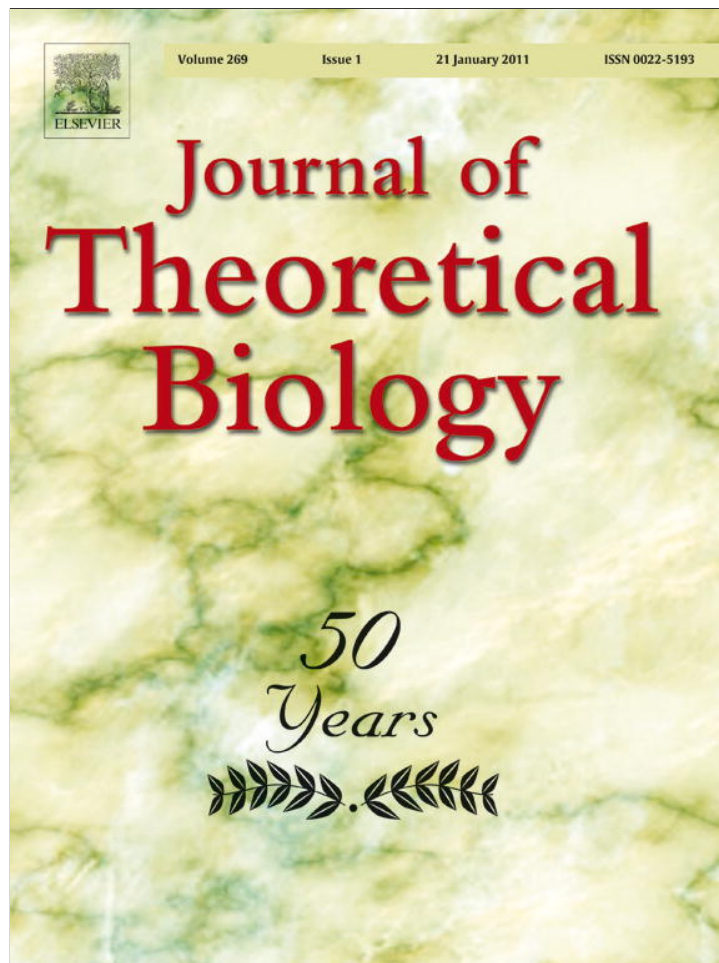


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



ELSEVIER

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/jtbi

Neuraminidase inhibitors for treatment of human and avian strain influenza: A comparative modeling study

Hana M. Dobrovolny^a, Ronald Gieschke^b, Brian E. Davies^c, Nelson L. Jumbe^b, Catherine A.A. Beauchemin^{a,*}

^a Department of Physics, Ryerson University, Toronto, Ontario, Canada

^b F. Hoffmann-La Roche, Ltd., Basel, Switzerland

^c F. Hoffmann-La Roche, Inc., Nutley, New Jersey, USA

ARTICLE INFO

Article history:

Received 20 February 2010

Received in revised form

9 July 2010

Accepted 11 October 2010

Available online 21 October 2010

Keywords:

Avian influenza

Neuraminidase inhibitor

Mathematical modeling

Antiviral treatment

Infectious disease

ABSTRACT

Treatment of seasonal influenza viral infections using antivirals such as neuraminidase inhibitors (NAIs) has been proven effective if administered within 48 h post-infection. However, there is growing evidence that antiviral treatment of infections with avian-derived strains even as late as 6 days post-infection (dpi) can significantly reduce infection severity and duration. Using a mathematical model of in-host influenza viral infections which can capture the kinetics of both a short-lived, typical, seasonal infection and a severe infection exhibiting sustained viral titer, we explore differences in the effects of NAI treatment on both types of influenza viral infections. Comparison of our model's behavior against experimental data from patients naturally infected with avian strains yields estimates for the times at which patients were infected that are consistent with those reported by the patients, and estimates of drug efficacies that are lower for patients who died than for those who recovered. In addition, our model suggests that the sustained, high, viral titers often seen in more severe influenza virus infections are the reason why antiviral treatment delayed by as much as 6 dpi will still lead to reduced viral titers and shortened illness. We conclude that NAIs may be an effective and beneficial treatment strategy against more severe strains of influenza virus characterized by high, sustained, viral titers. We believe that our mathematical model will be an effective tool in guiding treatment of severe influenza viral infections with antivirals.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Over the course of history there have been several influenza pandemics that have sickened and killed millions (Hsieh et al., 2006; Kobasa and Kawaoka, 2005). Since 1997, when the first cases of avian (H5N1) influenza appeared in humans (Claas et al., 1998; Subbarao et al., 1998), health authorities have feared that avian strain influenza will cause a big pandemic. With the mortality rate of avian strain influenza at 60% (Gambotto et al., 2008), there is a need for effective treatment and control strategies in the event of a pandemic.

One such strategy is treatment of affected patients with antiviral medication. In the case of influenza virus, two classes of antiviral drugs are available: M2 channel blockers, such as amantadine and rimantadine, and neuraminidase inhibitors (NAIs), such as oseltamivir and zanamivir. Unfortunately, there is widespread resistance to amantadine (Bright et al., 2006) among circulating

strains, including many of the avian-derived strains isolated from humans (Cheung et al., 2006; He et al., 2008). Although NAI resistance to seasonal influenza virus is on the rise (Dharan et al., 2009), and NAI resistant strains of avian-derived influenza virus have occasionally been isolated (de Jong et al., 2005; Le et al., 2005), most circulating strains of avian influenza virus appear to be sensitive to NAIs (Rameix-Welti et al., 2008). Thus, health authorities have recommended NAIs for the treatment of avian-derived influenza virus (Schunemann et al., 2007).

NAIs are known to be effective against seasonal influenza (Hayden et al., 1999; Whitley et al., 2001), but it is unclear whether NAIs are an effective treatment against infections with avian strain influenza. Studies show that NAIs have similar *in vitro* inhibitory activity against the neuraminidase of human- and avian-derived influenza virus strains (Duwe and Schweiger, 2008; Hurt et al., 2007). Animal studies also indicate that NAIs are effective against avian influenza virus, showing decreased viral loads and increased survival in mice (Govorkova et al., 2001; Leneva et al., 2000, 2001; Gubareva et al., 1998; Yun et al., 2008), ferrets (Le et al., 2005; Yun et al., 2008), and macaques (Stittelaar et al., 2008). However, some studies indicate that delayed treatment of avian influenza viral infections (Govorkova et al., 2007; Zheng et al., 2008;

* Corresponding author. Tel.: +1 416 979 5000x2508; fax: +1 416 979 5343.

E-mail addresses: hdrobrovo@ryerson.ca (H.M. Dobrovolny), ronald.gieschke@roche.com (R. Gieschke), brian.davies@roche.com (B.E. Davies), shasha.jumbe@roche.com (N.L. Jumbe), cbeau@ryerson.ca (C.A.A. Beauchemin).

Boltz et al., 2008a) or treatment with an insufficient dose (Boltz et al., 2008; Yen et al., 2005) can lead to poor survival outcomes.

Data on NAI treatment of humans infected with avian strain influenza virus is limited and it is difficult to draw conclusions on the effectiveness of NAIs against avian influenza in humans. In one report, 4 of 8 avian influenza virus-infected patients treated with oseltamivir subsequently died, although sequencing revealed two of the fatal cases were infected with an oseltamivir-resistant strain of H5N1 (de Jong et al., 2005). One retrospective study determined that avian influenza virus-infected patients who were treated with oseltamivir had a 20% mortality rate as compared to a 50% mortality rate in untreated cases (Hien et al., 2009). A second retrospective study found that oseltamivir treatment initiated within 5–6 dpi increased survival rates to 37.5% from 18.5% for patients whose treatment was initiated 7 dpi or later (Kandun et al., 2008). While promising, such limited data makes it difficult to determine how effective NAIs are against avian influenza virus and given the lethality of avian influenza in humans, it is also difficult to clinically establish dosage and treatment guidelines.

In this paper, we use an in-host model of influenza viral infections that captures the kinetics of human infections with both human- and avian-derived influenza viral strains. We set the model parameters to reflect the case of infections with either a human or avian influenza viral strain and simulate NAI treatment to study the effect of NAIs on the course of the illness. The results of this modeling study provide front-line health care personnel with guidance regarding the treatment of avian influenza viral infections with NAIs. In particular, this study suggests that treatment of an avian-derived infection, even when delayed up to six days post-infection, can have an important impact on disease severity and duration.

2. Methods

2.1. Mathematical model

We use a model in which the standard target-cell limited viral infection model (Baccam et al., 2006) is extended to consider two distinct cell populations, a default and a secondary cell population (Dobrovolny et al., in press). In our model, the two cell populations differ only in their susceptibility to infection, and in their rate of viral production. While the reasons for sustained viral titer in avian influenza infections are unclear, our model which allows for different infectivity and viral production rates for each of the two cell populations can be adapted to the proposed hypotheses. For example, cell tropism is thought by some to be the underlying cause of H5N1 virulence (Pekosz et al., 2009; Stevens et al., 2008; Matrosovich et al., 2004, 2007). Nonciliated cells predominantly express sialic acid α -2,6 galactose terminated saccharides on their surface, which are the preferred binding sites for human strain influenza virus (Matrosovich et al., 2004) while ciliated cells express sialic acid α -2,3 galactose terminated saccharides receptors (Thompson et al., 2006; Ibrecovic et al., 2006; Kogure et al., 2006), which are the preferred binding sites for avian strain influenza virus (Matrosovich et al., 2004). In our model, ciliated cells can represent the default cell population for avian strain influenza, and nonciliated cells can be the secondary cell population. Alternatively, the secondary cells in our model could also represent cells that are protected by the elevated cytokine response (de Jong et al., 2006; Kaiser et al., 2001; Seo and Webster, 2002; Cheung et al., 2002; Chan et al., 2005) seen in avian strain infections. The secondary cell population could also represent cells located in the lower respiratory tract, which are physically less accessible to the virus, and have also been implicated in the virulence of H5N1 (van Riel et al., 2006; Uprasertkul et al., 2007).

