Virology 393 (2009) 338–345

Contents lists available at ScienceDirect

Virology

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

# An early 'classical' swine H1N1 influenza virus shows similar pathogenicity to the 1918 pandemic virus in ferrets and mice

Matthew J. Memoli [a], Terrence M. Tumpey [b], Brett W. Jagger [a], Vivien G. Dugan [a], Zong-Mei Sheng [a], Li Qi [a], John C. Kash [a], Jeffery K. Taubenberger [a,*]

[a] Viral Pathogenesis and Evolution Section, Laboratory of Infectious Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

[b] Immunology and Pathogenesis Branch, Influenza Division, National Center for Immunization and Respiratory Diseases, Collaborating Centers for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

ARTICLE INFO

*Article history:*
Received 6 July 2009
Returned to author for revision
11 August 2009
Accepted 14 August 2009
Available online 5 September 2009

*Keywords:*
Viruses
Influenza
Pandemic
Ferrets
Viral pathogenesis
Animal models
Swine influenza

ABSTRACT

The 1918 pandemic influenza virus has demonstrated significant pathogenicity in animal models and is the progenitor of 'classical' swine and modern seasonal human H1N1 lineages. Here we characterize the pathogenicity of an early 'classical' swine H1N1 influenza A virus isolated in 1931 compared to the pathogenicity of the 1918 pandemic virus and a seasonal H1N1 virus in mice and ferrets. A/Swine/Iowa/31 (Sw31) and the 1918 influenza viruses were uniformly lethal in mice at low doses and produced severe lung pathology. In ferrets, Sw31 and 1918 influenza viruses caused severe clinical disease and lung pathology with necrotizing bronchiolitis and alveolitis. The modern H1N1 virus caused little disease in either animal model. These findings revealed that in these models the virulence factors of the 1918 influenza virus are likely preserved in the Sw31 virus and suggest that early swine viruses may be a good surrogate model to study 1918 virulence and pathogenesis.

Published by Elsevier Inc.

## Introduction

Influenza A viruses have unpredictably caused pandemics throughout human history (Taubenberger and Morens, 2009) and today cause approximately 36,000 deaths in the United States annually in inter-pandemic form (Thompson et al., 2003). In addition to humans, influenza A viruses infect multiple avian and mammalian species, including horses, swine, and poultry (Webster et al., 1992). Swine have been frequently infected with different strains of influenza globally (Alexander and Brown, 2000; Brown, 2000; Brown et al., 1998; Thacker and Janke, 2008; Van Reeth, 2007; Vincent et al., 2008). Interestingly, the first recognized swine influenza infections were identified clinically in 1918, during the largest known influenza pandemic in humans (Dimoch, 1918–1919; Koen, 1919). Since 1918 swine influenza viruses have continued to cause disease in populations of pigs (Vincent et al., 2008) worldwide, and much like human influenza (Rambaut et al., 2008), swine influenza viruses have been shown to be evolving dynamically by both antigenic drift and frequent reassortment (Brown et al., 1997; Dunham et al., 2009).

The human 1918 pandemic influenza virus has been reconstructed and characterized (Taubenberger et al., 2007; Taubenberger and Morens, 2006; Taubenberger et al., 2005; Tumpey et al., 2005). Its pathogenicity has been studied in mice (Kash et al., 2006; Pappas et al., 2008; Tumpey et al., 2005), macaques (Kobasa et al., 2007), guinea pigs (Van Hoeven et al., 2009), swine (Weingartl et al., 2009), and ferrets (Tumpey et al., 2007). In comparison to contemporary human H1N1 viruses, this virus has been shown to be extremely virulent in mice and macaques causing severe disease and high mortality. It also causes significant disease in ferrets and pigs, although it has been reported to be less lethal in these models. The virulence factors that increased the lethality of the 1918 virus in humans during the pandemic are the subject of continued investigation (Morens et al., 2008). Characterizing these virulence factors is essential to anticipating how current and future pandemic influenza viruses arise and cause significant human disease, as well as to provide a basis for development of novel therapeutics.

