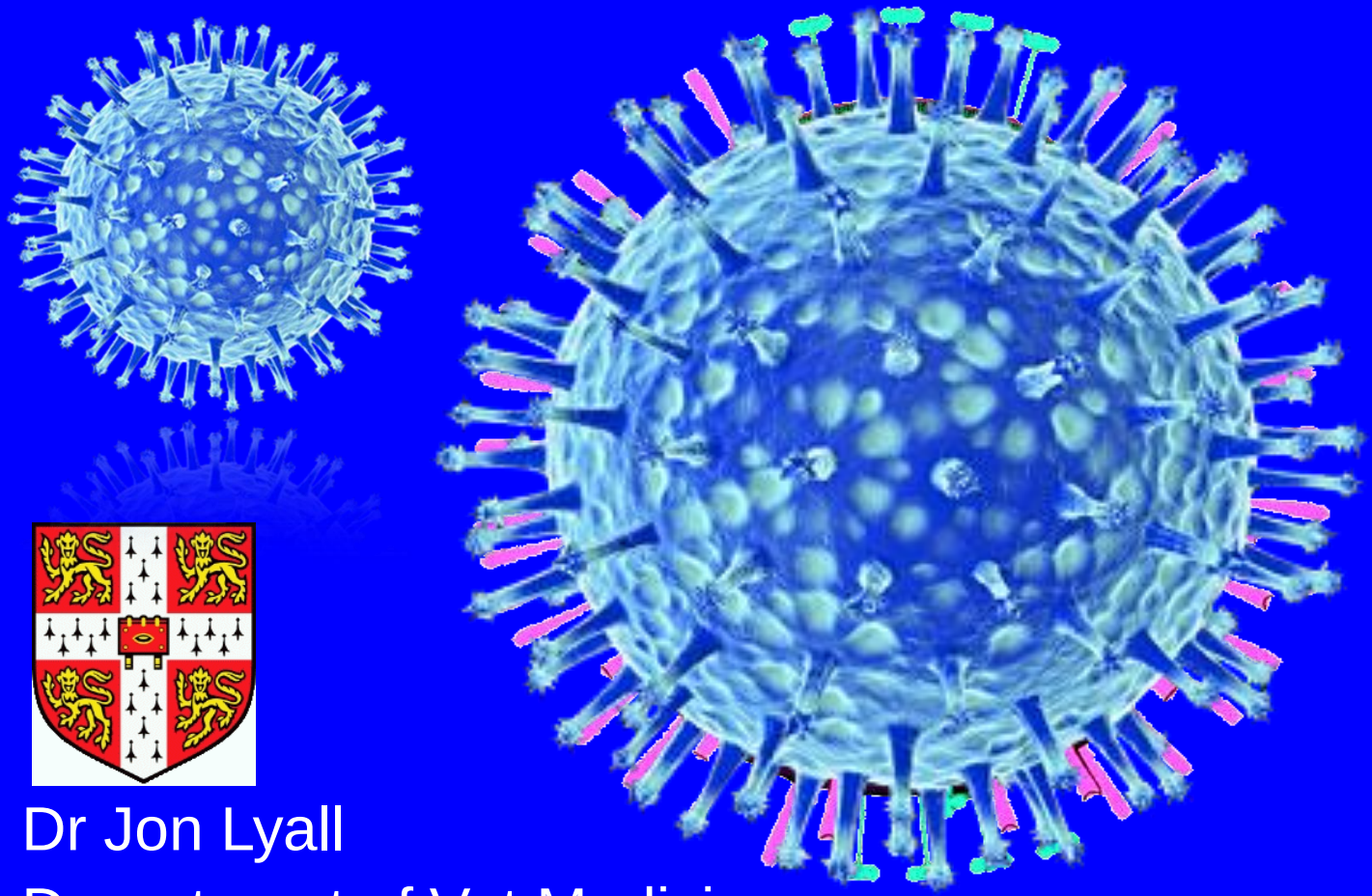
Transgenic Approach to Avian Influenza Control in Chickens



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Chickens on a Global Scale

Global environmental changes and population growth reinforce the need to:

- **produce more**
- **waste less**
- **protect the supply**
- **mitigate the environmental impact**