In the model,

$$\frac{dT_d}{dt} = -\beta_d T_d V, \quad \frac{dT_s}{dt} = -\beta_s T_s V$$

$$\frac{dE_d}{dt} = \beta_d T_d V - kE_d, \quad \frac{dE_s}{dt} = \beta_s T_s V - kE_s$$

$$\frac{dI_d}{dt} = kE_d - \delta I_d, \quad \frac{dI_s}{dt} = kE_s - \delta I_s$$

$$\frac{dV}{dt} = (1-\varepsilon)p_d I_d + (1-\varepsilon)p_s I_s - cV$$

infection proceeds as susceptible default target cells, T_d , (or secondary target cells, T_s) are infected by virus, V , at a rate β_d (or β_s), and as a result become latently infected E_d (or E_s). Latently infected cells become productively infected after a time $1/k$ on average. Once productively infected, I_d (or I_s), these cells produce virus at a rate p_d (or p_s), until they die after a time $1/\delta$ on average. Virions are cleared from the system at a rate c .

Neuraminidase inhibitors, such as oseltamivir or zanamivir, inhibit neuraminidase activity thereby blocking the release of newly produced virus particles from productively infected cells. The effect of NAIs is incorporated into our model by introducing the efficacy of the drug, $\varepsilon(t) = \varepsilon$, and using it to adjust the viral production rates, p_d and p_s . Thus, $\varepsilon(t)$ is the drug's efficacy in blocking viral release. Note that we assume that the drug efficacy at blocking viral release is the same for both nonciliated and ciliated cell types. The efficacy of the drug is tied to actual drug concentrations through the E_{max} model (Holford and Sheiner, 1981), which defines efficacy as

$$\varepsilon(t) = \varepsilon_{max} \frac{D(t)^n}{D(t)^n + IC_{50}^n}, \quad (1)$$

where $D(t)$ is the drug concentration, ε_{max} is the maximum effect of the drug such that $0 < \varepsilon_{max} \leq 1$, IC_{50} is the concentration of drug necessary to inhibit the response by 50%, and the parameter n , called the Hill coefficient, controls the steepness of the sigmoidal function. Since we have limited data on the actual values of IC_{50} , ε_{max} , and n , and for simplicity, we set $\varepsilon(t) = \varepsilon$, where $0 \leq \varepsilon < 1$ is a constant. In other words, we assume that drug concentration is held constant over the course of treatment. It is important to note, however, that with knowledge of the parameters in the E_{max} model, a specific value of ε can be related back to a specific drug concentration. Unfortunately, given the lack of experimental data and the difficulty in relating the dose administered to the drug concentration at the site of infection, it is not possible to do so at this time. We do, however, hope to address this issue in future work.

2.2. Selection of the base parameters

Since the model has more parameters than there are data points, some had to be fixed. Parameters $k = 4 \text{ d}^{-1}$, $c = 2 \text{ d}^{-1}$, $\delta = 5.2 \text{ d}^{-1}$, for both human and avian strains were fixed from values determined by fitting the simple, single target cell model to infections with influenza A/HK/123/77 (H1N1) in six human volunteers (Baccam et al., 2006). These values are in line with that reported elsewhere by others (Beauchemin et al., 2008; Handel et al., 2010; Bocharov and Romanyukha, 1994; Miao et al., 2010). Although these parameters may be different for infection with human and avian influenza viral strains, we believe that differences in cell infection rates (β_d , β_s) and viral productivity (p_d , p_s) are most critical to differences in dynamics between influenza viral strains of human and avian origin. While we do not present these results here, we have also considered the effect of allowing k or δ to differ between the two cell types rather than p or β and found that it did not lead to the delayed peak and sustained high viral titer characteristic of infections with avian influenza

strains (de Jong et al., 2006; Perrone et al., 2008; Tumpey et al., 2007; Maines et al., 2006). The results of the fits of the single target cell model (Baccam et al., 2006), which describes an influenza viral infection in a homogeneous cell population, to infection with a human influenza viral strain indicate that human influenza virus is adequately described by the single target cell eclipse model. Thus we assume that human influenza virus does not infect secondary cells, i.e. we set $\beta_s = p_s = 0$ for infection with human-derived strains. At time $t = 0$, the infection is initiated when a viral inoculum of $V(0) = V_0$ ($E(0) = I(0) = 0$) is introduced into a heterogeneous population of target cells composed of 70% default cells and 30% secondary cells. This is based on an assumption that cell tropism is the underlying cause of sustained viral titer and comes from studies of lung physiology indicating that the epithelium of the upper airway (up to the fifth generation) comprises 50–85% nonciliated cells (Crystal and West, 1991). Note that this can be done without loss of generality since a different choice of r_d (the fraction of the cell population which is of the default type) would simply re-scale p_d and p_s , but would not change the underlying dynamics (Dobrovolny et al., in press).

The remaining parameters, β_d, p_d for human influenza virus, and $\beta_d, p_d, \beta_s, p_s$ for avian influenza virus, as well as the initial viral inoculum, V_0 , were manually adjusted to match the experimental data presented in Fig. 1 and are listed in Table 1. The experimental data actually corresponds to the geometric mean of multiple viral titer points presented in de Jong et al. (2005) for each day. Manual adjustment was necessary since least-squares fitting could not converge due to the overparametrization of the model and the limited amount of experimental data available (Miao et al., in press). Note that the behavior of the model, and our results and conclusions, are robust over a wide range of parameter values for all the manually fitted parameters, including V_0 and allowing either $p_d > p_s$ or $p_s > p_d$, as the model's behavior relies on the ratio of the parameter values rather than on their absolute values as explained in Dobrovolny et al. (in press).

2.3. Fit of the single and two target-cell models to patient treatment data

Given the restrictive size of the data set (de Jong et al., 2005), the number of free parameters in the models had to be kept at a

Table 1

Default two target cell model parameters for human- and avian-strain infections.

<i>Infections with human strains</i>	
β_d	$1.6 \times 10^{-4} \text{ (cDNA/mL)}^{-1} \text{ d}^{-1}$
p_d	$3.3 \times 10^6 \text{ cDNA/mL d}^{-1}$
<i>Infections with avian strains</i>	
β_d	$1.1 \times 10^{-7} \text{ (cDNA/mL)}^{-1} \text{ d}^{-1}$
p_d	$4.8 \times 10^8 \text{ cDNA/mL d}^{-1}$
β_s	$1.3 \times 10^{-4} \text{ (cDNA/mL)}^{-1} \text{ d}^{-1}$
p_s	$8.6 \times 10^6 \text{ cDNA/mL d}^{-1}$
<i>Shared initial conditions</i>	
V_0	$7.5 \times 10^{-2} \text{ cDNA/mL}$
E_0, I_0	0

minimum. In the case of the two target cell model, all parameters were fixed to the values for a typical influenza viral infection with an avian strain presented in Table 1. Only the drug efficacy, ϵ , and the time at which treatment is initiated, τ , were allowed to vary between the different patients, and were determined through a least-square regression of the model to the data using the `leasqr` function in Octave. For the single target cell model, we assume that avian strain influenza virus only infects default cells during the infection (i.e., $\beta_s = p_s = 0$) and determined $\beta_d = 3.96 \times 10^{-7} \text{ (cDNA/mL)}^{-1} \text{ d}^{-1}$ and $p_d = 1.666 \times 10^8 \text{ (cDNA/mL)} \text{ d}^{-1}$, as well as the drug efficacy and the time at which treatment is initiated for each patient, through a least-square regression of the model to the data.

3. Results

3.1. Capturing infection dynamics for human and avian strains

To assess the effects of treatment with NAI on the severity and outcome of human infections with human vs. avian strains, a reasonable mathematical model able to capture the dynamics of both infection profiles is required. Human infections with avian strains appear to be characterized by higher viral loads sustained over longer periods of time, and peaking later than for infections with human-adapted strains (de Jong et al., 2006).

The high viral load sustained over longer periods of time seen with avian-derived influenza viral infections in human could either be the result of hindered clearance of already secreted virus particles due to a poor immune response to the novel strain, or it could be due to a sustained viral production over longer periods. In the former scenario, intervention with NAI treatment during the sustained viral titer phase would have little effect on infection severity and outcome as the free lingering viral particles have already been released. However, experimental data suggests that even late oseltamivir treatment (> 3 days post-infection (dpi)) of avian strain-infected patients is effective in reducing viral titer and can lead to disease resolution (de Jong et al., 2005; McGeer et al., 2007; Kandun et al., 2008). This suggests that the sustained viral titer seen in human infections with avian influenza virus strains is instead the result of sustained viral production over times longer than for infections with human-adapted strains.

While the reason for this sustained viral production is not clear, possible explanations include an excessive cytokine response (de Jong et al., 2006; Kaiser et al., 2001; Seo and Webster, 2002; Cheung et al., 2002; Chan et al., 2005), a poor immune response due to the strain's novelty (Hsieh et al., 2006; Seo et al., 2002), infection of the lower respiratory tract which normally has limited involvement in influenza infections (van Riel et al., 2006; Uiprasertkul et al., 2007), and differences in target cell receptor affinity (cell tropism) between the two strains (Pekosz et al., 2009; Stevens et al., 2008;

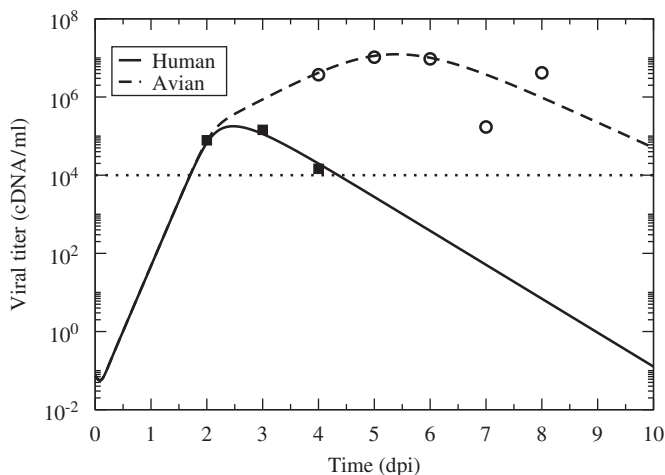


Fig. 1. Viral titer curves representative of the course of untreated human influenza viral infections with either human (solid) or avian (dashed) strains. Both infections are captured using the two target cell eclipse model with the parameter values set to match patient data (squares and circles) (de Jong et al., 2006) (Table 1). The estimated viral threshold below which the infection is asymptomatic is indicated with a horizontal dashed line.

