# An Overview of the Pathogenicity of the 1918 H1N1 Virus and Virulence Factors

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# Antigenic analysis of H1N1 viruses isolated the last century

| | HI titer with ferret antisera | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Virus** | **1918** | **Sw/Ia/30** | **WS/33** | **PR/8/34** | **USSR/77** | **Chili/83** | **Tx/91** | **N.Cal/99** |
| **1918 HA/NA** | **2560** | 1280 | 320 | 40 | <10 | 10 | 80 | 20 |
| **Sw/Ia/30** | 1280 | **2560** | 20 | 320 | 80 | 10 | 80 | 20 |
| **WS/33** | <10 | <10 | **640** | 40 | <10 | <10 | <10 | 40 |
| **PR/8/34** | 20 | <10 | 160 | **2560** | 10 | <10 | 10 | 10 |
| **USSR/77** | <10 | <10 | 10 | <10 | **1280** | 20 | <10 | <10 |
| **Chili/83** | <10 | <10 | 10 | <10 | 40 | **320** | 20 | 10 |
| **Tx/91** | <10 | <10 | 20 | <10 | <10 | <10 | **2560** | 40 |
| **N.Cal/99** | 10 | <10 | 10 | 20 | <10 | <10 | 40 | **1280** |

# Antigenic analysis of H1N1 viruses isolated the last century

| | HI titer with chicken sera | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Virus** | 1918 | Sw/Ia/30 | WS/33 | PR/8/34 | USSR/77 | Chili/83 | Tx/91 | N.Cal/99 |
| **1918 HA/NA** | **2560** | 1280 | 160 | 80 | 20 | 10 | 160 | 20 |
| Sw/Ia/30 | 640 | **2560** | <10 | 40 | 10 | 10 | 80 | 10 |
| WS/33 | <10 | <10 | **320** | <10 | <10 | <10 | 10 | <10 |
| PR/8/34 | 10 | <10 | 80 | **2560** | <10 | <10 | 10 | 20 |
| USSR/77 | <10 | <10 | <10 | <10 | **320** | 20 | 10 | <10 |
| Chili/83 | <10 | <10 | <10 | <10 | 40 | **320** | <10 | <10 |
| Tx/91 | 80 | <10 | 80 | <10 | <10 | <10 | **640** | <10 |
| N.Cal/99 | 10 | <10 | 10 | 320 | <10 | <10 | 80 | **2560** |

# Vaccine Protocol

**Vaccinate BALB/c mice 1x (10 ug/i.m.) with FI whole virus H1N1 vaccine**

1. **Mock PBS**
2. **X-31 (H3N2)**
3. **1918 HA/NA**
4. **PR/8/34**
5. **New Caladonia/99**
6. **Human influenza vaccine (7.5 ug)***

•**Contains *15* µg of haemagglutinin (HA) per 0.5ml of A/New Caledonia/20/99(H1N1)-like antigen.**

