

# Nasal Vaccination with a Recombinant H5 Antigen in Nanoemulsion Adjuvant Potentiates Immune Response Against Pandemic Avian Influenza

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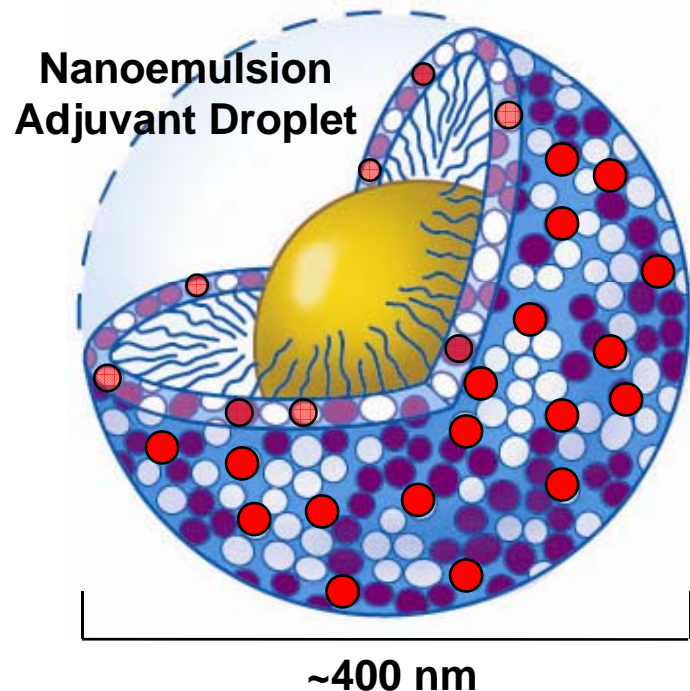
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# Pandemic Avian Influenza

- Large avian reservoir, but inefficient transmitted
- Mortality rate is very high (>60%)
- One currently approved vaccine in the US
  - limited availability, high antigen dose (90  $\mu$ g HA)
- Concerns about rapid manufacturing necessary quantities necessary for a pandemic scenario