Matrosovich et al., 2004, 2007). A model which extends the standard single target-cell limited viral infection model (Baccam et al., 2006) to include two distinct cell populations, captures the high, sustained viral titers seen in avian-strain infections (Dobrovoly et al., in press). While the original impetus for the extended model was cell tropism, with the default and secondary cell types representing cells which predominantly express SA α 2–3 Gal receptors and cells that predominantly express SA α 2–6 Gal receptors, the default and secondary cell types could, in fact, represent other biological processes. For example, the secondary population could represent cells protected by an immune response or cells located in the lower respiratory tract rather than in the upper respiratory tract. The Baron model (known as the two target cell model), simply requires that the secondary cells differ from the default cells in their susceptibility to infection by the virus and their rate of viral production once successfully infected. Under certain parameters, the model produces long-lasting high levels of viral titer, reminiscent of viral titers measured in humans and pigs infected with avian influenza virus strains (de Jong et al., 2006; Seo and Webster, 2002). The details of the mathematical model and our choices for parameter values are described in the Methods section.

We use data collected by de Jong et al. (2006) to determine model parameters that characterize the “typical” human influenza A viral infection with either a human or avian strain. The de Jong data consists of a single measurement of viral titer in pharyngeal and nasal swabs collected from each of 18 patients infected with avian (H5N1) influenza virus and 6 patients infected with human influenza virus (either H1N1 or H3N2) upon admission to hospital; we have used the geometric mean of all samples collected on a specific day. The parameters determined from fitting the data are such that human-derived influenza virus strains can only infect default cells, essentially reducing the model to the standard single target-cell limited model (Baccam et al., 2006), while avian-derived strains can infect both default and secondary cells. The parameters controlling the two target cells' susceptibility to infection and viral production rate for infection with a human- or avian-origin strain are described in Methods.

The viral titer profiles for our example human and avian strain influenza viral infections in the absence of treatment are shown in Fig. 1 along with the averaged patient data (de Jong et al., 2006). We can see that while the infection with a human strain is captured by a single target cell model, the shape of the viral titer curve for infection with an avian strain could not be captured by the single target cell model without using a very large initial viral titer (see Dobrovoly et al., in press) but is captured well by the two target cell model using a reasonable initial viral inoculum.

While the reasons behind the differences in dynamics of human infections with human and avian strains remain unclear, Fig. 1 shows that the two target cell model can yield good agreement with available experimental data. We believe that the additional terms and parameters of the two target-cell model can serve as generic dials to represent a range of plausible causes for differences in dynamics. Thus, we feel it reasonable to use this model with its selected parameters (see Table 1) to assess the effect of treatment with NAI on human disease severity and outcome for infection with human- and avian-derived strains for different treatment scenarios.

3.2. Assessing the effect of treatment with neuraminidase inhibitors

In order to better characterize patient discomfort and infection severity, we establish a definition for a symptomatic infection. The onset of symptoms in a human influenza viral infection with a human-adapted strain appears to take place, on average, around 1–2 dpi and subside 4–5 dpi (Hayden et al., 1998; Fritz et al., 1999;

Lau et al., 2010). In Fig. 1, the viral titer for infection with a human influenza virus strain crosses 10^4 cDNA/mL around these times. Thus, we use this titer level as the symptomatic viral titer threshold and indicate it with a horizontal dashed line on viral titer graphs. Note that the correlation level between viral titer and symptom score varies between individuals, but on average, viral titer should provide a good surrogate for symptom score. Thus, we take the time of symptom onset to correspond to the time at which the viral titer first crosses the symptomatic threshold, i.e., the time elapsed between infection and the appearance of symptoms.

For the sake of simplicity, we assume that treatment with the NAI is applied at time τ , spontaneously reaches the desired concentration, and remains at this concentration through the remainder of the infection. Here and elsewhere, drug efficacy (ε) is used to mean both actual efficacy in terms of the drug's IC₅₀, as well as the drug concentration. In other words, an increase in drug efficacy should be interpreted either as a decrease in IC₅₀ or as an increase in the administered drug concentration. Fig. 2 shows changes in the viral titers predicted by the model as the efficacy of the drug, ε , is varied for NAI treatment applied prophylactically, or at either 2 dpi or 3 dpi.

To better understand the impact of treatment efficacy and treatment delays on the disease burden experienced by a patient, a few markers are important. The time at which the viral titer curve peaks is a good measure of the infection's growth speed, but does not present a complete picture of the infection. In order to distinguish between infections growing rapidly due to high viral loads that are sustained over long periods of time leading to a late viral titer peak, and infections growing slowly and thus peaking later, it is important to also consider the area under the viral titer curve (AUC) which we will refer to as the viral titer burden. The viral titer burden not only helps delineate between a late peaking infection which grows slowly and one which grows rapidly and plateaus, but it is also an important consideration in itself since viral titer is associated with patient symptom scores, oral temperatures, weight of nasal discharges, and levels of IL-6 and IFN- α (Hayden et al., 1998; Handel et al., 2007).

In order to characterize disease burden and how it is affected by NAI treatment, we retain the time of viral titer peak and the viral titer burden as the two key measures of disease burden on a patient. Fig. 3 explores the effect of NAI treatment on these two measures of disease severity.

In the case of both avian and human strains, when NAI is applied prophylactically the growth of the infection is completely suppressed at a drug efficacy $\sim 98\%$, i.e., the initial viral titer does not grow, but simply decays to zero. The minimum drug efficacy required to suppress infection when NAI treatment is applied prophylactically is found through stability analysis (see Appendix) and is $\varepsilon^* = 0.972$ for both human and avian influenza virus strains for the parameters used in this paper. This is interesting given that drug efficacy for a typical zanamivir treatment course was estimated to be $\sim 97\%$ from fits of a mathematical model (Baccam et al., 2006) to experimental infections with influenza virus A/Texas/91 (H1N1) treated with zanamivir (Hayden et al., 1996).

When NAIs are applied prophylactically, the viral titer for infection with a human strain peaks later and at a lower level as treatment efficacy increases, leading to a reduced viral titer burden and a delayed viral titer peak. Prophylactic treatment for infection with an avian strain leads to more complex changes in the viral titer curves. In particular, viral titer for infection with an avian strain peaks later than for infection with a human strain until it drops to nearly the same level as that for infection with a human strain at a drug efficacy $\sim 72\%$. For the parameter values used here, a drug efficacy of 72% marks the point at which growth of the infection is halted in the secondary cells, proceeding only in the default cells (see Appendix). At drug efficacies $\geq 72\%$, prophylactic treatment

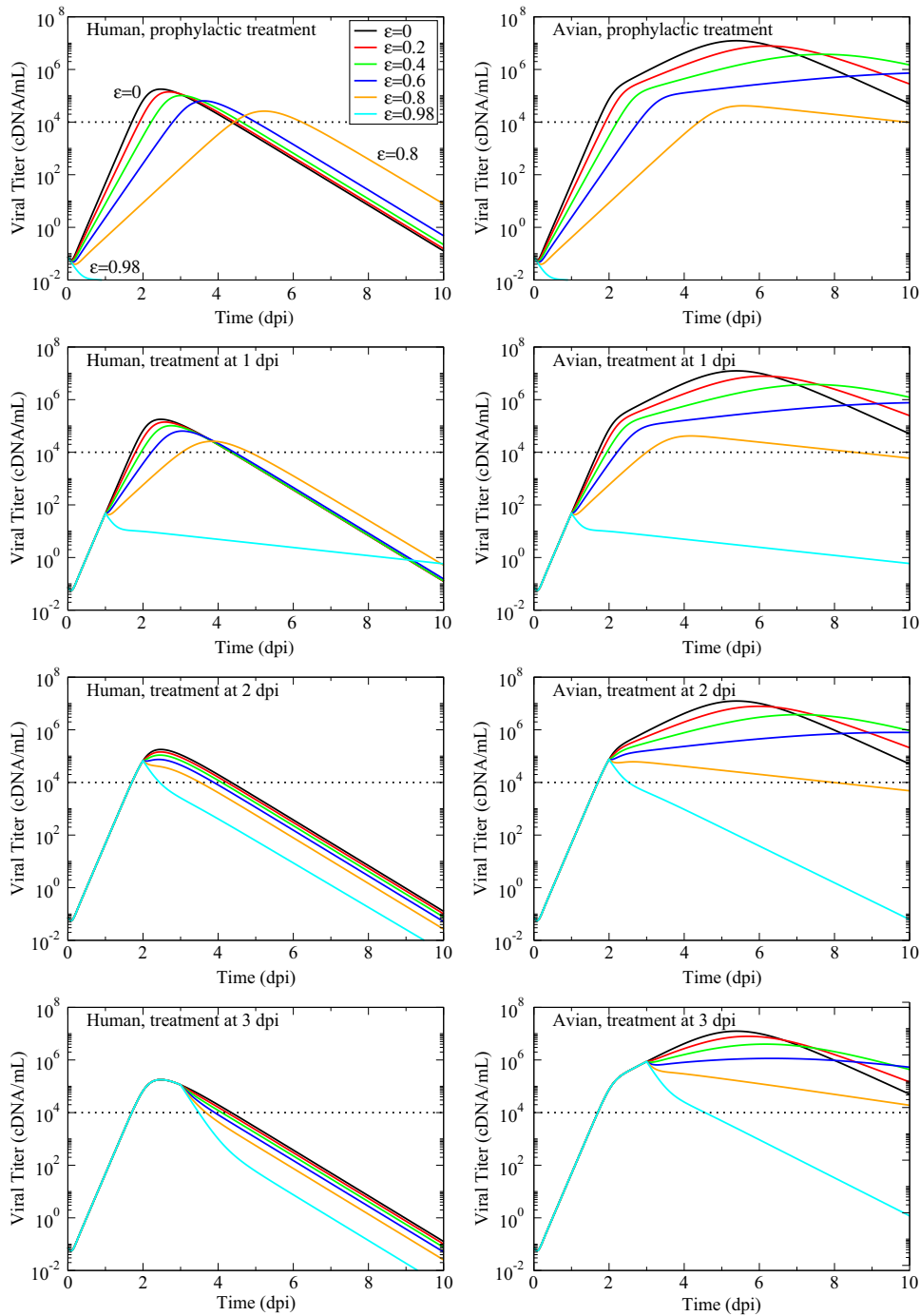


Fig. 2. Effect of NAI treatment on human and avian strain influenza viral infections. Treatment is applied either prophylactically (top row); at 1 dpi (second row); at 2 dpi (third row); or at 3 dpi (fourth row), and drug concentration is assumed to be constant ($\varepsilon(t) = \varepsilon$) from the time treatment begins. The resulting time course of viral titer is shown for several values of drug efficacy or dosage (ε) for human infections with either human (left column) or avian (right column) influenza virus strains.

with a NAI restricts the avian strain influenza viral infection to only take hold in the default cell population, and effectively behave similarly to a typical seasonal influenza viral infection.

