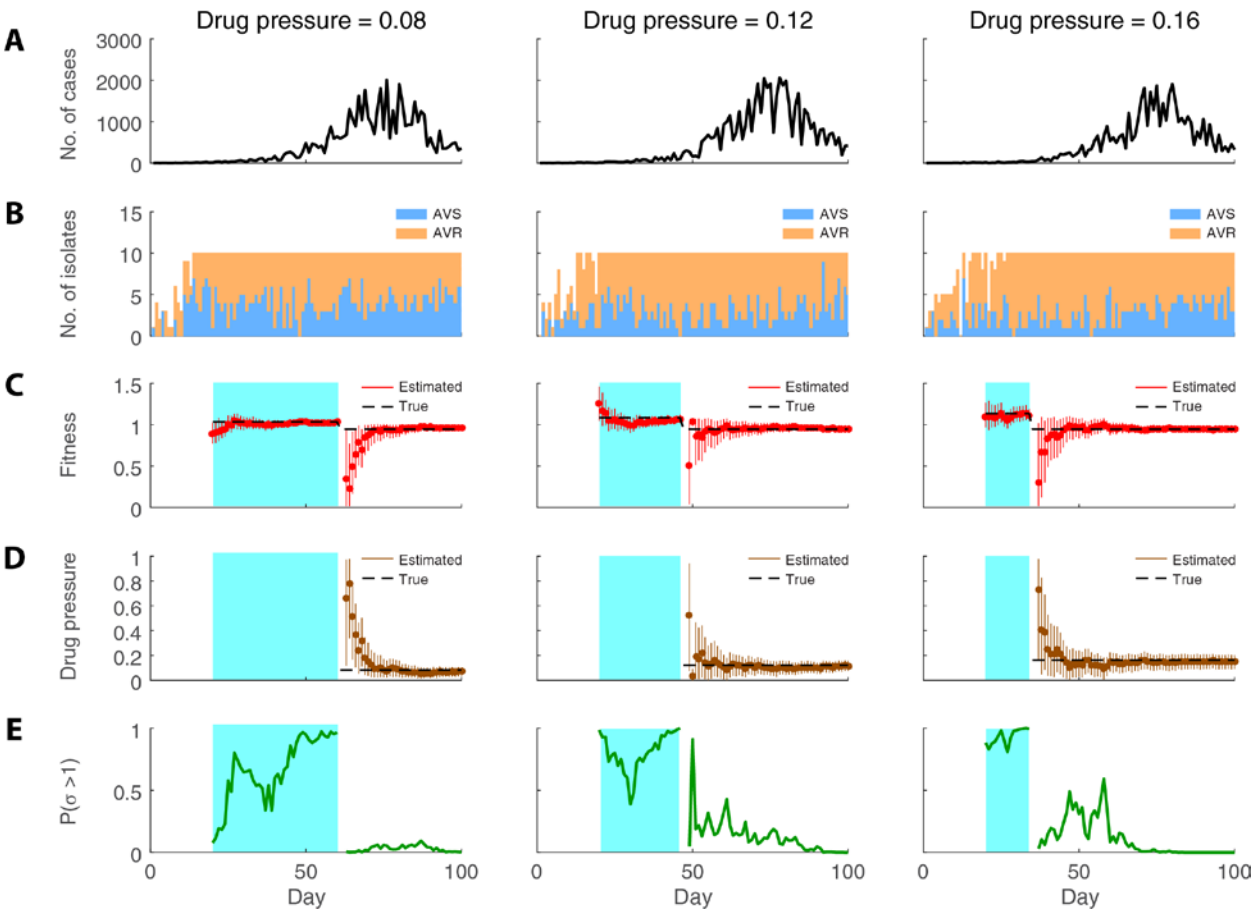

Figure 5. Estimating AVR fitness and drug pressure on the AVS strain posed by large-scale antiviral prophylaxis. The epidemic parameters are the same as that in Figure 2 with intrinsic AVR fitness $\sigma_0 = 0.95$. We assume that antiviral prophylaxis reduces susceptibility by 81% and the prophylaxis coverage is 10%, 15% and 20% so that the drug pressure μ is 0.08, 0.12 and 0.16, respectively. Large-scale antiviral intervention is suspended after the posterior probability of $\sigma > 1$ is greater than 0.9 for seven consecutive days. Cyan shade indicates the time period during which large-scale antiviral intervention is implemented. **A** The daily number of reported cases. **B** The daily number of influenza-positive isolates that are AVS and AVR with a testing capacity of 10 samples per day. **C** Posterior distribution of the AVR fitness estimate on each day. Circles and error bars indicate the posterior medians and the 95% credible intervals, respectively. **D** Posterior distribution of the estimates for drug pressure on the AVS strain at the baseline level (i.e. before large-scale antiviral interventions is suspended). **E** The posterior probability that AVR fitness is above 1.

References

1. World Health Organization. WHO Guidelines for Pharmacological Management of Pandemic (H1N1) 2009: Influenza and Other Influenza Viruses: World Health Organization; 2009.
2. National Audit Office. Access to clinical trial information and the stockpiling of Tamiflu. 2013.
3. Ives J, Carr J, Mendel D, Tai C, Lambkin R, Kelly L, et al. The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and in vivo. Antiviral research. 2002;55(2):307-17.