# Nanoemulsion (NE) Intranasal Vaccine Adjuvant Platform

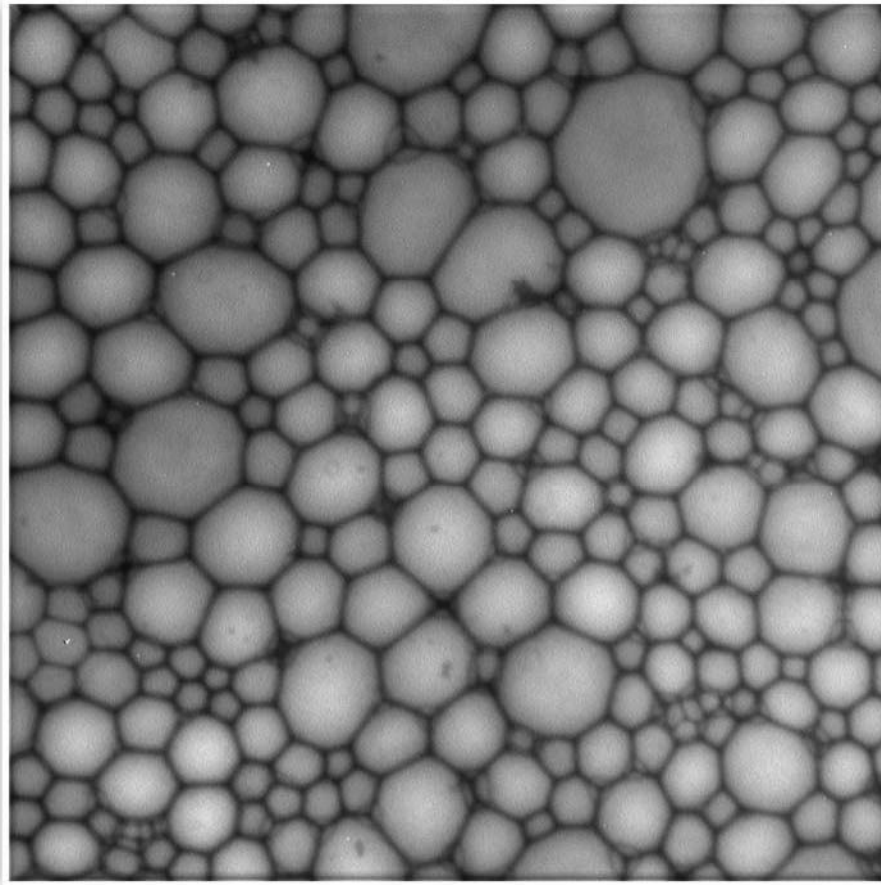


- Cetylpyridinium chloride
- Surfactant
- Oil
- Antigen

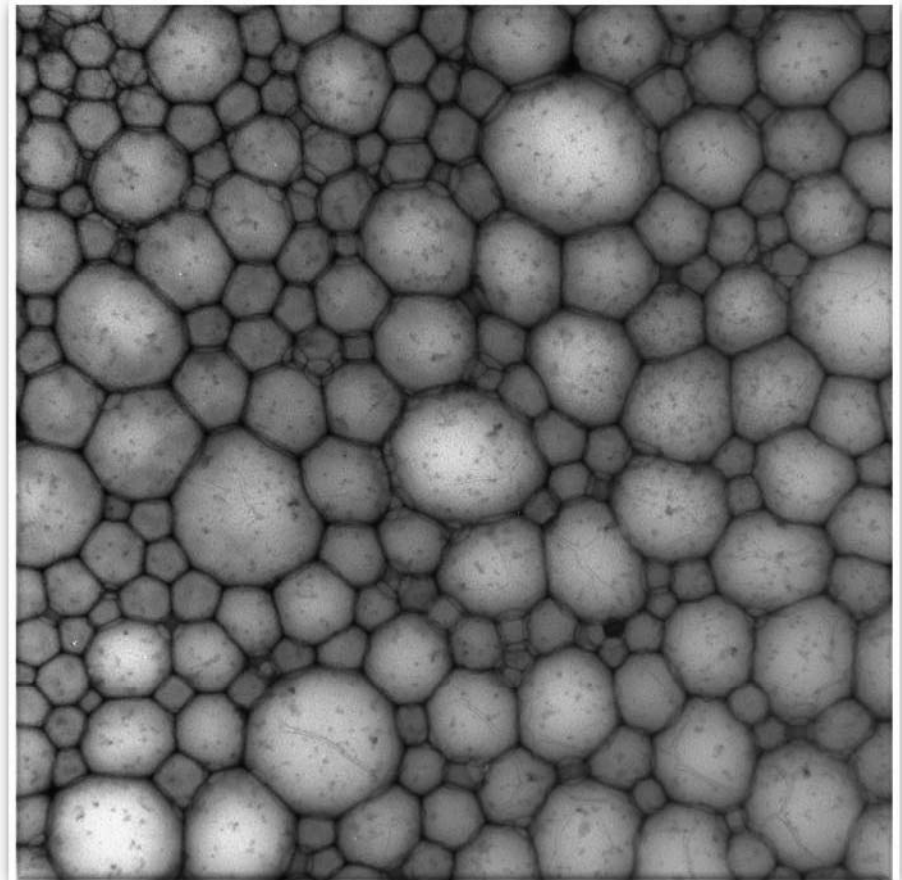
- **Universal** adjuvant platform
- Significant advantages:
  - Robust systemic & mucosal immunity
  - Antigen-sparing
  - Cross protection
  - Adjuvants multiple antigen types
  - Non-inflammatory
  - Needle-free
- Potential to convert live vaccines to highly effective inactivated vaccines