When NAI treatment is delayed and applied 2–3 dpi, the differences between human- and avian-strain infections are more marked. Even at high drug efficacies, NAI treatment of infections with a human influenza virus strain has little effect on infection severity and duration. This is in line with experimental data of delayed treatment of influenza viral infections with zanamivir (Hayden et al., 1996). For infections with avian strains, however, even a treatment delay of 3 dpi significantly decreases viral load. This is in line with experimental data of delayed

treatment with oseltamivir of human infections with avian strains (de Jong et al., 2005). This may be explained by the difference in the time of viral titer peak between infections with human and avian strains. The time at which the viral titer curve peaks corresponds to the time when most of the target cells have already been infected and have already released most of their virus particles (Baccam et al., 2006; Dobrovolny et al., in press). Intervention with NAIs close to or after viral titer peak has little effect on disease dynamics as there is little viral production left to block. Thus, the delayed peak seen in infections with avian influenza virus strains provides an increased window of opportunity for treatment of patients. Even late treatment with NAIs of patients infected with avian-adapted

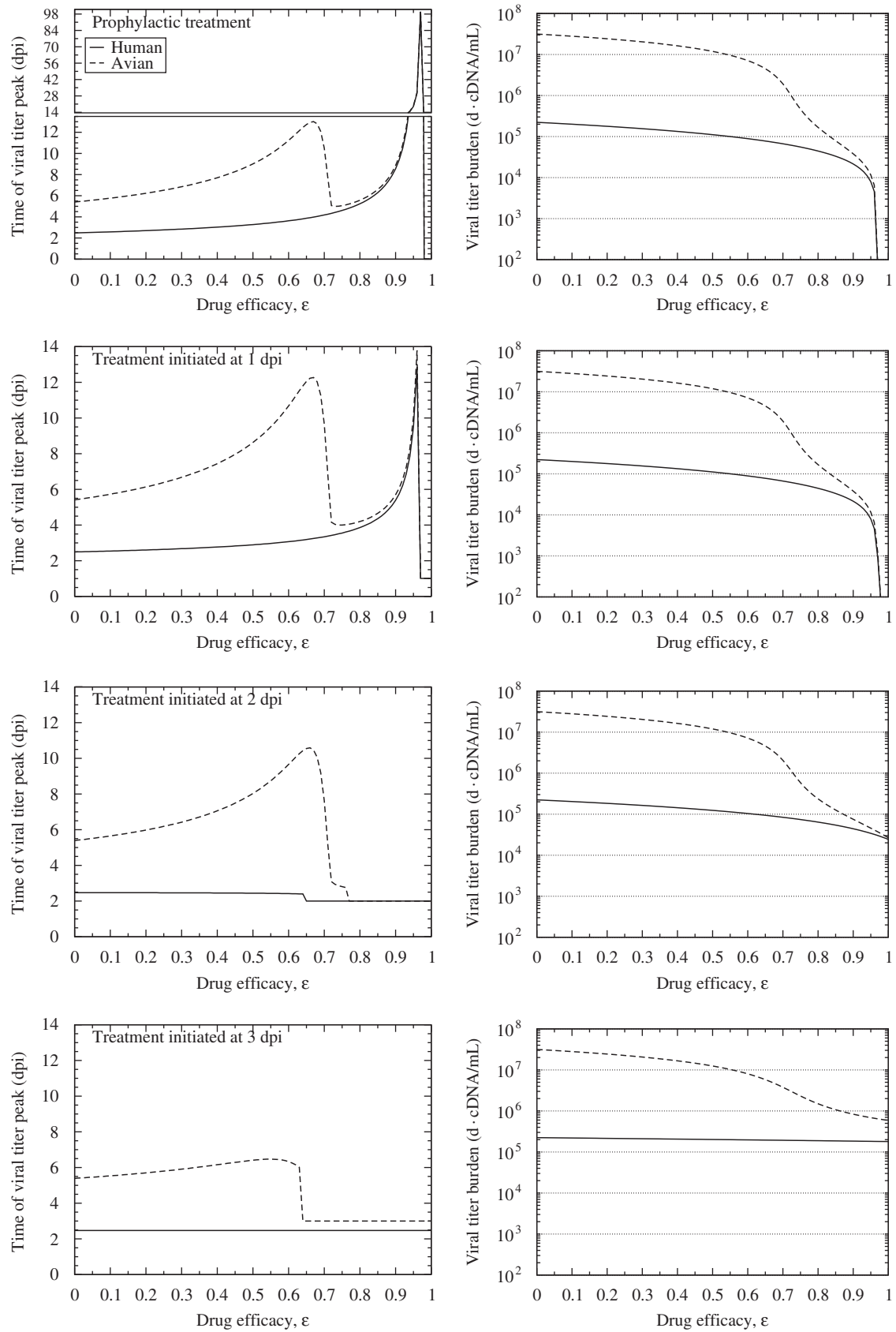


Fig. 3. Effect of NAI treatment on the disease burden of human influenza viral infections with human (solid) and avian (dashed) strains. Here, disease burden is characterized by the time of viral titer peak (left column) and the area under the viral titer curve or viral titer burden (right column). Treatment with NAI is initiated at the time of infection (top row); at 1 dpi (second row); at 2 dpi (third row); or at 3 dpi (bottom row), and is administered at a range of concentrations corresponding to the drug efficacies (ϵ) indicated on the x-axis. Drug concentration is assumed to be constant from the start of treatment.

strains should have a significant impact on disease severity and duration.

This is echoed in the plots of the viral titer burden (Fig. 3, right column) which show increasingly modest decreases in the viral titer burden as drug efficacy is increased for the treatment of infections with a human strain, but show continued and significant decrease for infections with avian strains. Remarkably, as drug efficacy increases, the viral titer burden decreases more rapidly for infections with an avian strain than for those with a human strain. For example, when NAI treatment is administered prophylactically a 10-fold decrease in the viral titer burden (a 90% reduction) requires a drug efficacy of $\sim 90\%$ for infection with a human strain, but only $\sim 68\%$ for infections with an avian strain. However, since the viral titer burden is much higher in infections with avian strains than in those with human strains, a ~ 140 -fold reduction in the viral titer burden of an infection with an avian strain is required to match an untreated infection with a human strain. This is achieved with a drug efficacy of $\sim 79\%$ for prophylactic NAI treatment, $\sim 81\%$ if treatment is initiated at 2 dpi, and cannot be achieved for treatment initiated at 3 dpi. But at a drug efficacy of 98%, treatment initiated at 3 dpi still reduces the viral titer burden of infections with avian strains by 50-fold (a 98% reduction), whereas the reduction for human strains is only 1.2-fold (a 19% reduction).

Altogether, these measures indicate that while delayed NAI treatment of infections with human influenza virus strains has little impact on disease morbidity, the model predicts that in the case of infections with avian strains, even long treatment delays (3 dpi) and modest drug efficacies (80%) lead to a significant reduction in disease morbidity.

3.3. Comparing the model against data from NAI-treated patients

In order to validate our findings, we used both the single target cell and two target cell models to fit viral titer measurements from patients (Patients 3–8 in Fig. 3 of de Jong et al. (2005)) naturally

infected with various H5N1 strains and treated with 75 mg of oseltamivir twice daily for 5 days (de Jong et al., 2005). The best fits of the two target cell (solid line) and single target cell (dashed line) models to the patient data are shown in Fig. 4. The sharp jumps in the viral titer appearing at the start and end of drug treatment is due to the discontinuous (on-off) manner in which treatment endpoints are implemented in the models for simplicity. This can easily be remedied with the use of a more sophisticated pharmacokinetic model of drug absorption in the body.

While the data is limited, the models both generally agree well with the patient data. The sum of squared residuals (SSR), a measure of how well the model fits the data, is 6.4 for the two target cell model and 7.0 for the single target cell model. However, since the models have differing numbers of parameters, we use small-sample size (second order) Aikake's "an information criterion" (AIC_C) (Burnham and Anderson, 2002) to compare the model fits. The model with the smallest AIC_C represents the model which is best supported by the limited amount of data and still provides a reasonable fit. The AIC_C for the fit using the single target cell model is 85, while the AIC_C for the fit using the two target cell model is 44, suggesting that the two target cell model provides a better fit for the experimental data.

The fitting allowed us to obtain estimates of the efficacy of the treatment with oseltamivir in each patient. For both models, the drug efficacies obtained for patients who recovered from the infection (91–99%) are higher than those for patients 3 and 4 who died (51% and 79% for the single target cell model, and 76% and 82% for the two target cell model). In the case of patient 4, this can easily be attributed to the high level of oseltamivir resistance which was confirmed through viral sequencing (de Jong et al., 2005). Generally, a poor drug efficacy can also be attributed to differences in how the drug is metabolized in individual patients and variations in the dose reaching the site of infection.

