

# Severity of pneumonia in ferrets due to pandemic H1N1 influenza virus is intermediate between that of seasonal H1N1 and highly pathogenic avian influenza H5N1 virus

Judith MA van den Brand<sup>1</sup>, Koert J Stittelaar<sup>2</sup>, Geert van Amerongen<sup>1</sup>, Guus F Rimmelzwaan<sup>1,2</sup>, James Simon<sup>2</sup>, Emmie de Wit<sup>1</sup>, Vincent Munster<sup>1</sup>, Theo Bestebroer<sup>1</sup>, Ron A.M. Fouchier<sup>1</sup>, Albert D.M.E. Osterhaus<sup>1,2</sup> and Thijs Kuiken<sup>1</sup>



<sup>1</sup>Department of Virology, Erasmus MC and <sup>2</sup>ViroClinics Biosciences BV, Rotterdam, The Netherlands.  
E-mail: j.vandenbrand@erasmusmc.nl

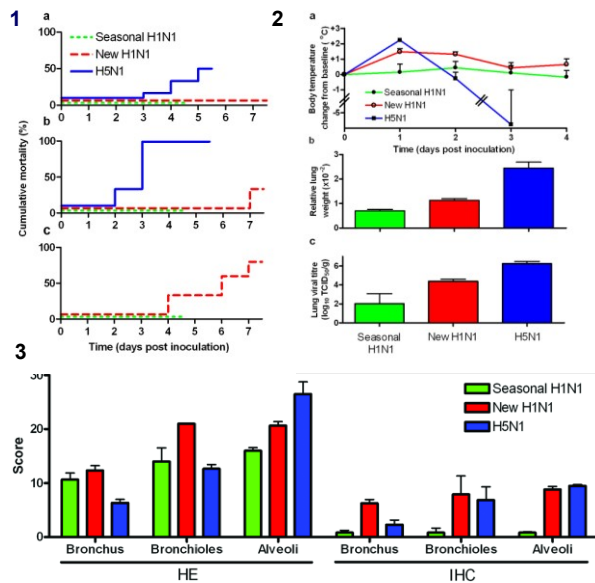


## Introduction

The newly emerged influenza A H1N1 virus (new H1N1 virus) is the first pandemic influenza virus of this century. Three influenza pandemics of the previous century caused variable mortality with the development of severe pneumonia<sup>1</sup>. However, the ability of the new H1N1 virus to cause pneumonia is poorly understood.

## Aim

To compare the pathology of new H1N1 influenza virus with highly pathogenic avian influenza virus (HPAI) H5N1 and seasonal H1N1 in ferrets.



**Figure 1-3.** 1. Cumulative mortality rates of ferrets inoculated with different influenza viruses. The cumulative mortality of new H1N1 was intermediate between that for seasonal H1N1 and HPAI H5N1. 2. Body temperatures, relative lung weights and lung viral titers of ferrets inoculated with different influenza viruses. The increase in body temperature (a), the relative lung weight (b), and lung viral titer (c) of the new H1N1 groups was intermediate between those of the seasonal H1N1 and HPAI H5N1 group. 3. Histological and immunohistochemical scoring of the lungs of ferrets inoculated with different influenza viruses. Staining with hematoxylin and eosin (HE) showed alveolar lesions intermediate in severity and more severe bronchiolar lesions of new H1N1 when compared to seasonal H1N1 and HPAI H5N1. In immunohistochemistry (IHC) influenza virus antigen expression in the lungs of the new H1N1 group was high at all three levels, while for HPAI H5N1 it was only high in bronchioles and alveoli and for the seasonal H1N1 it was low at all three levels.

## Methods

Three influenza viruses were used: new H1N1 virus (A/NL/602/2009), seasonal H1N1 virus (A/NL/26/2007) and HPAI H5N1 (A/Ind/5/2005).<sup>2</sup>

• Survival study: groups of 3 (seasonal H1N1) or 6 ferrets, intra-tracheally inoculated with either 10<sup>4</sup>, 10<sup>6</sup> or 10<sup>8</sup> TCID<sub>50</sub> virus, euthanized on day 7.

• Pneumonia study: groups of 3 (seasonal H1N1) or 6 ferrets, intra-tracheally inoculated with 10<sup>6</sup> TCID<sub>50</sub> virus, euthanized on day 4.

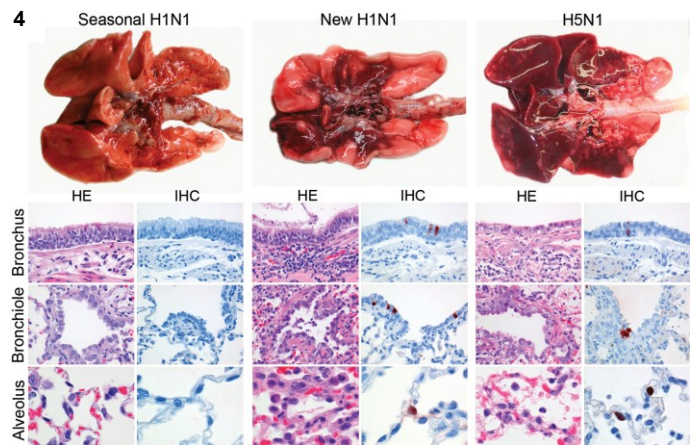
Both respiratory and extra-respiratory tissues were sampled for virological, pathological and immunohistochemical analyses.

## Results

Our results showed that the new H1N1 virus causes pneumonia in ferrets intermediate in severity between that of seasonal H1N1 virus and HPAI H5N1 virus.

The new H1N1 virus replicated well throughout the lower respiratory tract and more extensively than both seasonal H1N1 virus (mainly bronchi) and HPAI H5N1 virus (mainly alveoli).

High loads of new H1N1 virus in lung tissue were associated with diffuse alveolar damage and mortality.



**Figure 4.** Macroscopy, histopathology and immunohistochemistry in the lungs of ferrets inoculated with different influenza viruses. Macroscopic lung lesions in the new H1N1 virus group were intermediate between those in the seasonal H1N1 and the HPAI H5N1 virus groups. The new H1N1 virus group showed moderate influenza virus expression (IHC) in bronchi, bronchioles, and alveoli, associated with histological lesions (HE) characterized by inflammatory cell infiltrates and epithelial necrosis. In contrast, the seasonal H1N1 virus group showed minimal influenza virus expression and histological lesions. In the HPAI H5N1 virus group, there was more abundant influenza virus antigen expression in the alveoli associated with more severe histological lesions, but less abundant influenza virus antigen expression in the bronchi and bronchioles, associated with milder histological lesions.

## Discussion

Intra-tracheal inoculation of new H1N1 influenza virus causes severe pneumonia in ferrets.

Pneumonia caused by new H1N1 influenza virus is intermediate in severity between that of HPAI H5N1 and seasonal H1N1.

The new H1N1 influenza virus replicates well in epithelial cells in the lower respiratory tract, which is also seen in HPAI H5N1 but not in seasonal H1N1.

Therefore, the new H1N1 virus may be intrinsically more pathogenic for humans than seasonal H1N1 virus.

## References

1. Kuiken & Taubenberger. *Vaccine* 26 Suppl 4:D59-66 (2008)
2. van den Brand et al. *J Infect Dis* In press (2010)