# NE Adjuvant with Hepatitis B Antigen

Nanoemulsion Adjuvant



Nanoemulsion Adjuvant Mixed With Hepatitis B Antigen



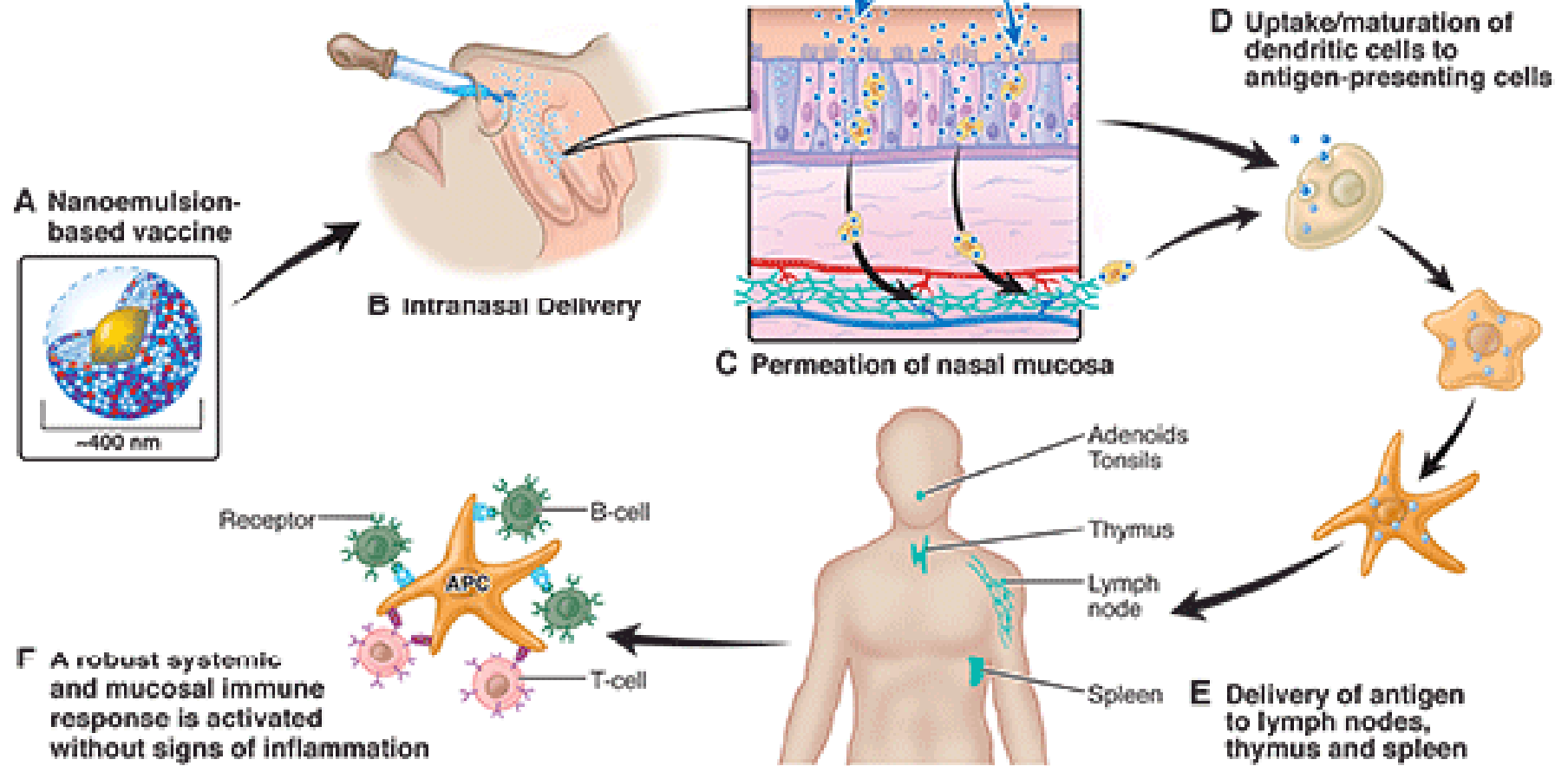
# Recombinant H5 Antigen – Influenza A/Indonesia/05/05

- Yusibov and colleagues reported in *Vaccine* 27 (2009) 1087-1092
- Produced in *Nicotiana benthamiana* by agroinfiltration
- Poly-histadine tagged, purified by Ni-column chromatography
- Current requires relatively high doses, multiple injections, and an inflammatory adjuvant (Quil A)