The treatment delays obtained through fitting of the single target cell model were fairly consistent, ranging from 6.2 dpi to 7.3 dpi. This is consistent with the median treatment delay of 6 dpi

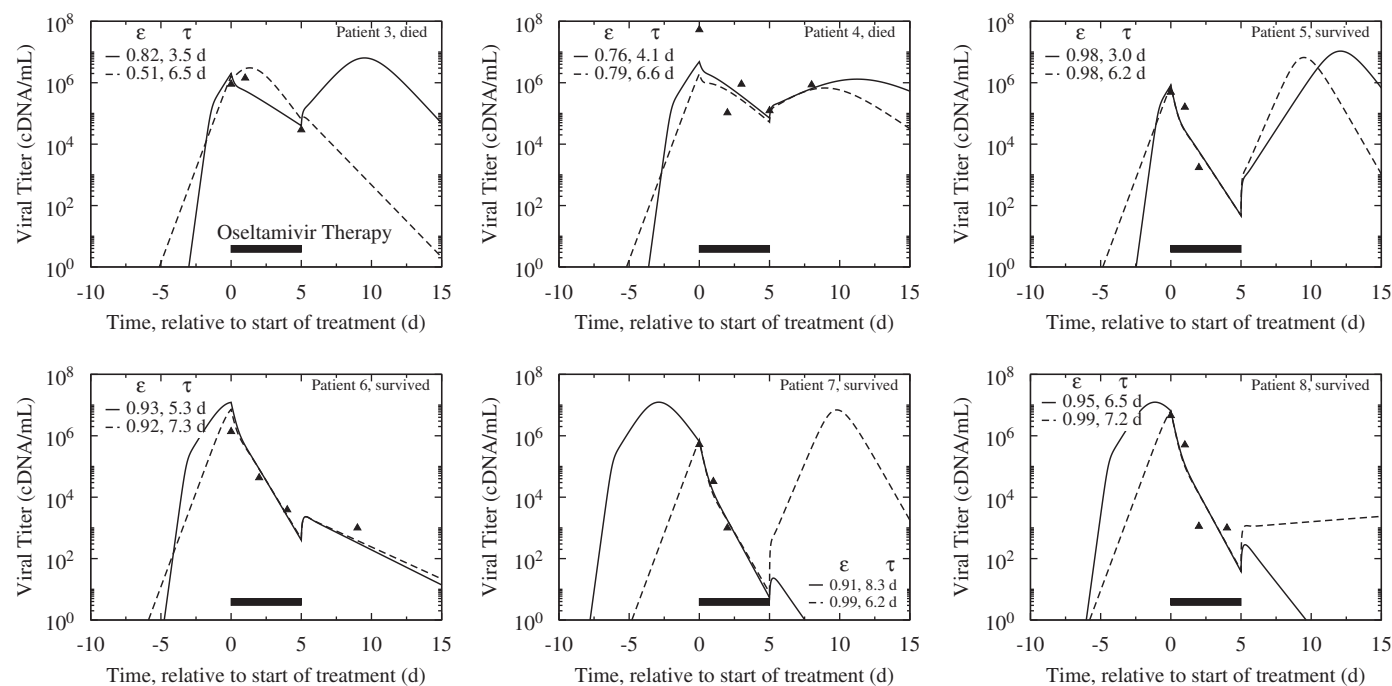


Fig. 4. Fits of the two target cell mathematical model (solid lines) and the single target cell model (dashed lines) with oseltamivir treatment to data for treated avian-influenza virus infected patients (de Jong et al., 2005). For the two target cell model, patients are assumed to be infected with the "typical" avian influenza virus whose parameters are given in Table 1, differing only in the time at which treatment is initiated and the efficacy of the treatment. For the single target cell model, it is assumed that the avian influenza virus only infects default cells ($\beta_s = p_s = 0$) with β_d and p_d remaining as free parameters. Patients 3–8 correspond to those in Fig. 3 of de Jong et al. (2005).

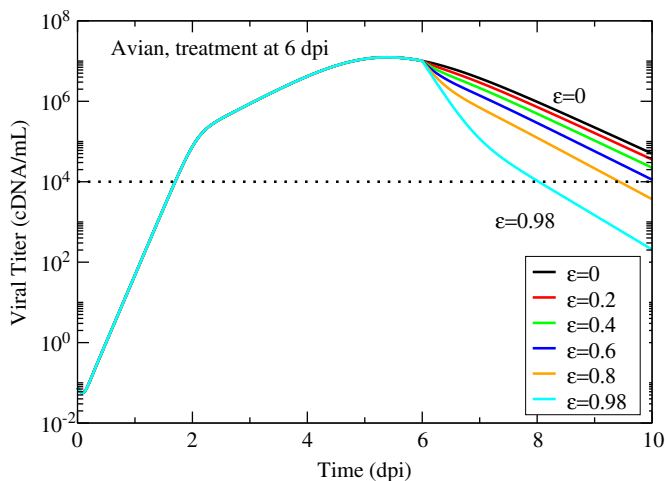


Fig. 5. Effect of NAI treatment initiated at 6 dpi on avian strain influenza viral infections. The resulting time course of viral titer is shown for several values of drug efficacy or dosage (ϵ).

found in several studies (Hien et al., 2009; de Jong et al., 2005; Buchy et al., 2007; Oner et al., 2006; Hien et al., 2004) although it does not show the range of treatment delays seen in these studies. Treatment delays obtained through fitting of the two target cell model varied between 3.0 dpi and 8.3 dpi. The delays found with the two target model are consistent with those reported by the six patients of this study (de Jong et al., 2005), and by patients of another study (Hien et al., 2009), namely 3–8 dpi. At a treatment delay of 6 dpi, our model predicts that there is no reduction in peak viral titer, since 6 dpi is past the viral titer peak, but treatment with a drug efficacy of $\epsilon = 0.98$ shows a marked decrease in the duration of the illness, as seen in Fig. 5.

Once treatment is stopped, some viral titer curves show continued decrease in viral titer while others show a rebound of the infection. While the titer rebound is credible in patients 3 and 4 who died from the infection, it is probably inaccurate in the case of patients 5 and 7, who survived the infection. It is probably a consequence of the absence of an explicit immune response in our model which likely played an important role in infection resolution in these patients. This is especially likely given that the rebound is seen once treatment has been terminated, and occurs at > 5 dpi which is shortly after the adaptive immune system would begin to respond (Doherty et al., 2006; Miao et al., 2010). So while it is reasonable to neglect the effect of the adaptive immune response when considering uncomplicated, seasonal influenza infections which are resolved within 5–6 dpi (Miao et al., 2010), our fits indicate that this assumption is poor when considering longer-lasting, severe infections with an avian influenza strain. Thus, one should be cautious in drawing conclusions from our model's prediction for late viral titer dynamics at > 5 –6 dpi.

4. Discussion

In preparation for a possible human influenza pandemic with an avian-derived strain, it is important to understand the kinetics of human infections with avian-origin influenza virus strains, identify how they differ from seasonal infections, and determine how their kinetics may be affected by treatment with NAIs. To that aim, we have adapted an existing mathematical model (Dobrovoly et al., in press) to match viral loads collected from patients naturally infected with either seasonal or avian-adapted influenza virus strains (de Jong et al., 2006). The model we used was an extension to the traditional in-host viral kinetics model used for a seasonal

influenza viral infection (Baccam et al., 2006), but included two distinct cell populations. By restricting the human influenza virus strains to infecting only default cells and allowing the avian strains to preferentially infect default cells but also to a lesser extent secondary cells, we were able to match the typical course of infections with either human or avian influenza virus strains suggested by patient data (de Jong et al., 2006). Our model clearly shows the benefit of treating avian influenza patients, even several days post-infection and can quantify the effects of NAI treatment as compared to the untreated case.

Using the model thus calibrated for the typical untreated human infection with either human or avian influenza virus strains, we investigated the effect of NAI treatment on the course of the infection. Given the chosen parameters, we found that a drug efficacy of 97.2% is sufficient to suppress symptomatic infection in both human- and avian-strain infections when treatment is administered prophylactically. Although we found that both human and avian strain influenza virus require the same drug efficacy to halt the infection, it must be noted that the dosage needed to reach this efficacy may be different for the two strains. The equation used to characterize drug efficacy (Eq. (1)) is such that the efficacy is determined not only by drug concentration, but also by the IC_{50} and by the maximum (saturation) efficacy of the drug which likely differ for avian and human influenza virus strains. Recent in vitro data shows that the IC_{50} for the treatment of different avian influenza viral infections with either oseltamivir or zanamivir varies widely (Govorkova et al., 2001). Determining the correct dosage is crucial, particularly for avian strain influenza virus, as the prolonged infection with sustained viral production which appears to be characteristic of infections with avian strains (de Jong et al., 2006; Seo and Webster, 2002) offers an increased opportunity for drug resistance to develop.

Our model predicts that while late treatment (beyond ~ 48 h) of an infection with a human strain has little effect on disease duration and severity, even very late treatment of an infection with an avian strain significantly shortens the duration and reduces the severity of the infection. In particular, a 90% reduction in peak viral titer can be achieved with NAI treatment up to 3 days post-infection with avian influenza virus. This is fortunate since there is often a significant delay in seeking medical treatment—a median of 6 dpi (Hien et al., 2009; de Jong et al., 2005; Buchy et al., 2007; Oner et al., 2006; Hien et al., 2004)—in regions that have been hardest hit by avian influenza. Our model suggests that even these patients will see a reduction in the severity and duration of their illness if treated with NAIs. Unfortunately, there is no clinical data available which can be used to support our prediction that NAI treatment of avian-derived strains of influenza as late as 6 dpi reduces severity and duration of the infection compared to an untreated case in humans. The main difficulty lies in obtaining data from an untreated case of avian strain influenza. The high mortality rate of H5N1 (Gambotto et al., 2008) means that any person infected with H5N1 must be treated wherever treatment is available, and when a facility does not have antivirals available, it seems it also will not have the resources to collect and culture patient swabs. However, the clinical data from treated patients collected by de Jong et al. (2005) and presented in this paper suggests that treatment even past 6 dpi is beneficial since four of the six patients survived, although we cannot quantify the reduction in severity or duration. Other researchers have found similar results: typically, H5N1-infected patients seek treatment late (often a week after the onset of symptoms) resulting in a late start of treatment, yet many seem to benefit from treatment with NAIs (Hien et al., 2004, 2009; de Jong et al., 2005; Buchy et al., 2007; Oner et al., 2006; McGeer et al., 2007; Kandun et al., 2008). It is also clear that if viral production continues several days after infection, then NAIs still have an opportunity to act to block this production and contribute

to reducing the viral titer. The inability to quantify experimentally or clinically the benefits of NAI treatment in avian influenza patients highlights the need to develop appropriate models. Our model predicts benefit in treating avian influenza-infected patients, even several days post-infection and can quantify the effects of NAI treatment as compared to the untreated case.