4. Meijer A, Lackenby A, Hungnes O, Lina B, Van Der Werf S, Schweiger B, et al. Oseltamivir-resistant influenza virus A (H1N1), Europe, 2007–08 season. Emerging infectious diseases. 2009;15(4):552.
5. Kramarz P, Monnet D, Nicoll A, Yilmaz C, Ciancio B. Use of oseltamivir in 12 European countries between 2002 and 2007--lack of association with the appearance of oseltamivir-resistant influenza A(H1N1) viruses. Euro Surveill. 2009;14(5):854-8.
6. Stilianakis NI, Perelson AS, Hayden FG. Emergence of Drug Resistance during an Influenza Epidemic: Insights from a Mathematical Model. Journal of Infectious Diseases. 1998;177(4):863-73.
7. Wu JT, Leung GM, Lipsitch M, Cooper BS, Riley S. Hedging against Antiviral Resistance during the Next Influenza Pandemic Using Small Stockpiles of an Alternative Chemotherapy. PLoS Med. 2009;6(5):e1000085.
8. Takashita E, Meijer A, Lackenby A, Gubareva L, Rebelo-de-Andrade H, Besselaar T, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013–2014. Antiviral Research. 2015;117:27-38.
9. Wu WL, Lau S-Y, Chen Y, Wang G, Mok BW-Y, Wen X, et al. The 2008–2009 H1N1 influenza virus exhibits reduced susceptibility to antibody inhibition: Implications for the prevalence of oseltamivir resistant variant viruses. Antiviral Research. 2012;93(1):144-53.
10. Mossong J, Hens N, Jit M, Beutels P, Auranen K, Mikolajczyk R, et al. Social Contacts and Mixing Patterns Relevant to the Spread of Infectious Diseases. PLoS Med. 2008;5(3):e74.
11. Wu JT, Ho A, Ma ESK, Lee CK, Chu DKW, Ho P-L, et al. Estimating Infection Attack Rates and Severity in Real Time during an Influenza Pandemic: Analysis of Serial Cross-Sectional Serologic Surveillance Data. PLoS Med. 2011;8(10):e1001103.
12. Chunara R, Aman S, Smolinski M, Brownstein JS. Flu near you: an online self-reported influenza surveillance system in the USA. Online Journal of Public Health Informatics. 2013;5(1).