↓

**Day 21**

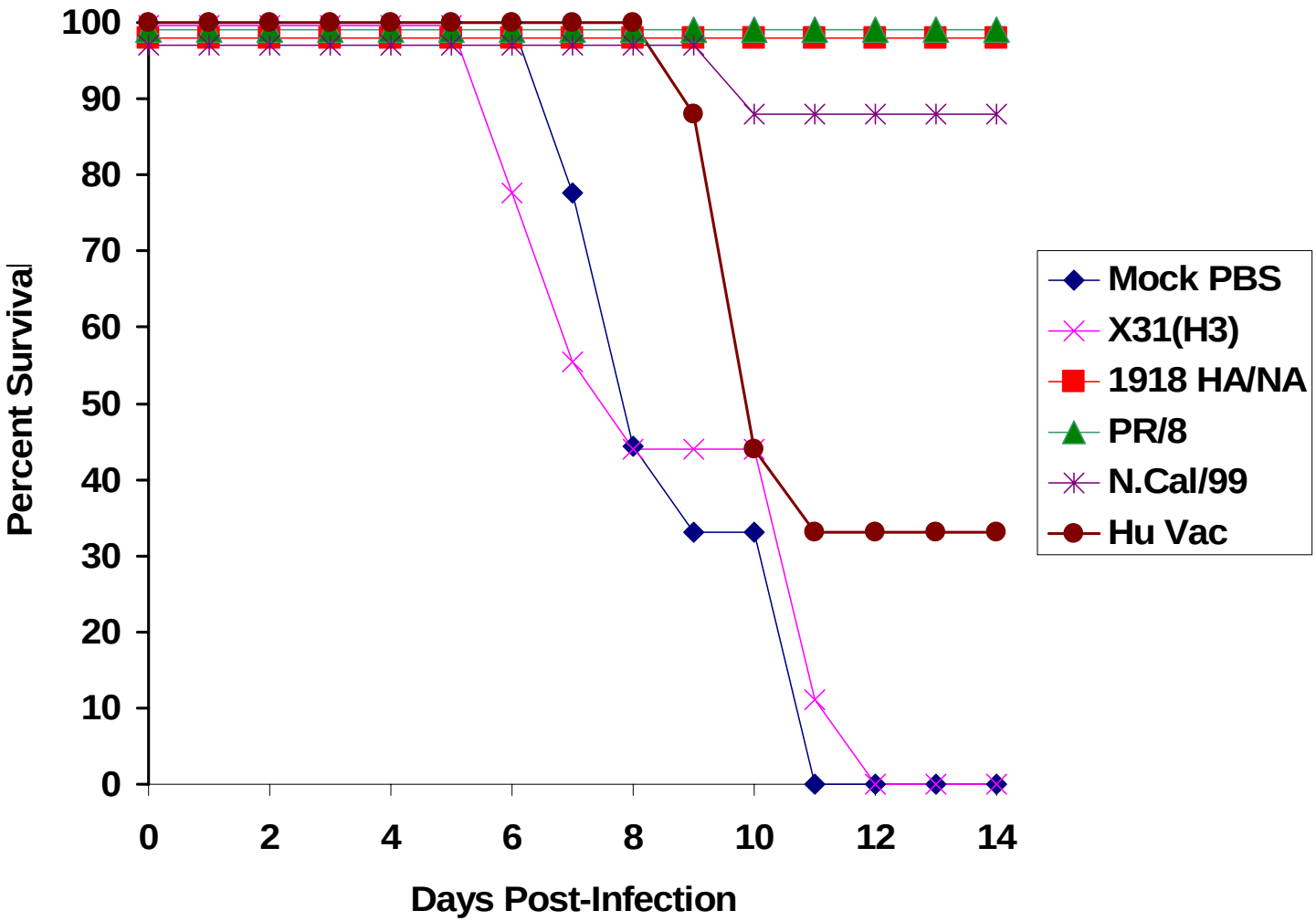
↓

**Challenge i.n. with 100 $LD_{50}$ of 1918 recombinant virus**

↓

**Morbidity/Mortality and virus titers**

# Protective efficacy of H1N1 vaccines against lethal infection with 1918 recombinant virus



# Use the 1918 virus a model for pandemic influenza

## Main Objective

- Identify the properties that are responsible for the virulence of the 1918 influenza virus

- Identify the genetic determinants responsible for transmissibility of the 1918 virus

# Characterize 1918 recombinant viruses in BALB/c mice

## Experimental Protocol

**Inoculate 50 ul i.n. and determine:**

- 50% lethal dose ($LD_{50}$) by inoculating groups of 3-5 mice with serial 10-fold dilutions ($10^6$-$10^0$ PFU) of recombinant viruses.
- Weight loss - 14 day observation period.
- Virus replication in lung and extrapulmonary tissues at the peak (days 4 p.i.) of virus replication.