There are some animal studies that suggest that delayed treatment of avian influenza with neuraminidase inhibitors may be beneficial. Govorkova et al. (2001) found that survival of mice infected with A/HK/156/97 (H5N1) went from 0% in untreated mice to ~60% in mice treated with oseltamivir started at 2.5 dpi. A second NAI, RWJ-270201, was slightly less beneficial increasing survival to only ~40% when initiated at 2.5 dpi. A slightly longer delay was studied by Boltz et al. (2008) in mice infected with A/Vietnam/1203/04 (H5N1) and treated with peramivir. They found that treatment initiated at 3 dpi did not increase survival, but did decrease weight loss and the number of animals showing neurological symptoms. Neither of these studies presents the change in viral titer over time between treated and untreated cases so it is difficult to completely assess the effect of delayed treatment on the entire course of the infection and whether our model agrees with their experiments. It is also important to note that our model does not predict that simply giving any amount of drug will benefit the patient; the drug dosage should be above the critical drug efficacy, ε^* , to substantially shorten the duration of the infection, particularly when treatment is initiated at 3 or 6 dpi.

We analyzed data collected from six patients infected with avian influenza virus and treated with oseltamivir using the two target cell model as well as the single target cell model. Although both models captured well the viral titer from the six patients, the AIC_c indicates that the two target cell model is better supported by the experimental data. The fit of the model to the data yielded estimates for the time post-infection at which treatment was initiated, and the drug efficacy. The treatment start times were all consistent with those reported by the patients (de Jong et al., 2005) and elsewhere in the literature (Hien et al., 2009). In addition, the drug efficacies obtained were smaller for the two patients who died compared to those for the four patients who survived.

In our analysis, we have not explicitly considered the possible impact infection with a drug-resistant strain might have on treatment efficacy. However, we have considered the effect of treatment at low drug efficacies ($\varepsilon < 0.5$) which could correspond to the efficacy of a NAI against a drug-resistant strain (i.e., one with a much larger IC₅₀). At low efficacies, our model predicts that treatment has a minimal impact on time of viral titer peak or cumulative viral titer. In such a case, NAI treatment would mostly only contribute to increasing the production and shedding of drug-resistant virus by treated patients through drug pressure, as discussed in Moghadas (2008). With respect to the development of resistance over the course of treatment, it is clear that the prolonged viral shedding seen in infections with avian influenza strains increases the window of opportunity for drug resistance to emerge. The emergence of resistance to oseltamivir is difficult to predict or model because the main mutation conferring resistance to oseltamivir (H274Y in the neuraminidase) has been shown in some strains to greatly reduce the replicative efficacy of the virus both in vivo and in vitro (Yen et al., 2005; Baz et al., 2010), whereas in others it has a negligible effect (Yen et al., 2007; Baz et al., 2010). Thus, surveillance of avian influenza strains and studies of their replicative efficacy with the introduction of the H274Y mutation is crucial to improving the models so as to refine treatment recommendations.

The two target cell model as presented here assumes that differences in infection dynamics between human-strain and avian-strain infections are due to the existence of a second cell population that only the avian-strain virus can access. We have

indicated several possible scenarios for the two cell populations consistent with current hypotheses of the biological cause of sustained viral titer. We can interpret the two cell populations as cells with different cell receptors if cell tropism is the underlying cause of severe infection (Pekosz et al., 2009; Stevens et al., 2008; Matrosovich et al., 2007, 2004). We can interpret the two cell populations as those protected by an immune response and those left unprotected if either increased immune response (de Jong et al., 2006; Kaiser et al., 2001; Seo and Webster, 2002; Cheung et al., 2002; Chan et al., 2005) or lack of an immune response (Hsieh et al., 2006; Seo et al., 2002) are responsible for severe infections. Finally, we can interpret the two cell populations as cells located either in the upper respiratory tract and cells located in the lower respiratory tract which are not normally involved in human-strain infections (van Riel et al., 2006; Uiprasertkul et al., 2007). In reality, however, it is likely that all of these mechanisms play a role in the severity of avian-strain infections and the two target cell model is a simplified view of the infection process incorporating many of these mechanisms implicitly.

While it would be interesting to isolate the effect of the immune response by including it explicitly into our model, it is simply not possible at this time due to the very limited amount of data available (Miao et al., in press). And although it is the lack of an explicit immune response in our model which is responsible for the unrealistic rebound in viral titer seen in some of our fits to patient data, we still believe that our model's results and predictions are to a large extent independent of the model's implementation details. This is because our conclusions ultimately rely on the fact that the late viral titer peak and prolonged viral production period seen in influenza viral infections with avian strains (de Jong et al., 2006; Seo and Webster, 2002) is the reason why a delayed intervention with NAI is still very much effective in controlling these infections. Thus, we believe that our conclusions generally hold regardless of the model's implementation details so long as the proposed model can capture sustained viral production. One should keep in mind, however, that our model's predictions represent an average effect or benefit, and that treatment efficacy and infection severity in a given patient will vary due to factors beyond our model's description (e.g., pre-existing conditions, prior exposure, bacterial co-infection).

In conclusion, we have adapted the two target cell model (Dobrovolny et al., in press) to match the dynamics of human infections with either a human- or avian-adapted influenza virus strain. The model captured the literature data for both infections (de Jong et al., 2006), and was shown to provide a good match to data collected from patients infected with avian strains and treated with oseltamivir (de Jong et al., 2005). In addition, our findings that NAIs can control human- and avian-strain infections with similar drug efficacies and that NAIs will generally reduce viral titer and illness duration even with delayed treatment lead us to conclude that NAI treatment will be an effective tool in controlling human infections with avian-derived influenza virus strains.

Acknowledgments

This work was supported in part by the Natural Sciences and Engineering Research Council of Canada through a Discovery Grant (CAAB), with additional support provided by F. Hoffmann-La Roche, Ltd.

Appendix A. Stability analysis

We found two drug efficacies at which there were distinct changes in behavior of the infections. In addition to the arrested growth of the infection when treatment is administered above the

critical drug efficacy, human infections with avian strains showed another marked change in behavior for prophylactic treatment using NAIs at lower efficacies $\sim 70\%$. These points can be predicted through stability analysis. The system has an infinite number of fixed points of the form $(T_d = [0, r_d], E_d = 0, I_d = 0, T_s = [0, 1 - r_d], E_s = 0, I_s = 0, V = 0)$, where r_d is the fraction of the cell population which is of the default type. That is, there is no chronic state of infection where the viral titer remains constant at some non-zero value. We are particularly interested in the stability of the fixed point $(r_d, 0, 0, 1 - r_d, 0, 0, 0)$ since this determines whether any target cells become infected. The stability of this fixed point is determined using the condition

$$\varepsilon > \varepsilon^* = 1 - \frac{c\delta}{T_0[\beta_s p_s(1-r_d) + \beta_d p_d r_d]}$$

When $\varepsilon < \varepsilon^*$, the system undergoes a transcritical bifurcation, the fixed point $(r_d, 0, 0, 1 - r_d, 0, 0, 0)$ becomes unstable and the system tends to another fixed point where both T_d and T_s are lower than their initial values, indicating that the infection has killed some of the target cells.

The bifurcation diagram (Fig. 6) shows a fairly sharp transition from no target cells infected when $\varepsilon > \varepsilon^*$ to infection of target cells when $\varepsilon < \varepsilon^*$, particularly for the default cell population. The secondary cell population is not affected much by the infection until a second transition point,

$$\varepsilon > \varepsilon_s^* = 1 - \frac{c\delta}{\beta_s p_s T_0(1-r_d)}$$

when it too starts to participate in the infection although this transition is not as sharp. For the parameters we chose to capture infection with an avian strain, this corresponds to a drug efficacy of $\varepsilon_s^* = 0.72$ which is in line with the features of Fig. 3 for prophylactic treatment.

When NAI treatment is initiated with a delay, the drug efficacy required to stop the progression of the infection takes on a different meaning and a different value. For delayed treatment, this represents an arrest in the progression of the infection resulting in a monotonic decay in viral titer from the time treatment is initiated. Since the number of available target cells when late treatment is initiated is less than T_0 , the drug efficacy required to halt the infection is less than that required for halting the progression of the infection when the drug is administered prophylactically. This is also reflected in Fig. 3 where the abrupt drop in the time of viral

titer peak occurs at smaller drug efficacies for larger delays in treatment.