## MECHANISM OF ACTION FOR NANOEMULSION-BASED VACCINES

Illustrations by Holly R. Fischer, MFA

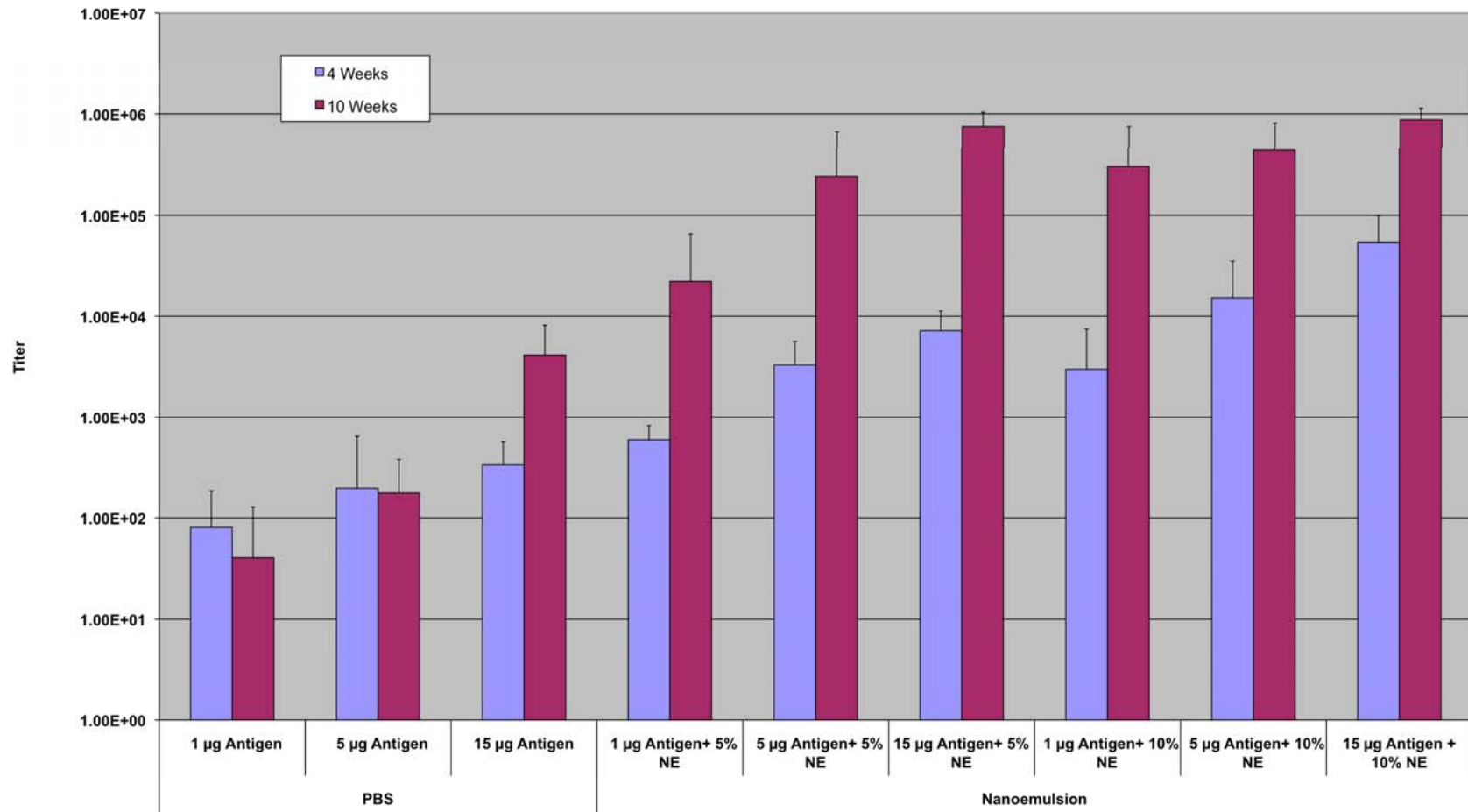
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# Study Design