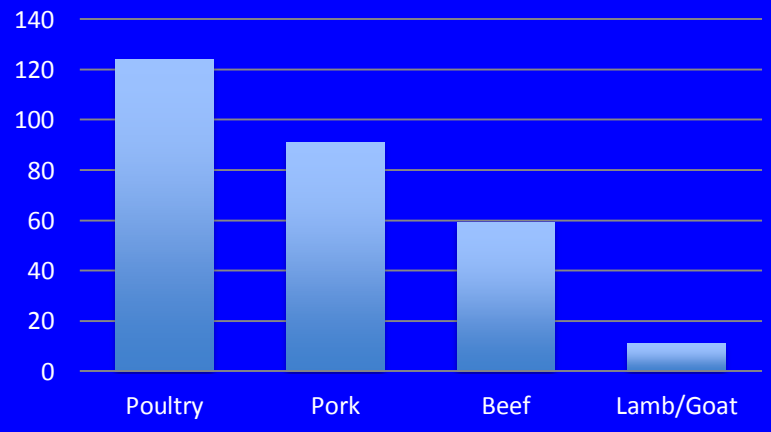
50 billion chickens

- **12 billion backyard/small scale**
- **38 billion intensively reared**
- **12 billion eggs produced in UK (2015)**
- **Set to double by 2030**

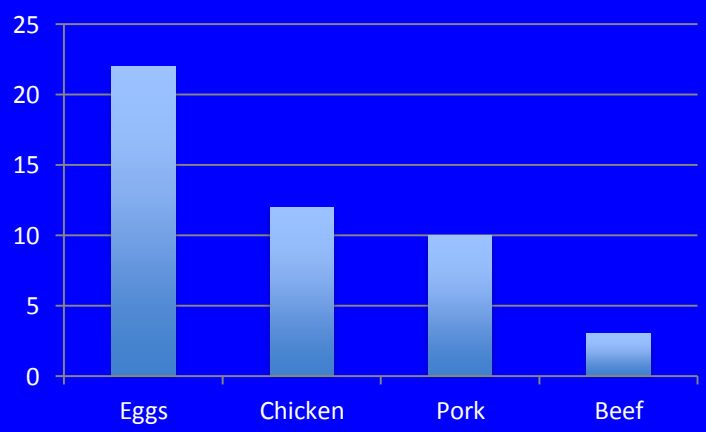
Carbon footprint of Livestock per Kg protein

Poultry	**3.7 Kg**
Pork	**24 Kg**
Beef	**58-1000 Kg**

Global food production (millions of tons)



% of calories converted to food



Why is Avian Influenza a problem?