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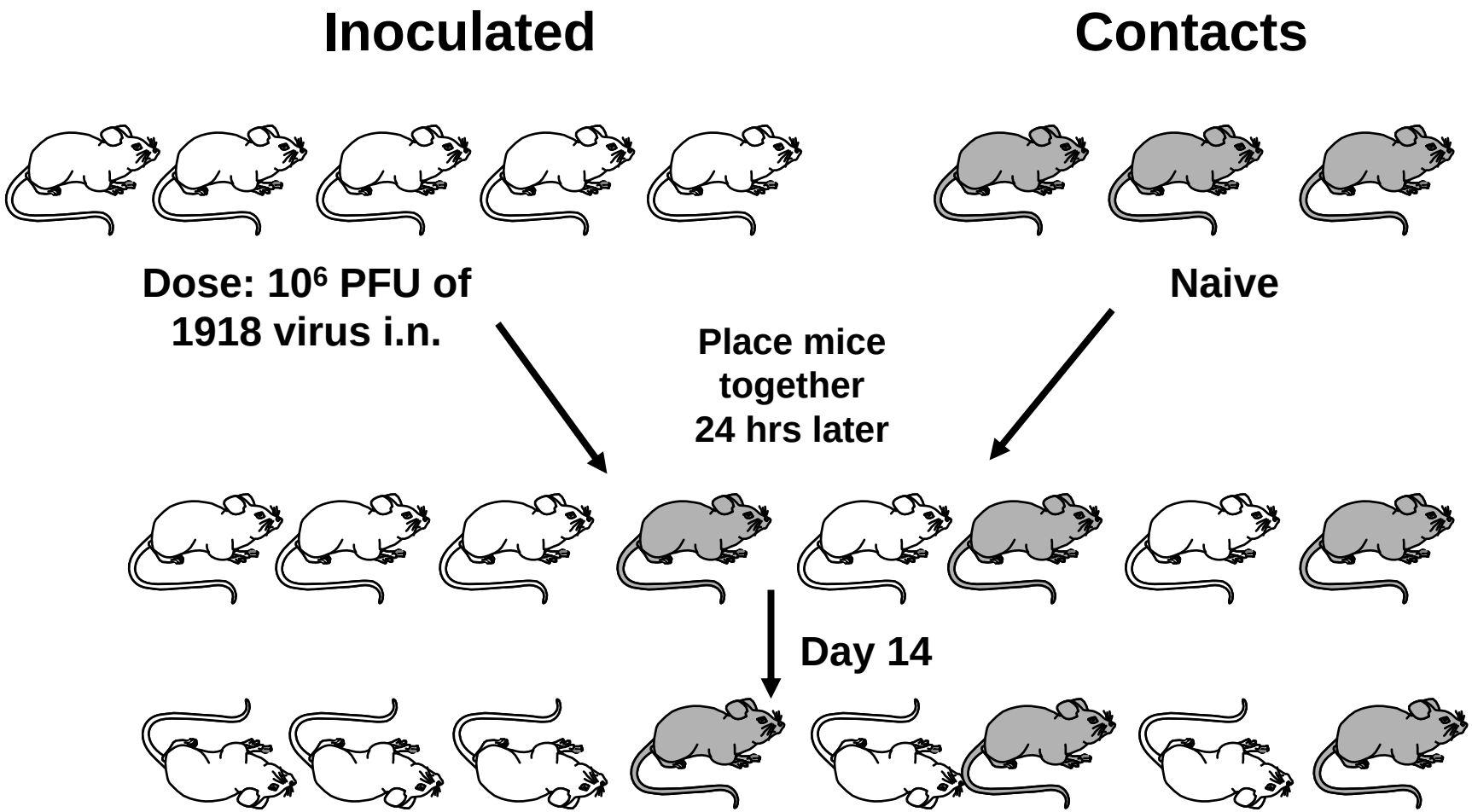
# Does the 1918 virus transmit in mice?

**Eight mice used for direct transmission**




**No seroconversion among contact animals**

# Pathogenicity of the 8-gene 1918 virus in mammalian species

**Intranasal inoculation with $10^6$ PFU**

| Species | % Mortality | Manuscript Status |
|---|---|---|
| *Mice (Mx1-/-)* | 100 | Published |
| Ferret | 50 | Some Published |
| Guinea pig | Not lethal | Submitted |
| Chickens | Not lethal | In preparation |

**Intranasal inoculation with $10^5$ PFU**

| | | |
|---|---|---|
| *Mx1-/-* mice | 100 | Published |
| *Mx1+/+* mice | Not lethal | Published |
| Ferret | Not lethal | Unpublished |
| Guinea pig | Not lethal | Submitted |
| Chicken | Not lethal | In preparation |
| Pig | Not lethal | Submitted |