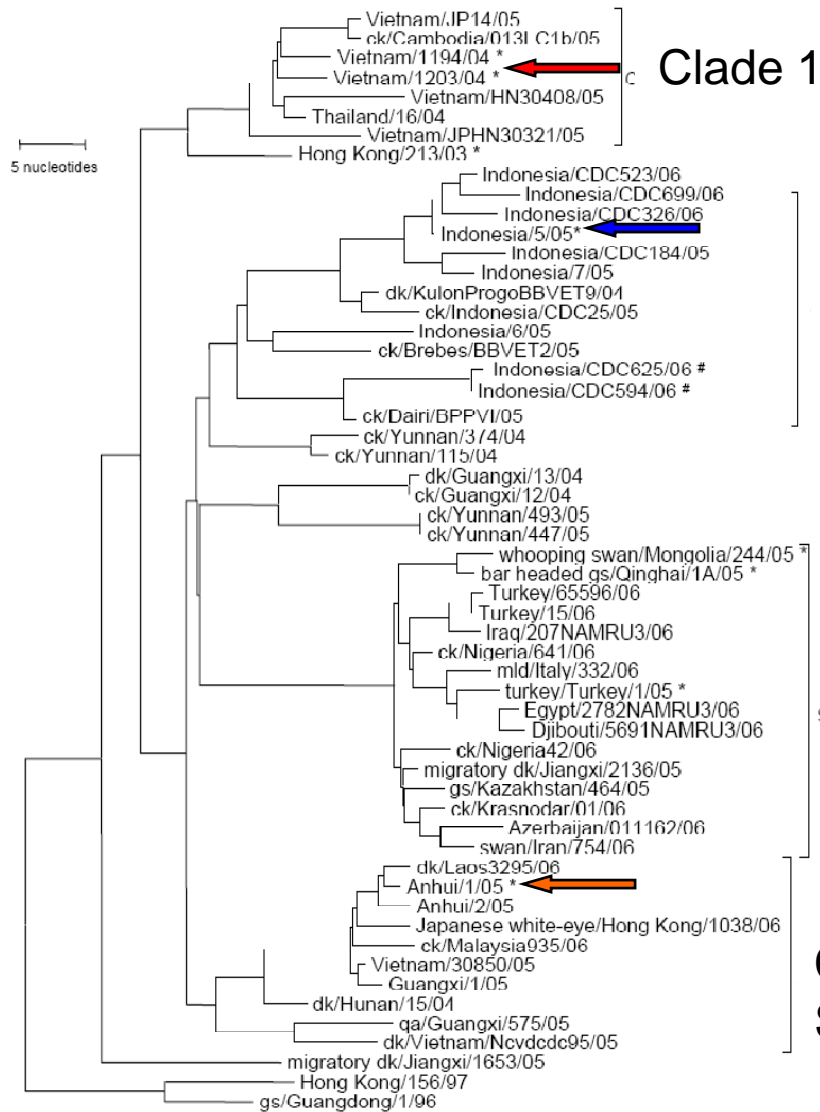
- CD-1 out-bred mice were nasally immunized with 12  $\mu$ L containing either
  - Either 1, 5 or 15 ug of H5 antigen
  - In either PBS, 5% or 10% NE
- 5 mice in each group
- Immunized and boosted once at 4 weeks
- Bled at 4 weeks (before boost) and 10 weeks after initial immunization