13. Wong JY, Wu P, Nishiura H, Goldstein E, Lau EHY, Yang L, et al. Infection Fatality Risk of the Pandemic A(H1N1)2009 Virus in Hong Kong. American Journal of Epidemiology. 2013;177(8):834-40.
14. Vink MA, Bootsma MCJ, Wallinga J. Serial Intervals of Respiratory Infectious Diseases: A Systematic Review and Analysis. American Journal of Epidemiology. 2014;180(9):865-75.
15. Hurt AC, Hardie K, Wilson NJ, Deng YM, Osbourn M, Leang SK, et al. Characteristics of a Widespread Community Cluster of H275Y Oseltamivir-Resistant A(H1N1)pdm09 Influenza in Australia. Journal of Infectious Diseases. 2012;206(2):148-57.
16. Takashita E, Kiso M, Fujisaki S, Yokoyama M, Nakamura K, Shirakura M, et al. Characterization of a Large Cluster of Influenza A(H1N1)pdm09 Viruses Cross-Resistant to Oseltamivir and Peramivir during the 2013-2014 Influenza Season in Japan. Antimicrobial Agents and Chemotherapy. 2015;59(5):2607-17.
17. Govorkova EA, Baranovich T, Seiler P, Armstrong J, Burnham A, Guan Y, et al. Antiviral resistance among highly pathogenic influenza A (H5N1) viruses isolated worldwide in 2002–2012 shows need for continued monitoring. Antiviral Research. 2013;98(2):297-304.
18. Hai R, Schmolke M, Leyva-Grado VH, Thangavel RR, Margine I, Jaffe EL, et al. Influenza A(H7N9) virus gains neuraminidase inhibitor resistance without loss of in vivo virulence or transmissibility. Nat Commun. 2013;4.
19. Lipsitch M, Cohen T, Murray M, Levin BR. Antiviral Resistance and the Control of Pandemic Influenza. PLoS Med. 2007;4(1):e15.
20. McCaw JM, Wood JG, McCaw CT, McVernon J. Impact of emerging antiviral drug resistance on influenza containment and spread: influence of subclinical infection and strategic use of a stockpile containing one or two drugs. PLoS ONE. 2008;3.
21. Alexander ME, Bowman CS, Feng Z, Gardam M, Moghadas SM, Röst G, et al. Emergence of drug resistance: implications for antiviral control of pandemic influenza. Proceedings of the Royal Society B: Biological Sciences. 2007;274(1619):1675-84.
22. Halloran ME, Hayden FG, Yang Y, Longini IM, Monto AS. Antiviral effects on influenza viral transmission and pathogenicity: observations from household-based trials. Am J Epidemiol. 2007;165.
23. Wu JT, Lee CK, Cowling BJ, Yuen KY. Logistical feasibility and potential benefits of a population-wide passive-immunotherapy program during an influenza pandemic. Proceedings of the National Academy of Sciences. 2010;107(7):3269-74.

24. World Health Organization. Influenza A(H1N1) virus resistance to oseltamivir: World Health Organization; 2008 [Available from: http://www.who.int/influenza/patient_care/antivirals/oseltamivir_summary/en/.
25. Tashiro M, McKimm-Breschkin JL, Saito T, Klimov A, Macken C, Zambon M, et al. Surveillance for neuraminidase-inhibitor-resistant influenza viruses in Japan, 1996–2007. Antivir Ther. 2009;14(6):751-61.
26. Wu JT, Leung K, Perera RAPM, Chu DKW, Lee CK, Hung IFN, et al. Inferring Influenza Infection Attack Rate from Seroprevalence Data. PLoS Pathog. 2014;10(4):e1004054.
27. World Health Organization. Laboratory methodologies for testing the antiviral susceptibility of influenza viruses [Available from: http://www.who.int/influenza/gisrs_laboratory/antiviral_susceptibility/en/.
28. Bloom JD, Gong LI, Baltimore D. Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. Science. 2010;328(5983):1272-5.
29. Chao DL, Bloom JD, Kochin BF, Antia R, Longini IM. The global spread of drug-resistant influenza. Journal of The Royal Society Interface. 2012;9(69):648-56.
30. McCaw JM, McVernon J. Prophylaxis or treatment? Optimal use of an antiviral stockpile during an influenza pandemic. Mathematical Biosciences. 2007;209(2):336-60.