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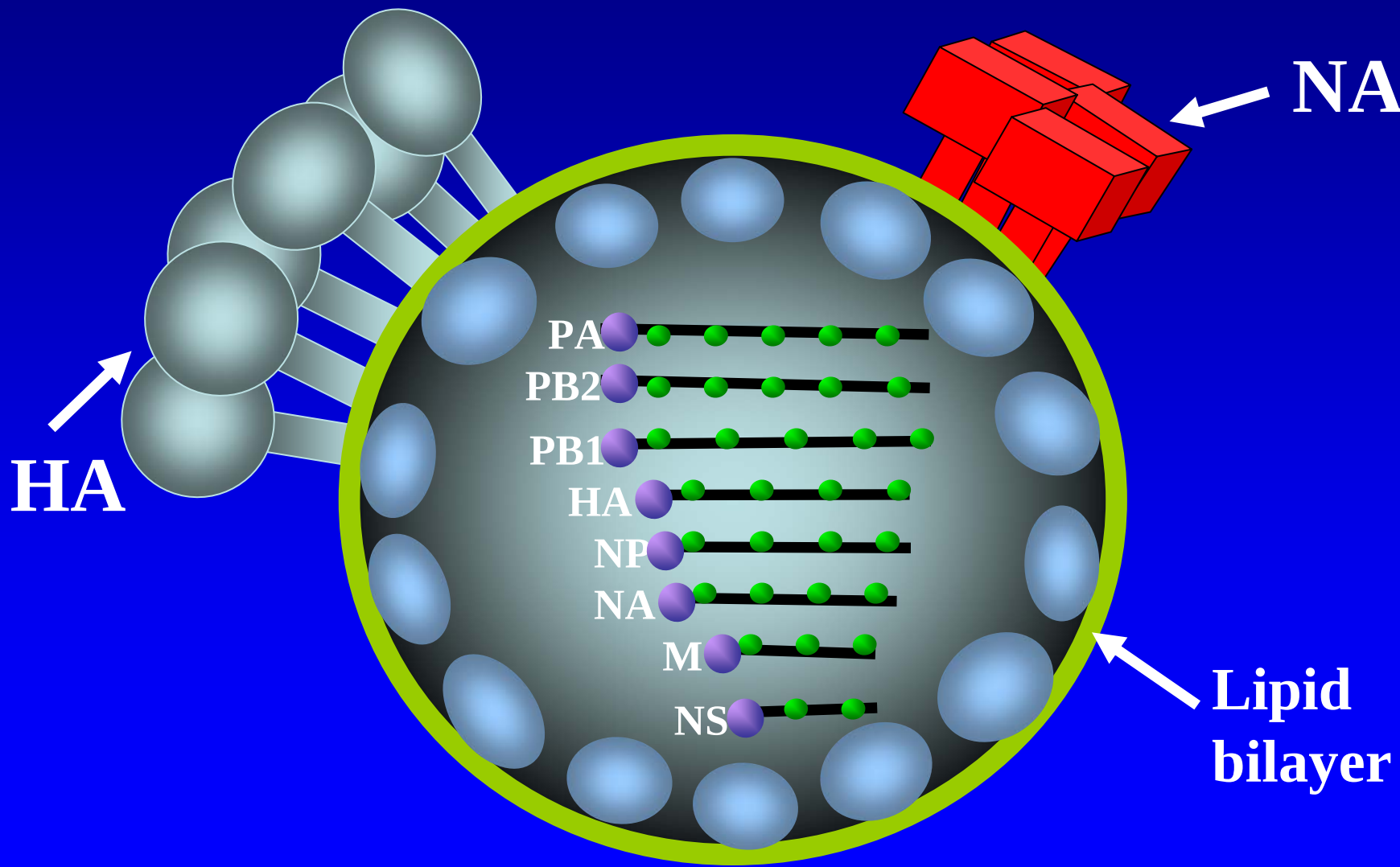
# The hemagglutinin (HA) and neuraminidase (NA) are the major viral surface proteins



# Pathogenicity of the 1918 HANA recombinant virus in mammalian species

| Species | Inoculating Dose (PFU) | % Mortality | Manuscript Status |
|---|---|---|---|
| *Mx1-/-* mice | 10^6 | 100 | Published |
| Ferret | 10^6 | Not lethal | Submitted |
| Macaque | 10^7 | Not lethal | Submitted |

# The Lethality of 1918 virus versus other viruses in mice

| Virus (subtype) | $LD_{50}$* | Amount of 1918 virus (times) required to kill mice |
|---|---|---|
| 1918 (H1N1) | 3.5 | - |
| A/Thailand/16/2004 (H5N1) | 1.7 | 63 |
| A/Chicken/Korea/IS/2006 (H5N1) | 0.8 | 500 |
| A/Netherlands/219/03 (H7N7) | 2.5 | 10 |
| A/Swine/Iowa/30 (H1N1) | 2.2 | 20 |
| A/WSN/33 (H1N1) | 2.5 | 10 |