While clinically identified in pigs in 1918 (Dimoch, 1918-1919; Koen, 1919), swine influenza viruses were first isolated from pigs in 1930 and until 1998 had been primarily of the 'classical' H1N1 lineage. Similar disease to what was observed in humans was seen in pigs

* Corresponding author. Laboratory of Infectious Diseases, NIAID, NIH, 33 North Drive, MSC 3203, Bethesda, MD 20892-3203, USA. Fax: +1 301 480 1696.
*E-mail address:* taubenbergerj@niaid.nih.gov (J.K. Taubenberger).

0042-6822/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.virol.2009.08.021

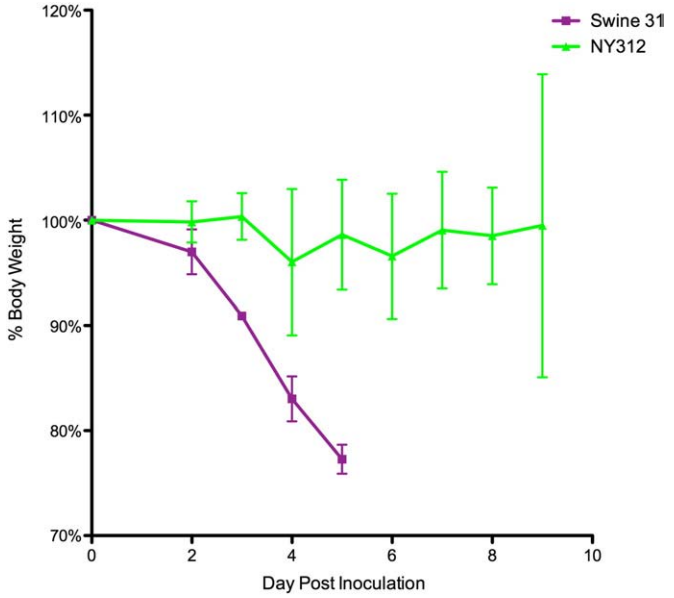


**Fig. 1.** Mean percentage body weight loss from mean baseline weight of mice in each inoculated with A/Iowa/Swine/31 [Sw31] and A/NY/312/2001/H1N1 [NY312] from 0 to 14 dpi. Sw31 inoculated mice lost a significant amount of weight by day 2 and reached 100% mortality by 6 dpi. Mice inoculated with NY312 demonstrated 100% survival and minimal weight loss. Error bars represent SEM.

during the 1918 pandemic, possibly due to concurrent infection of pigs and humans. Sequence analysis has shown that the classical swine influenza viruses first isolated in 1930s were direct descendants of the 1918 pandemic virus (Taubenberger and Morens, 2006; Taubenberger et al., 2000). Thus, swine H1N1 influenza viruses isolated in the 1930s are the closest naturally occurring relatives to the 1918 pandemic virus. Patrick Laidlaw, one of the co-authors of the 1933 paper describing the isolation of the first human influenza A virus (Smith et al., 1933), wrote in 1935, "the virus of swine influenza is really the virus of the great pandemic of 1918, adapted to the pig and persisting in that species ever since" (Laidlaw, 1935).

Swine influenza viruses have caused sporadic human infections over the past decades (Gray et al., 2007; Myers et al., 2007; Vincent et al., 2009). A classical swine H1N1 virus caused the well-known outbreak at Ft. Dix in New Jersey in 1976 (Gaydos et al., 2006). Recently in 2009, a large-scale human outbreak of a novel swine influenza lineage has developed into the first pandemic of the 21st century (Garten et al., 2009; Smith et al., 2009; WHO, 2009). Thus, a better understanding of the role that influenza A virus lineages and evolution in swine play in the formation of strains capable of infecting humans remains vitally important for pandemic preparedness (Dunham et al., 2009; Morens et al., 2009).

To determine if H1N1 viruses isolated in the 1930s still retain some of the virulence properties of the 1918 influenza virus, we compared the pathogenicity of the reconstructed 1918 influenza virus with a classical swine H1N1 virus isolated in 1931, A/Iowa/swine/31 (Sw31), in ferrets and mice.

