

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Lee VJ, Yap J, Cook AR, et al. Oseltamivir ring prophylaxis for containment of 2009 H1N1 influenza outbreaks. *N Engl J Med* 2010;362:2166-74.

SUPPLEMENTARY APPENDIX

Oseltamivir ring prophylaxis for containment of Influenza A (H1N1-2009) outbreaks

Vernon Lee et al

Laboratory Methods

Molecular Diagnosis

Nucleic acid material for each specimen was extracted using the DNA minikit (Qiagen, Inc, Valencia, CA, USA) according to manufacturer's instructions. Five μ l of nucleic acid was subjected to PCR testing for H1N1-2009, according to the SWH1 Forward/Reverse primer set and probe (1). This one-step PCR was performed with the Superscript III RT/Platinum Taq mix (Invitrogen Corporation, CA, USA) on the real-time PCR system (Applied Biosystems 7500, USA).

The PCR thermocycling conditions were 50⁰C for 30mins, 95⁰C for 2min, followed by 45 cycles of PCR amplification of 95⁰C for 15sec and 55⁰C for 30sec. A florescence growth curve crossing the threshold line within 40 cycles is indicative of a positive result.

Molecular Sequencing

For the whole genome sequencing, viral RNA from the diagnostic swabs or RNA extracted from MDCK cell cultures was reverse-transcribed to cDNA and then amplified by PCR using H1N1-2009 specific primers. PCR products were sequenced using GIS flu-resequencing microarrays manufactured by Roche Nimblegen in an approach described previously (2,3). These sequences were used to generate phylogenetic trees and genetic relatedness using the Neighbour-Joining algorithm and Maximum Composite Likelihood Nucleotide Substitution model with 10,000 bootstrap replicates using the MEGA 4.0 software (4). More information on the genetic analysis tool can be found at <http://mendel.bii.a-star.edu.sg/METHODS/flumapIntro.html>.

Statistical Methods

Following the argument in Cauchemez et al (5), we assume that in the absence of control each case creates a number of new cases distributed as a Poisson variate with mean λ , and that the time between onset of the primary and all secondary cases are independently distributed with a discretised gamma distribution, which we parametrised from the posterior mean mean [sic] and variance of the gamma distribution fitted to the data provided by Moser (6). We further assume that after the intervention at time τ (which varies by outbreak), the mean number of cases is $\lambda\theta$. By Rényi's splitting theorem (7), the number of new cases with onset on day j is Poisson with mean

$$\sum_{i \leq j} c_i \lambda \theta^{1\{j > \tau\}} w(i - j)$$

where c_t is the number of cases with onset on day t , $w(t)$ is the probability mass function for the generation interval of length t , and $1\{A\} = 1$ if A is true and 0 otherwise. From this the likelihood function follows by taking the product of this over days and outbreaks. The posterior distribution of the parameters conditional on the data is taken to be proportional to this, i.e. a pseudo-objective improper flat prior on the parameters λ and $\lambda\theta$ is assumed. Therefore this analysis was performed within

the Bayesian paradigm using improper flat priors on the parameter space – ie. $p(\lambda, \theta) \propto 1$ if $\lambda > 0$ and $\theta > 0$, and 0 otherwise; the likelihood function has a finite integral and so the posterior is proper (8).

The posterior distribution is estimated via Markov chain Monte Carlo integration (9). The hypothesis of an effect, $\theta < 1$, is assessed via posterior hypothesis probabilities (10) by direct calculation from the posterior sample of $p(\theta > 1 | \text{data})$. In the same fashion, marginal 95% credible intervals are obtained.

To distinguish the effects of ring prophylaxis from that of sending soldiers home for outbreak IV, we calculate the probability distribution of the fitted model from the start of interventions in which only one subsequent generation of contacts is allowed. These use the posterior mean for the secondary infection rate, λ , in the absence of control, and are derived via Monte Carlo simulation.

References

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