* Expressed as the $\log_{10}$ PFU or $EID_{50}$ required to give 1 $LD_{50}$

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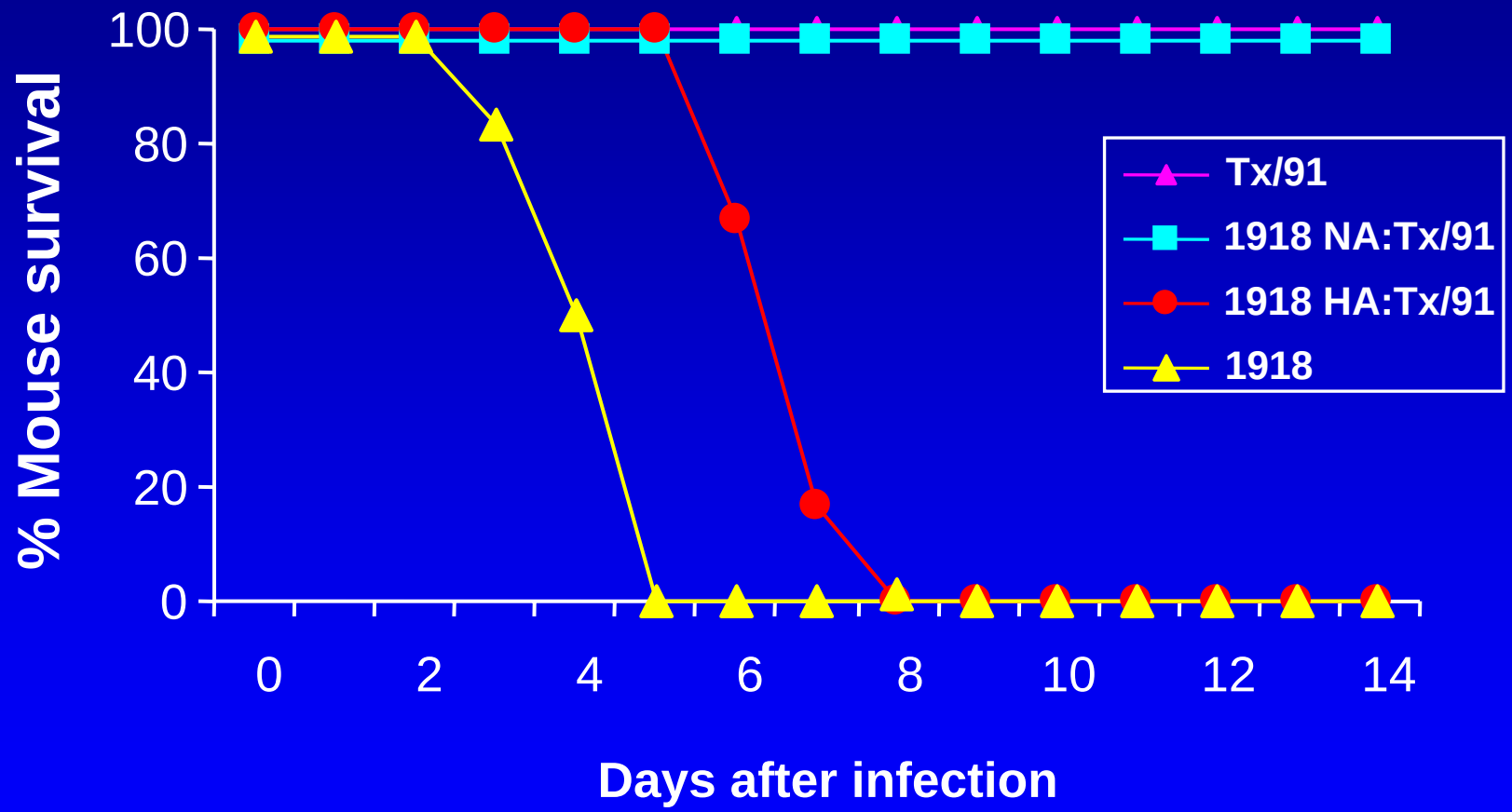
# 1918 1:7 recombinant viruses generated using reverse genetics