## Results

The ten major open reading frames of the influenza virus (PB2, PB1, PA, HA, NP, NA, M1, M2, NS1, NS2 (NEP)) consist in aggregate of 4468 codons. The Sw31 virus genome has a 96.3% amino acid identity with the 1918 virus genome.

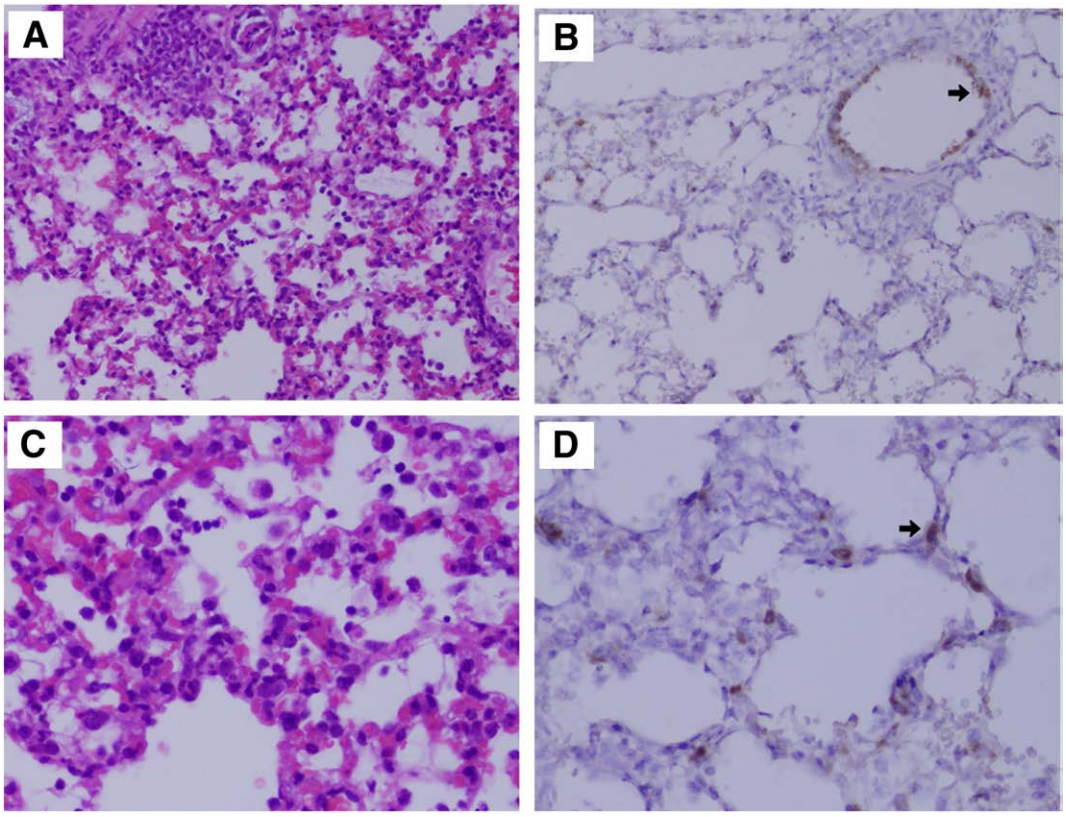


**Fig. 2.** Histopathology and immunohistochemistry of Sw31 influenza virus-infected mouse lung tissue. Photomicrographs of hematoxylin-and-eosin-stained tissue sections (A, C) and immunohistochemically stained sections to detect influenza viral antigen (B, D) from mice infected with the Sw31 influenza virus at 4 dpi. Viral antigen (B, D) is stained red brown on a hematoxylin-stained background. (A and C) Sections from mouse lungs show moderate-to-marked alveolitis with abundant neutrophils (original magnifications 20× and 40×, respectively). (B and D) Viral antigen (arrows) was observed in bronchiolar epithelial cells and alveolar cells (original magnifications 20× and 40×, respectively).