References

- Baccam, P., Beauchemin, C., Macken, C.A., Hayden, F.G., Perelson, A.S., 2006. Kinetics of influenza A virus infection in humans. *J. Virol.* 80 (15), 7590–7599.
- Baz, M., Abed, Y., Simon, P., Hamelin, M., Boivin, G., 2010. Effect of the neuraminidase mutation H274Y conferring resistance to oseltamivir on the replicative capacity and virulence of old and recent human influenza A(H1N1) viruses. *J. Infect. Dis.* 201 (5), 740–745.
- Beauchemin, C.A., McSharry, J.J., Drusano, G.L., Nguyen, J.T., Went, G.T., Ribeiro, R.M., Perelson, A.S., 2008. Modeling amantadine treatment of influenza A virus in vitro. *J. Theor. Biol.* 254 (2), 439–451.
- Bocharov, G.A., Romanyukha, A.A., 1994. Mathematical model of antiviral immune response III. Influenza A virus infection. *J. Theor. Biol.* 167 (4), 323–360.
- Boltz, D.A., Ilyushina, N.A., Arnold, C.S., Babu, Y.S., Webster, R.G., Govorkova, E.A., 2008a. Intramuscularly administered neuraminidase inhibitor peramivir is effective against lethal H5N1 influenza virus in mice. *Antiviral Res.* 80 (May), 150–157.
- Boltz, D.A., Rehg, J.E., McClaren, J., Webster, R.G., Govorkova, E.A., 2008. Oseltamivir prophylactic regimens prevent H5N1 influenza morbidity and mortality in a ferret model. *J. Infect. Dis.* 197 (May), 1315–1327.
- Bright, R.A., Shay, D.K., Shu, B., Cox, N.J., Klimov, A.I., 2006. Adamantane resistance among influenza A viruses isolated early during the 2005–2006 influenza season in the United States. *J. Am. Med. Assoc.* 295 (8), 891–894.
- Buchy, P., Mardy, S., Vong, S., Toyoda, T., Aubin, J.-T., Miller, M., Touch, S., Sovann, L., Dufourcq, J.-B., Richner, B., Tu, P.V., Tien, N.T.K., Lim, W., Peiris, J.M., Van der Werf, S., 2007. Influenza A/H5N1 virus infection in humans in Cambodia. *J. Clin. Virol.* 39 (April), 164–168.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, second ed. Springer-Verlag, New York, Inc., New York, USA.
- Chan, M., Cheung, C., Chui, W., Tsao, S., Nicholls, J., Chan, Y., Chan, R., Long, H., Poon, L., Guan, Y., Peiris, J., 2005. Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. *Respir. Res.* 6 (November), 135.
- Cheung, C.-L., Rayner, J.M., Smith, G.J.D., Wang, P., Naipospos, T.S.P., Zhang, J., Yuen, K.-Y., Webster, R.G., Peiris, J.S.M., Guan, Y., Chen, H., 2006. Distribution of amantadine-resistant H5N1 avian influenza variants in Asia. *J. Infect. Dis.* 193 (June), 1626–1629.
- Cheung, C.Y., Poon, L.L.M., Lau, A.S., Luk, W., Lau, Y.L., Shortridge, K.F., Gordon, S., Guan, Y., Peiris, J.S.M., 2002. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* 360 (December) 1831–1837.
- Claas, E.C.J., Osterhaus, A.D.M.E., van Beek, R., de Jong, J.C., Rimmelzwaan, G.F., Senne, D.A., Krauss, S., Shortridge, K.F., Webster, R.G., 1998. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet* 351 (February), 472–477.
- Crystal, R.G., West, J.B., 1991. *The Lung: Scientific Foundations*. Raven Press, Ltd., New York, USA.
- de Jong, M.D., Simmons, C.P., Thanh, T.T., Hien, V.M., Smith, G.J.D., Chau, T.N.B., Hoang, D.M., Chau, N.V.V., Khanh, T.H., Dong, V.C., Qui, P.T., Cam, B.V., Ha, D.Q., Guan, Y., Peiris, J.S.M., Chinh, N.T., Hien, T.T., Farrar, J., 2006. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat. Med.* 12 (10), 1203–1207.
- de Jong, M.D., Thanh, T.T., Khanh, T.H., Hien, V.M., Smith, G.J., Chau, N.V., Cam, B.V., Qui, P.T., Ha, D.Q., Guan, Y., Peiris, J.M., Hien, T.T., Farrar, J., 2005. Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N. Engl. J. Med.* 353 (25), 2667–2672.
- Dharan, N.J., Gubareva, L.V., Meyer, J.J., Okomo-Adhiambo, M., McClinton, R.C., Marshall, S.A., George, K.S., Epperson, S., Brammer, L., Klimov, A.I., Bresee, J.S., Fry, A.M., 2009. Local and systemic cytokine responses during experimental human influenza A virus infection. *J. Am. Med. Assoc.* 301 (10), 1034–1041.
- Dobrovolny, H.M., Baron, M.J., Gieschke, R., Davies, B.E., Jumble, N.L., Beauchemin, C.A.A. Exploring cell tropism as a possible contributor to influenza infection severity. *PLoS One*, in press, doi:10.1371/journal.pone.0013811.
- Doherty, P.C., Turner, S.J., Webby, R.G., Thomas, P.G., 2006. Influenza and the challenge for immunology. *Nat. Immunol.* 7 (5), 449–455.
- Duwe, S., Schweiger, B., 2008. A new and rapid genotypic assay for the detection of neuraminidase inhibitor resistant influenza A viruses of subtype H1N1, H3N2, and H5N1. *J. Virol. Methods* 153 (July), 134–141.
- Fritz, R.S., Hayden, F.G., Calfee, D.P., Cass, L.M.R., Peng, A.W., Alvord, W.G., Strober, W., Straus, W., 1999. Nasal cytokine and chemokine response in experimental influenza A virus infection: results of a placebo-controlled trial of intravenous zanamivir treatment. *J. Infect. Dis.* 180, 586–593.
- Gambotto, A., Barratt-Boyes, S.M., de Jong, M.D., Neumann, G., Kawaoka, Y., 2008. Human infection with highly pathogenic H5N1 influenza virus. *Lancet* 371 (April), 1464–1475.
- Govorkova, E.A., Ilyushina, N.A., Boltz, D.A., Douglas, A., Yilmaz, N., Webster, R.G., 2007. Efficacy of oseltamivir therapy in ferrets inoculated with different clades of H5N1 influenza virus. *Antimicrob. Agents Chemother.* 51 (4), 1414–1424.
- Govorkova, E.A., Leneva, I.A., Goloubeva, O.G., Bush, K., Webster, R.G., 2001. Comparison of efficacies of RWJ-270201, zanamivir, and oseltamivir against

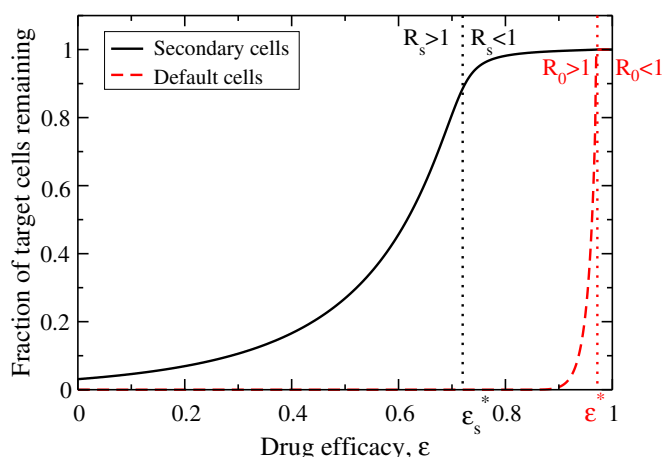


Fig. 6. Fraction of target cells remaining after an NAI-treated infection. Both default and secondary target cells become infected when $\varepsilon < \varepsilon^*$ although secondary target cells only play a small role in the infection unless $\varepsilon < \varepsilon_s^*$. Regions where the basic reproductive numbers for the entire population (R_0) or for the secondary population only (R_s) are either less or greater than one are indicated by the vertical lines.