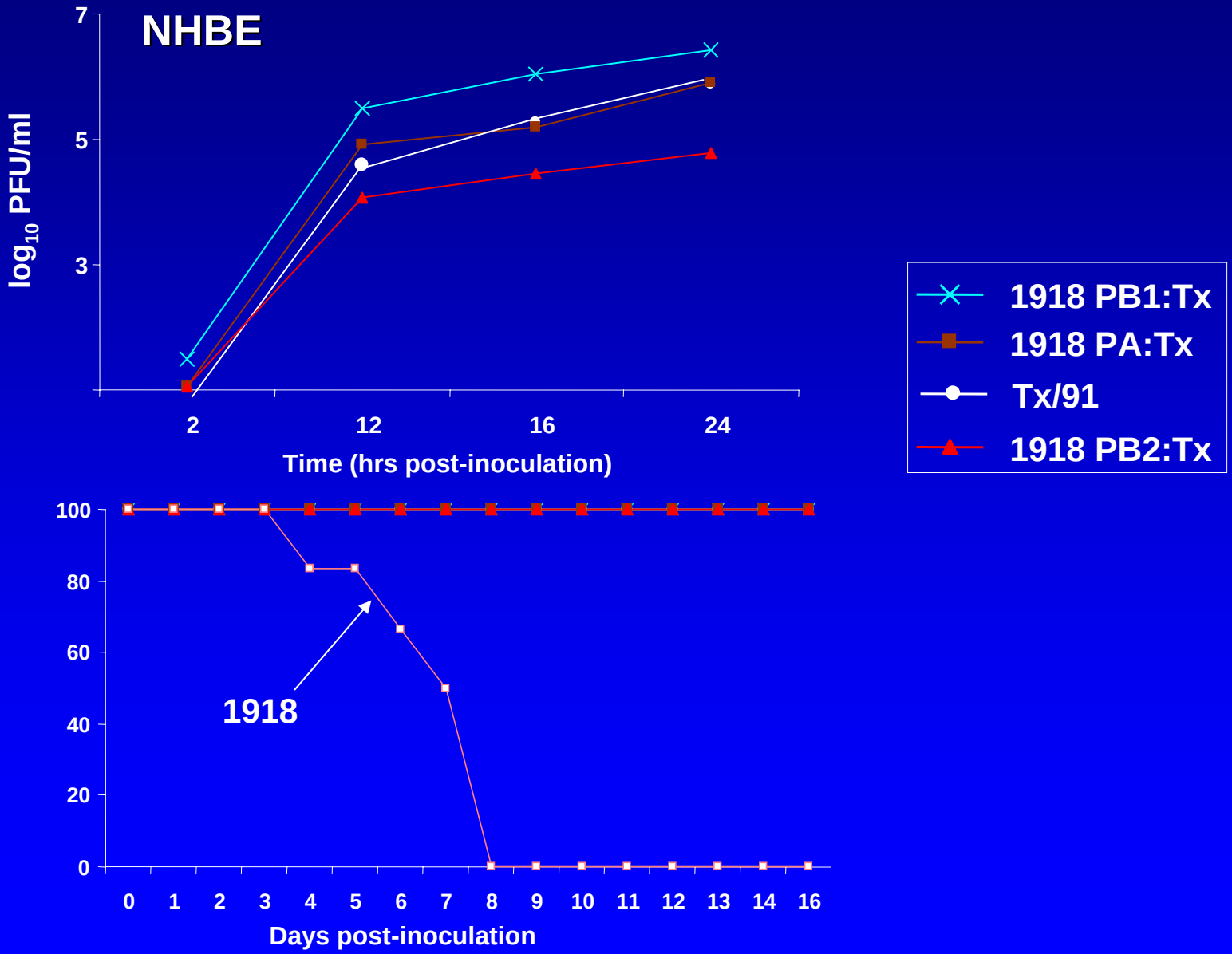
| Single 1918 gene segment | Virus Designation |
|---|---|
| PA | 1918 PA:Tx/91 |
| PB2 | 1918 PB2:Tx/91 |
| PB1 | 1918 PB1:Tx/91 |
| HA | 1918 HA:Tx/91 |
| NA | 1918 NA:Tx/91 |
| NS | 1918 NS:Tx/91 |
| NP | 1918 NP:Tx/91 |
| M | 1918 M:Tx/91 |
| - | Tx/91 |

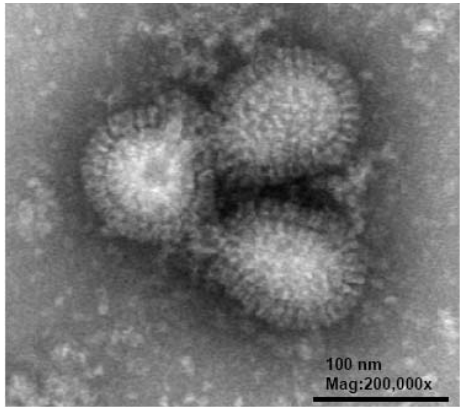
* The identity of the 1918 and Tx/91 influenza virus genes was confirmed by RT-PCR and sequence analysis.