# Serum IgG End-Titer by ELISA



# Avian Influenza – Classification

Fig. 1 Evolution of the H5N1 haemagglutinin gene



A/Indonesia/5/05

A/Anhui/1/05

A/Vietnam/1203/2004

Clade 2  
Subclade 1

Cross Clade Antigens for

- Clade 1
- Clade 2.1
- Clade 2.3

Clade 2  
Subclade 2

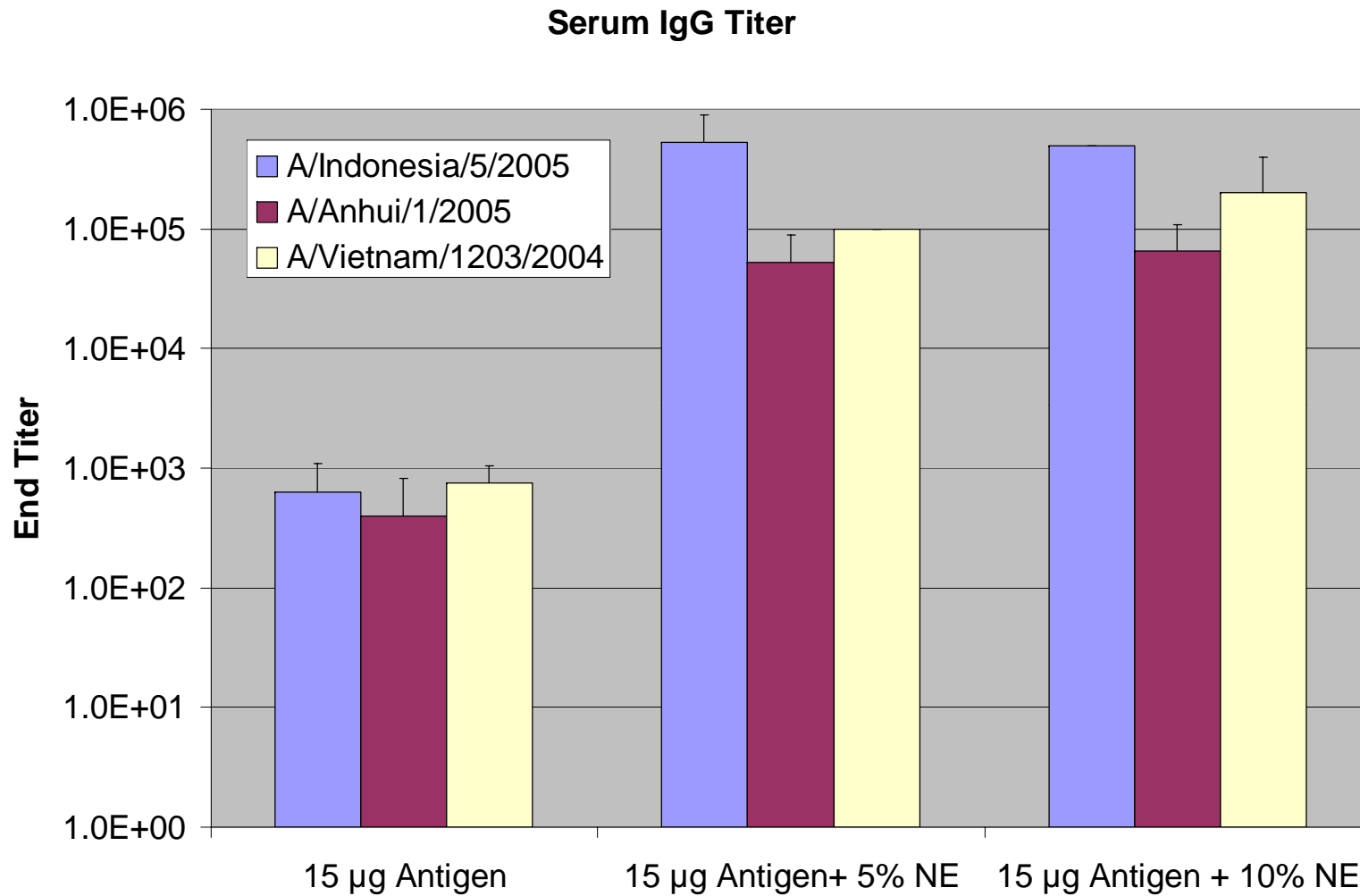
Clade 2  
Subclade 3

\* Candidate vaccine reference viruses

# Karo family cluster

[http://www.who.int/csr/disease/avian\\_influenza/guidelines/recommendationvaccine.pdf](http://www.who.int/csr/disease/avian_influenza/guidelines/recommendationvaccine.pdf)