- H5N1, H9N2 and other avian influenza viruses. *Antimicrob. Agents Chemother.* 45 (10), 2723–2732.
- Gubareva, L.V., McCullers, J.A., Bethell, R.C., Webster, R.G., 1998. Characterization of influenza A/HongKong/156/97 (H5N1) virus in a mouse model and protective effect of zanamivir on H5N1 infection in mice. *J. Infect. Dis.* 178 (6), 1592–1596.
- Handel Jr., A., Longini, I.M., Antia, R., 2007. Neuraminidase inhibitor resistance in influenza: assessing the danger of its generation and spread. *PLoS Comput. Biol.* 3 (12), 2456–2464.
- Handel, A., Longini, I.M., Antia, R., 2010. Towards a quantitative understanding of the within-host dynamics of influenza A infections. *J. R. Soc. Interface* 7 (42), 35–47.
- Hayden, F.G., Fritz, R.S., Lobo, M.C., Alvord, W.G., Strober, W., Straus, S.E., 1998. Local and systemic cytokine responses during experimental human influenza A virus infection. *J. Clin. Invest.* 101 (3), 643–649.
- Hayden, F.G., Treanor, J.J., Betts, R.F., Lobo, M., Esinhart, J.D., Hussey, E.K., 1996. Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza. *J. Am. Med. Assoc.* 275 (4), 295–299.
- Hayden, F.G., Treanor, J.J., Fritz, R.S., Lobo, M., Betts, R.F., Miller, M., Kinnersley, N., Mills, R.G., Ward, P., Straus, S.E., 1999. Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. *J. Am. Med. Assoc.* 282 (13), 1240–1246.
- He, G., Qiao, J., Dong, C., He, C., Zhao, L., Tian, Y., 2008. Amantadine-resistance among H5N1 avian influenza viruses isolated in northern China. *Antiviral Res.* 77, 72–76.
- Hien, N.D., Ha, N.H., Van, N.T., Ha, N.T.M., Lien, T.T.M., Thai, N.Q., Trang, V.D., Shimbo, T., Takahashi, Y., Kato, Y., Kawana, A., Akita, S., Kudo, K., 2009. Human infection with highly pathogenic avian influenza virus (H5N1) in Northern Vietnam, 2004–2005. *Emerg. Infect. Dis.* 15 (1), 19–23.
- Hien, T.T., Liem, N.T., Dung, N.T., San, L.T., Mai, P.P., van VinhChau, N., Suu, P.T., Dong, V.C., Mai, L.T.Q., Thi, N.T., Khoa, D.B., Phat, L.P., Truong, N.T., Long, H.T., Tung, C.V., Giang, L.T., Tho, N.D., Nga, L.H., Tien, N.T.K., San, L.H., Tuan, L.V., Dolecek, C., Thanh, T.T., de Jong, M., Schultz, C., Cheng, P., Lim, W., Horby, P., Farrar, J., 2004. Avian influenza A (H5N1) in 10 patients in Vietnam. *N. Engl. J. Med.* 350 (12), 1179–1188.
- Holford, N., Sheiner, L., 1981. Understanding the dose–effect relationship: clinical application of pharmacokinetic–pharmacodynamic models. *Clin. Pharmacokinet.* 6 (6), 429–453.
- Hsieh, Y., Wu, T., Liu, D., Shao, P., Chang, L., Lu, C., Lee, C., Huang, F., Huang, L., 2006. Influenza pandemics: past, present and future. *J. Formos. Med. Assoc.* 105 (1), 1–6.
- Hurt, A., Selleck, P., Komadina, N., Shaw, R., Brown, L., Barr, I., 2007. Susceptibility of highly pathogenic A(H5N1) avian influenza viruses to the neuraminidase inhibitors and adamantanes. *Antiviral Res.* 73, 228–231.
- Ibrecevic, A., Pekosz, A., Walter, M.J., Newby, C., Battaile, J.T., Brown, E.G., Holtzman, M.J., Brody, S.L., 2006. Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. *J. Virol.* 80 (15), 7469–7480.
- Kaiser, L., Fritz, R.S., Straus, S.E., Gubareva, L., Hayden, F.G., 2001. Symptom pathogenesis during acute influenza: Interleukin-6 and other cytokine responses. *J. Med. Virol.* 64 (3), 262–268.
- Kandun, I.N., Tresnaningsih, E., Purba, W.H., Lee, V., Samaan, G., Harun, S., Soni, E., Septiawati, C., Setiawati, T., Sariwati, E., Wandura, T., 2008. Factors associated with case fatality of human H5N1 virus infections in Indonesia: a case series. *Lancet* 372 (9640), 744–749.
- Kobasa, D., Kawaoka, Y., 2005. Emerging influenza viruses: past and present. *Curr. Mol. Med.* 5 (8), 791–803.
- Kogure, T., Suzuki, T., Takahashi, T., Miyamoto, D., Hidari, K.I., Guo, C.-T., Ito, T., Kawaoka, Y., Suzuki, Y., 2006. Human trachea primary epithelial cells express both sialyl(α 2–3)Gal receptor for human parainfluenza virus type 1 and avian influenza viruses, and sialyl(α 2–6)Gal receptor for human influenza virus. *Glycoconj. J.* 23 (1–2), 101–106.
- Lau, L.L.H., Cowling, B.J., Fang, V.J., Chan, K., Lau, E.H.Y., Lipsitch, M., Cheng, C.K.Y., Houck, P.M., Uyeki, T.M., Peiris, J.S.M., Leung, G.M., 2010. Viral shedding and clinical illness in naturally acquired influenza virus infections. *J. Infect. Dis.* 201 (10), 1509–1516.
- Le, Q.M., Kiso, M., Someya, K., Sakai, Y.T., Nguyen, T.H., Nguyen, K.H.L., Pham, N.D., Ngyen, H.H., Yamada, S., Muramoto, Y., Horimoto, T., Takada, A., Goto, H., Suzuki, T., Suzuki, Y., Kawaoka, Y., 2005. Isolation of drug-resistant H5N1 virus. *Nature* 437 (October), 1108.
- Leneva, I.A., Goloubeva, O., Fenton, R.J., Tisdale, M., Webster, R.G., 2001. Efficacy of zanamivir against avian influenza A viruses that possess genes encoding H5N1 internal proteins and are pathogenic in mammals. *Antimicrob. Agents Chemother.* 45 (4), 1216–1224.
- Leneva, I.A., Roberts, N., Govorkova, E.A., Goloubeva, O.G., Webster, R.G., 2000. The neuraminidase inhibitor GS4104 (oseltamivir phosphate) is efficacious against A/Hong Kong/156/97 (H5N1) and A/Hong Kong/1074/99 (H9N2) influenza viruses. *Antiviral Res.* 48 (2), 101–115.
- Maines, T.R., Chen, L.-M., Matsuoka, Y., Chen, H., Rowe, T., Ortin, J., Falcon, A., Hien, N.T., Mai, L.Q., Sedyaningsih, E.R., Harun, S., Tumpey, T.M., Donis, R.O., Cox, N.J., Subbarao, K., Katz, J.M., 2006. Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model. *Proc. Natl. Acad. Sci. USA* 103 (32), 12121–12126.
- Matrosovich, M.N., Matrosovich, T.Y., Gray, T., Roberts, N.A., Klenk, H.-D., 2004. Human and avian influenza viruses target different cell types in cultures of human airway epithelium. *Proc. Natl. Acad. Sci. USA* 101 (13), 4620–4624.
- Matrosovich, M.N., Matrosovich, T.Y., Uhlendorff, J., Garten, W., Klenk, H.-D., 2007. Avian-virus-like receptor specificity of the hemagglutinin impedes influenza virus replication in cultures of human airway epithelium. *Virology* 361 (2), 384–390.
- McGeer, A., Green, K.A., Plevneshi, A., Shigayeva, A., Siddiqi, N., Raboud, J., Low, D.E., for the Toronto Invasive Bacterial Diseases Network, 2007. Antiviral therapy and outcomes of influenza requiring hospitalization in Ontario, Canada. *Clin. Infect. Dis.* 45 (12), 1568–1575.
- Miao, H., Hollenbaugh, J.A., Zand, M.S., Holden-Wiltse, J., Mosmann, T.R., Perelson, A.S., Wu, H., Topham, D.J., 2010. Quantifying the early immune response and adaptive immune response kinetics in mice infected with influenza A virus. *J. Virol.* 84 (13), 6687–6698.
- Miao, H., Xia, X., Perelson, A.S., Wu, H., On identifiability of nonlinear ODE models and applications in viral dynamics. *SIAM Rev.*, in press. Available at: <http://www.urmc.rochester.edu/biostat/people/faculty/wusite/documents/2010-7.pdf>.
- Moghadas, S.M., 2008. Management of drug resistance in the population: influenza as a case study. *Proc. R. Soc. B* 275, 1163–1169.
- Oner, A.F., Bay, A., Arslan, S., Akdeniz, H., Sahin, H.A., Cesur, Y., Epcacan, S., Yilmaz, N., Deger, I., Kizilyildiz, B., Karsen, H., Ceyhan, M., 2006. Avian influenza A (H5N1) infection in eastern Turkey in 2006. *N. Engl. J. Med.* 355 (21), 2179–2185.
- Pekosz, A., Newby, C., Bose, P.S., Lutz, A., 2009. Sialic acid recognition is a key determinant of influenza A virus tropism in murine trachea epithelial cell cultures. *Virology* 386 (February), 61–67.
- Perrone, L.A., Plowden, J.K., Garcia-Sastre, A., Katz, J.M., Tumpey, T.M., 2008. H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS Pathog.* 4 (8), 1–11.
- Rameix-Welti, M.A., van der Werf, S., Naffakh, N., 2008. Sensitivity of H5N1 influenza viruses to oseltamivir: an update. *Future Virol.* 3 (2), 157–165.
- Schunemann, H.J., Hill, S.R., Kakad, M., Bellamy, R., Uyeki, T.M., Hayden, F.G., Yazdanpanah, Y., Beigel, J., Chotpitayasuonondh, T., Del Mar, C., Farrar, J., Hien, T.T., Ozbay, B., Sugaya, N., Fukuda, K., Shindo, N., Stockman, L., Vist, G.E., Croisier, A., Nagidaliyev, A., Roth, C., Thomson, G., Zucker, H., Oxman, A.D., 2007. WHO rapid advice guidelines for pharmacological management of sporadic human infection with avian influenza A (H5N1) virus. *Lancet Infect. Dis.* 7 (January), 21–31.
- Seo, S.H., Hoffmann, E., Webster, R.G., 2002. Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. *Nat. Med.* 8 (9), 950–954.
- Seo, S.H., Webster, R.G., 2002. Tumor necrosis factor alpha exerts powerful anti-influenza virus effects in lung epithelial cells. *J. Virol.* 76 (3), 1071–1076.
- Stevens, J., Blixt, O., Chen, L.-M., Donis, R.O., Paulson, J.C., Wilson, I.A., 2008. Recent avian H5N1 viruses exhibit increased propensity for acquiring human receptor specificity. *J. Mol. Biol.* 381 (April), 1382–1394.
- Stittelaar, K., Tisdale, M., van Amerongen, G., van Lavieren, R., Pistoro, F., Simon, J., Osterhaus, A., 2008. Evaluation of intravenous zanamivir against experimental influenza A (H5N1) virus infection in cynomolgus macaques. *Antiviral Res.* 80 (July), 225–228.
- Subbarao, K., Klimov, A., Katz, J., Regnery, H., Lim, W., Hall, H., Perdue, M., Swayne, D., Bender, C., Huang, J., Hemphill, M., Rowe, T., Shaw, M., Xu, X., Fukuda, K., Cox, N., 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* 279 (January), 393–396.
- Thompson, C.I., Barclay, W.S., Zambon, M.C., Pickles, R.J., 2006. Infection of human airway epithelium by human and avian strains of influenza A virus. *J. Virol.* 80 (16), 8060–8068.
- Tumpey, T.M., Maines, T.R., Hoeven, N.V., Glaser, L., Solorzano, A., Pappas, C., Cox, N.J., Swayne, D.E., Palese, P., Katz, J.M., Garcia-Sastre, A., 2007. A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission. *Science* 315 (February), 655–659.
- Uiprasertkul, M., Kitphatit, R., Piuthavathana, P., Kriwong, R., Kongchanagul, A., Ungchusak, K., Angkasekwinai, S., Chokephaibulkit, K., Srisook, K., Vanprapar, N., Auewarakul, P., 2007. Apoptosis and pathogenesis of avian influenza A (h5n1) virus in humans. *Emerg. Infect. Dis.* 13 (5), 708–712.
- van Riel, D., Munster, V.J., de Wit, E., Rimmelzwaan, G.F., Fouchier, R.A., Osterhaus, A.D., Kuiken, T., 2006. H5N1 virus attachment to lower respiratory tract. *Science* 312 (5772), 399 (originally published in Science Express on 23 March 2006).
- Whitley, R.J., Hayden, F.G., Reisinger, K.S., Young, N., Dutkowski, R., Ipe, D., Mills, R.G., Ward, P., 2001. Oral oseltamivir treatment of influenza in children. *Pediatr. Infect. Dis. J.* 20 (2), 127–133.
- Yen, H.-L., Herlocher, L.M., Hoffmann, E., Matrosovich, M.N., Monto, A.S., Webster, R.G., Govorkova, E.A., 2005. Neuraminidase inhibitor-resistant influenza viruses may differ substantially in fitness and transmissibility. *Antimicrob. Agents Chemother.* 49 (10), 4075–4084.
- Yen, H.-L., Ilyushina, N.A., Salomon, R., Hoffmann, E., Webster, R.G., Govorkova, E.A., 2007. Neuraminidase inhibitor-resistant recombinant A/Vietnam/1203/04 (H5N1) influenza viruses retain their replication efficiency and pathogenicity in vitro and in vivo. *J. Virol.* 81 (22), 12418–12426.
- Yun, N.E., Linde, N.S., Zacks, M.A., Barr, I.G., Hurt, A.C., Smith, J.N., Dziuba, N., Holbrook, M.R., Zhang, L., Kilpatrick, J.M., Arnold, C.S., Paessler, S., 2008. Injectable peramivir mitigates disease and promotes survival in ferrets and mice infected with the highly virulent influenza virus A/Vietnam/1203/04 (H5N1). *Virology* 374 (January), 198–209.
- Zheng, B.-J., Chan, K.-W., Lin, Y.-P., Zhao, G.-Y., Chan, C., Zhang, H.-J., Chen, H.-L., Wong, S.S.Y., Lau, S.K.P., Woo, P.C.Y., Chan, K.-H., Jin, D.-Y., Yuen, K.-Y., 2008. Delayed antiviral plus immunomodulator treatment still reduces mortality in mice infected by high inoculum of influenza A/H5N1 virus. *Proc. Natl. Acad. Sci.* 105 (23), 8091–8096.