# 1918 hemagglutinin (HA) confers a virulent phenotype in mice



# 1918 PB1 increases replication efficiency, but not lethality of Tx/91 H1N1 virus






1918 VLP

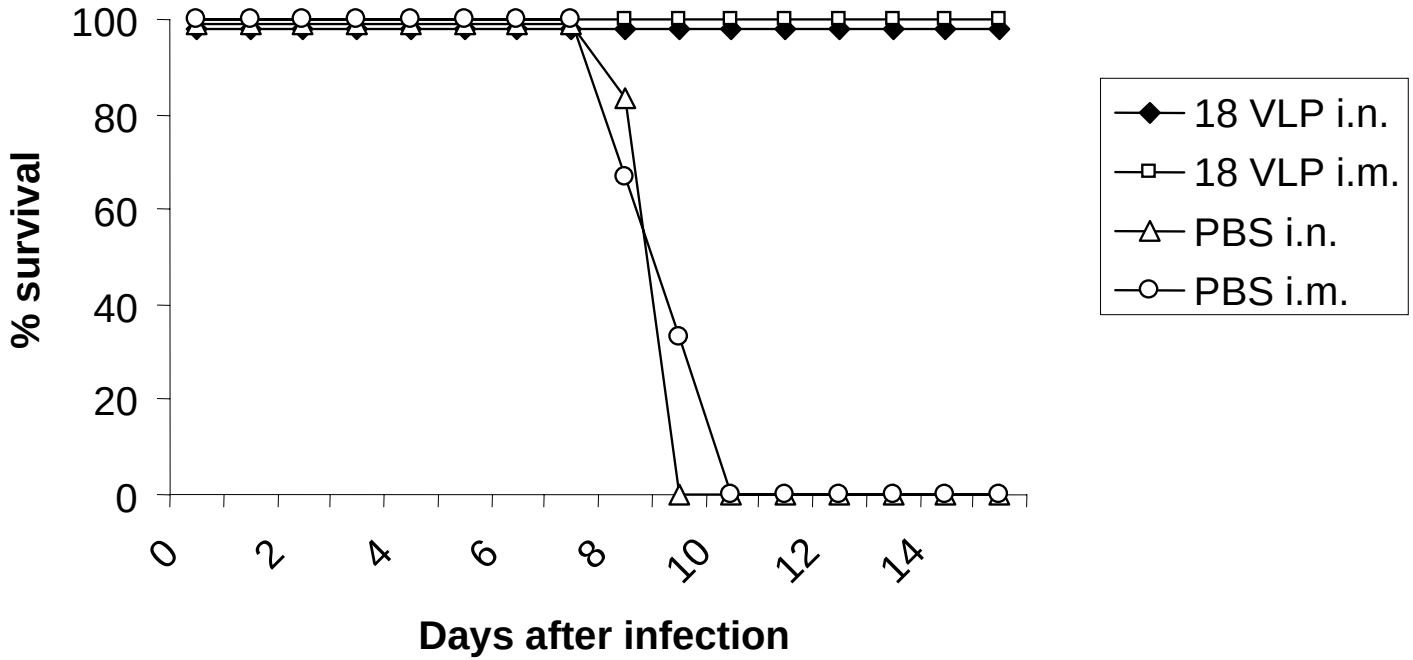
# Evaluation of influenza virus-like particles (VLP) as candidate vaccine for 1918 virus

| Vaccination | Route^ | % Survival |
|---|---|---|
| 1918 VLP | i.n. | 100 |
| 1918 VLP | i.m. | 100 |
| HIV VLP | i.n. | 0 |
| PBS | i.m. | 0 |
| PBS | i.n. | 0 |

^ Mice were vaccinated intra-nasally (i.n.) or intra-muscularly (i.m.) with 5 ug on days -28 and -14 with 1918 virus VLP's or given HIV VLP's or PBS as controls. N= 6 mice per group

* Mice were challenged with 50 LD50 of the 8-gene 1918 virus

## Protective efficacy of 1918 VLP against lethal infection with 8-gene 1918 virus



# Conclusions

- **Antigenic analysis with animal H1N1 antisera showed that the 1918 recombinant viruses antigenically resembled A/Swine/Iowa/30 virus, but differed from contemporary H1N1 viruses.**

- **Mice that received homologous or Sw/Iowa/30 inactivated vaccine demonstrated HI and neutralizing antibodies to the 1918 recombinant virus and were completely protected against lethal challenge.**

- **Mice that received PR/8, N.Cal/99 or human vaccine displayed partial protection against 1918 recombinant virus .**

# Conclusions-cont.

- In mice, the 8 gene 1918 virus does not spread to naïve cage mates.
- At 10^5 PFU, the 8 gene 1918 virus is not lethal in Ferrets, guinea pigs, chickens, pigs and mice with a functional Mx1 gene.
- Recombinant influenza viruses containing the 1918 HA/NA gene segments are not lethal in ferrets or macaques
- The 1918 virus is less lethal than contemporary H5N1 isolates, requiring 63 to 500 times more virus to kill mice.