Production losses

Food security

Public health risk

Economic impact

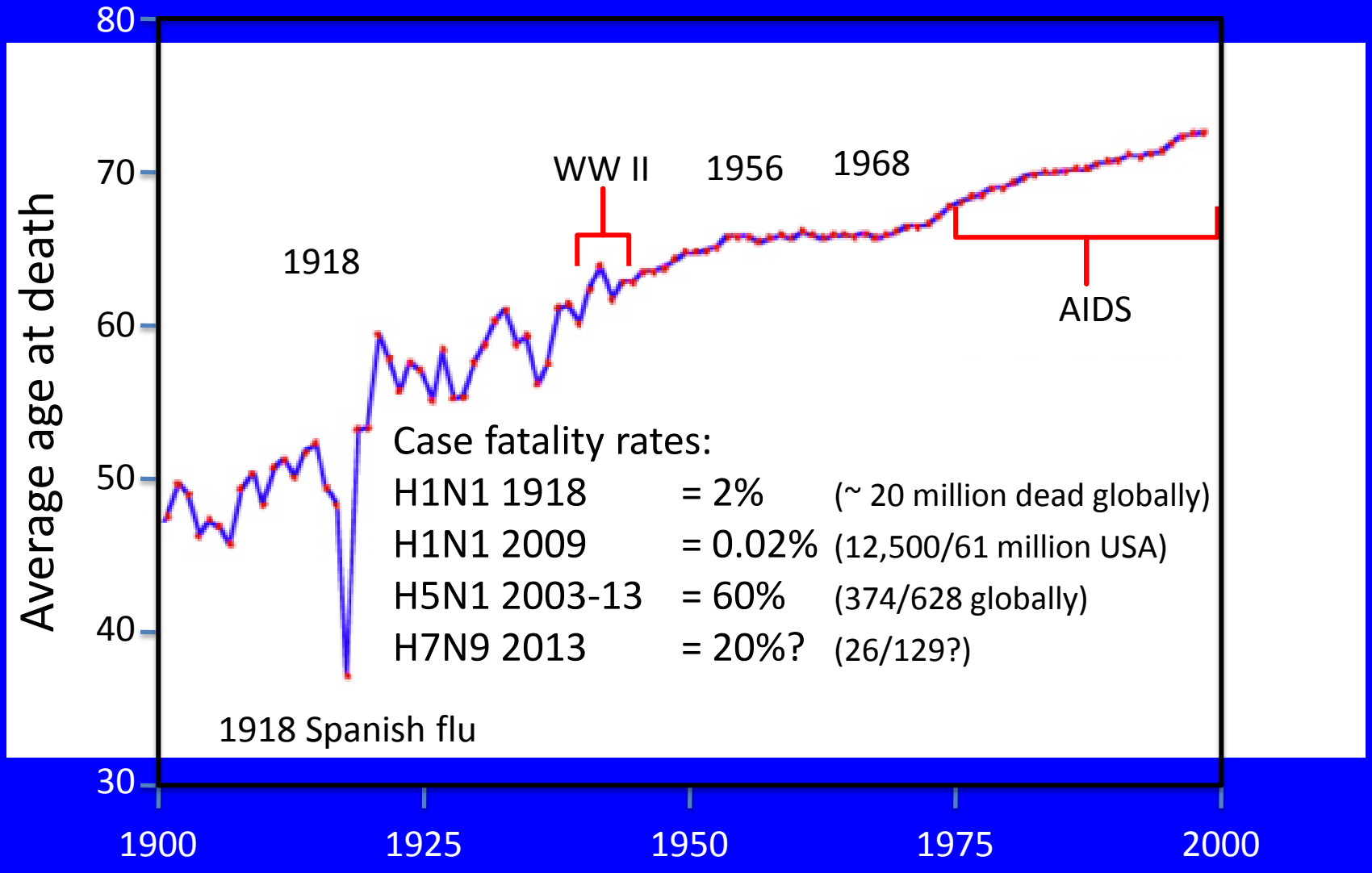
Cost of control measures

Collateral consequences for GDP

Impact of Avian Influenza

- Economic losses from poultry disease around 10-20%
- In 2004 H5N1 caused losses of 400 million chickens worldwide including 44 million birds (17.5% of population) in Viet Nam, 29 million birds (14.5%) in Thailand
- Poultry sector contributed 1.3% of GDP in China in 2004, 20-50% rural family incomes in Nigeria
- Pandemic threat from infected chickens (and pigs) to humans

Impact of pandemic flu



Public health risk

- Avian influenza is the ultimate source of all new strains of influenza.
  - Endemic in wild birds. Low risk of direct infection to humans. However…
- Chickens (and pigs) are a large susceptible population with close contact to humans and wild birds
  - Have the potential to amplify human exposure
  - Facilitate virus evolution
  - Act as a bridge for cross-species transmission.