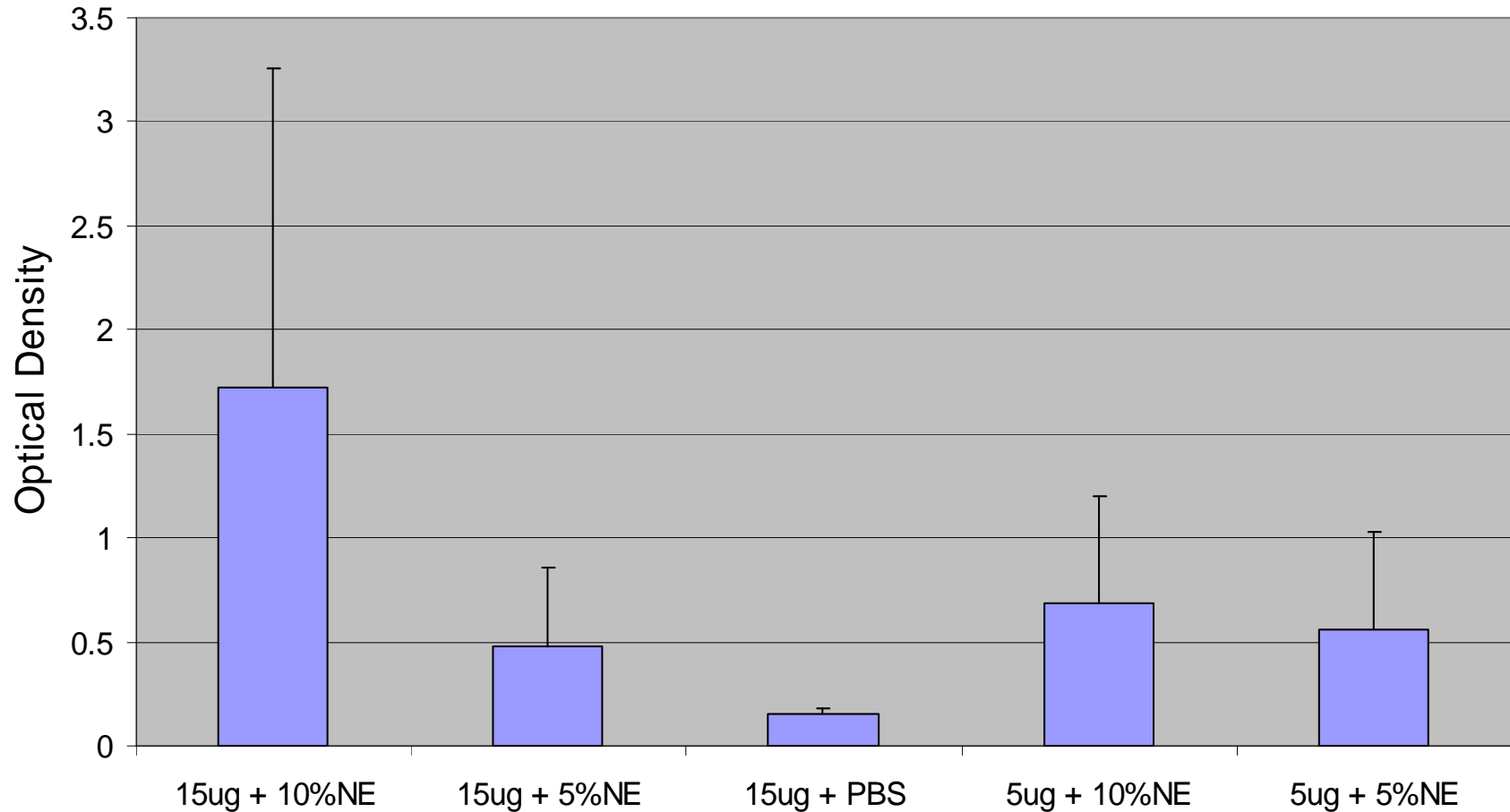
# Cross Clade Reactivity - ELISA



Commercial Antigens Produced in HEK293 cells

Antibodies were cross reactive to Clade 1 and Clade 2.3

# IgA in Bronchial Aveolar Lavage Fluid



Secretory IgA was found in Bronchial Aveolar Lavage fluid from nanoemulsion vaccinated mice

# Conclusions

Nanoemulsion H5 vaccine was able to

- Induce high titers of serum anti-H5 antibodies
- Produce antibodies that cross react with other H5 antigens
- Induce mucosal immunity at the highest antigen and nanoemulsion concentration

# Next Steps

- Work with Yusibov and colleagues to further characterize the murine immune response
- Evaluate the ability to immunize ferrets or another appropriate model for challenge
- Examine the toxicology of this unique vaccine