# Conclusions-cont.

- The 1918 virus is less lethal than some laboratory ABS2 isolates, requiring 10 to 20 times more virus to kill mice.
- Among the 8 1918 1:7 recombinant viruses generated, only the 1918 HA confers a virulent phenotype in mice.
- The 1918 PB1 increases replication efficiency in human airway cells.
- Intranasal or intramuscular vaccination with 1918 influenza virus-like particles protect mice from lethal 8-gene 1918 influenza virus challenge.

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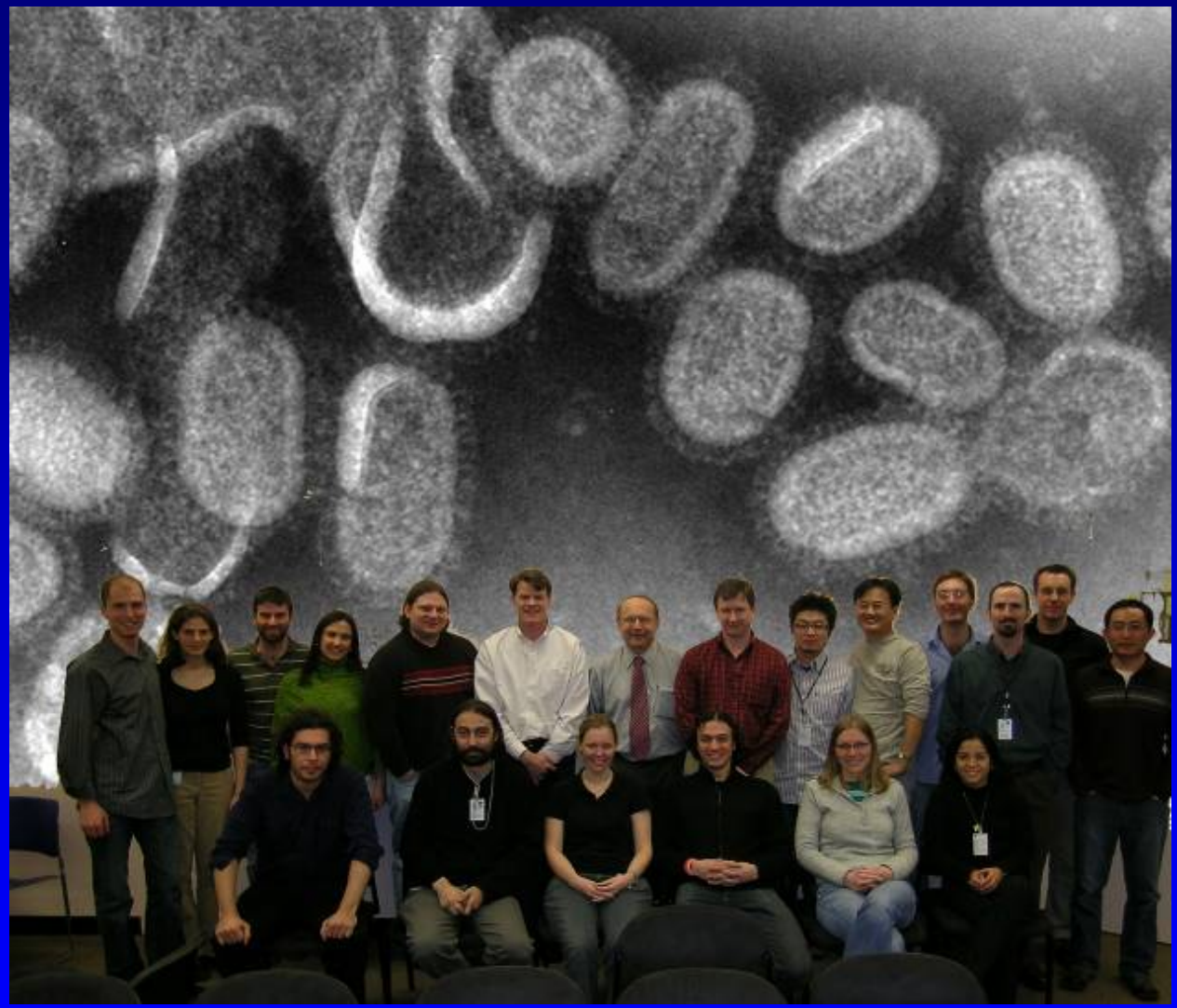


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