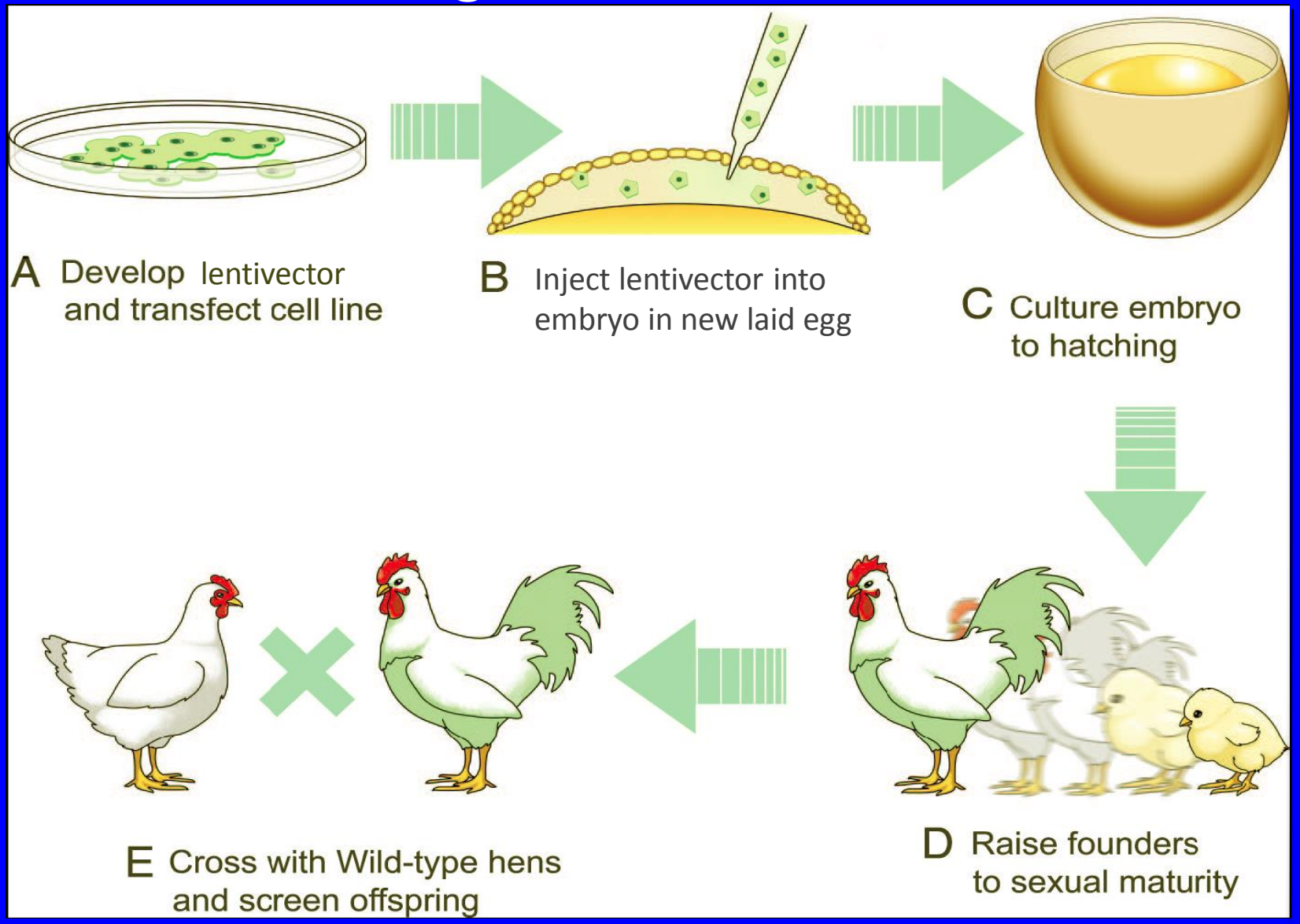
Control Measures For Avian Influenza

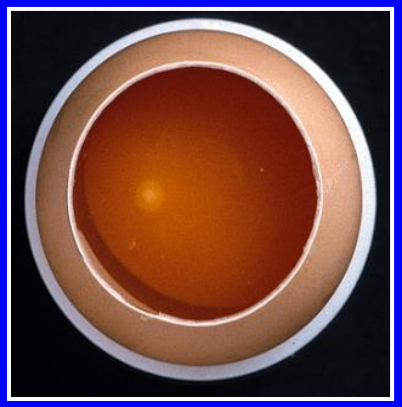
- Improve farming and market practices
  - Control mixed livestock production and wet markets
- Vaccination
  - Multiple subtypes/antigenic drift
    - Sub-clinical disease
    - Drives evolution of HA
- Biosecurity
  - Incompatible with free-range production
- Influenza resistance
  - Genetic Modification

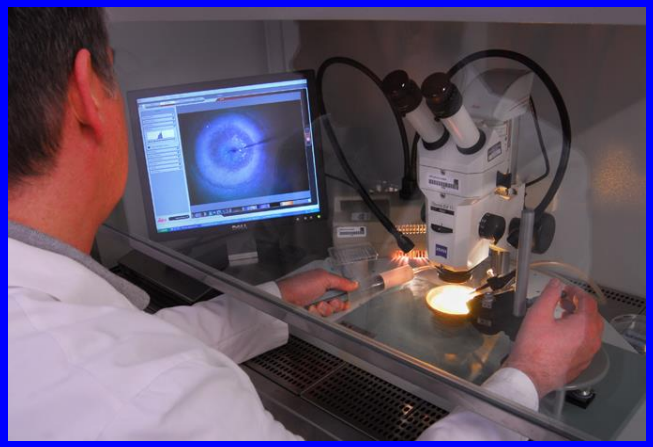
Genetic Modification

- Precise
  - Introduce only the gene of interest
- Versatile
  - Introduce unique combinations
  - Natural and artificial genes
- Maintain genetic diversity
  - Introduce same trait into multiple founder lines
  - No loss of valuable traits

Production of genetically-modified chickens using lentivectors







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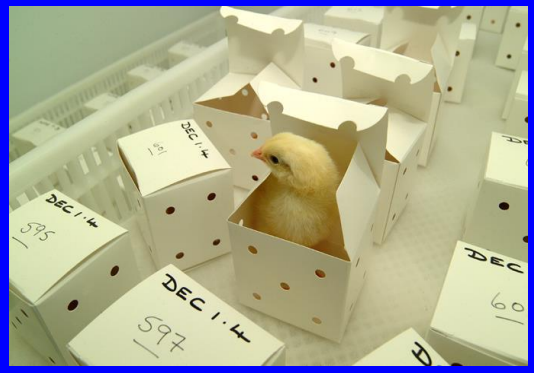
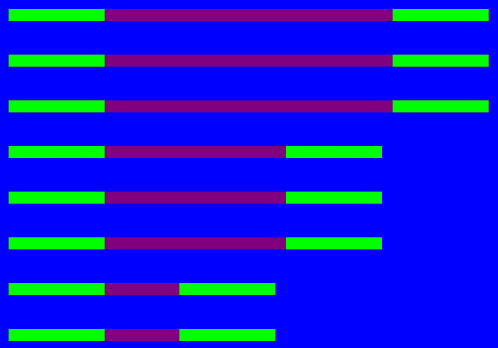


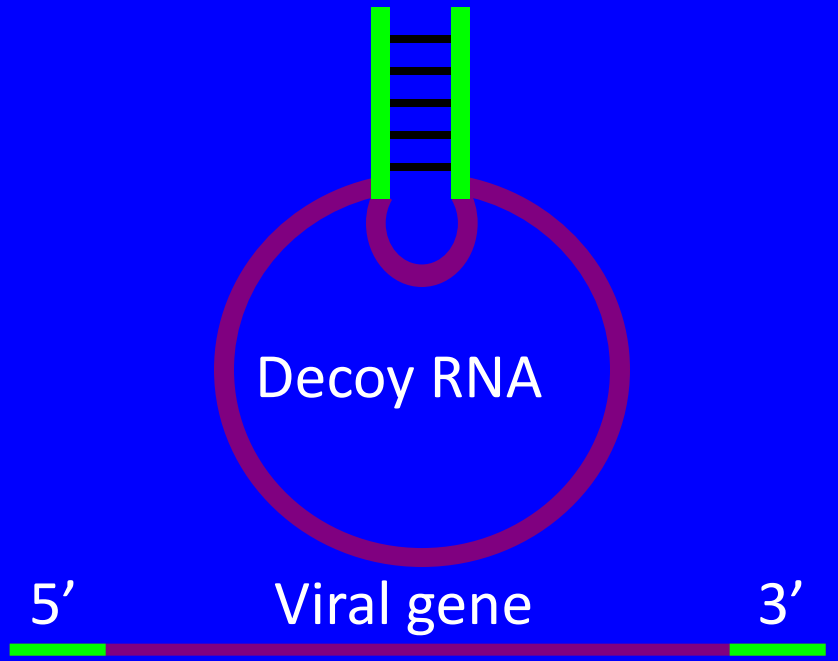
Our Inhibitors

- Pan subtype influenza virus decoy RNA
  - Non coding RNA
  - Binds viral polymerase
  - Replicated by viral polymerase
- Influenza virus driven expression of chicken interferon alpha
  - Antiviral gene expression directly driven by virus
  - Product is identical to endogenous chicken IFNa

Influenza Virus Decoy

Viral gene segments





Panhandle RNA structure

H5N1 Challenge of Decoy GM Chickens

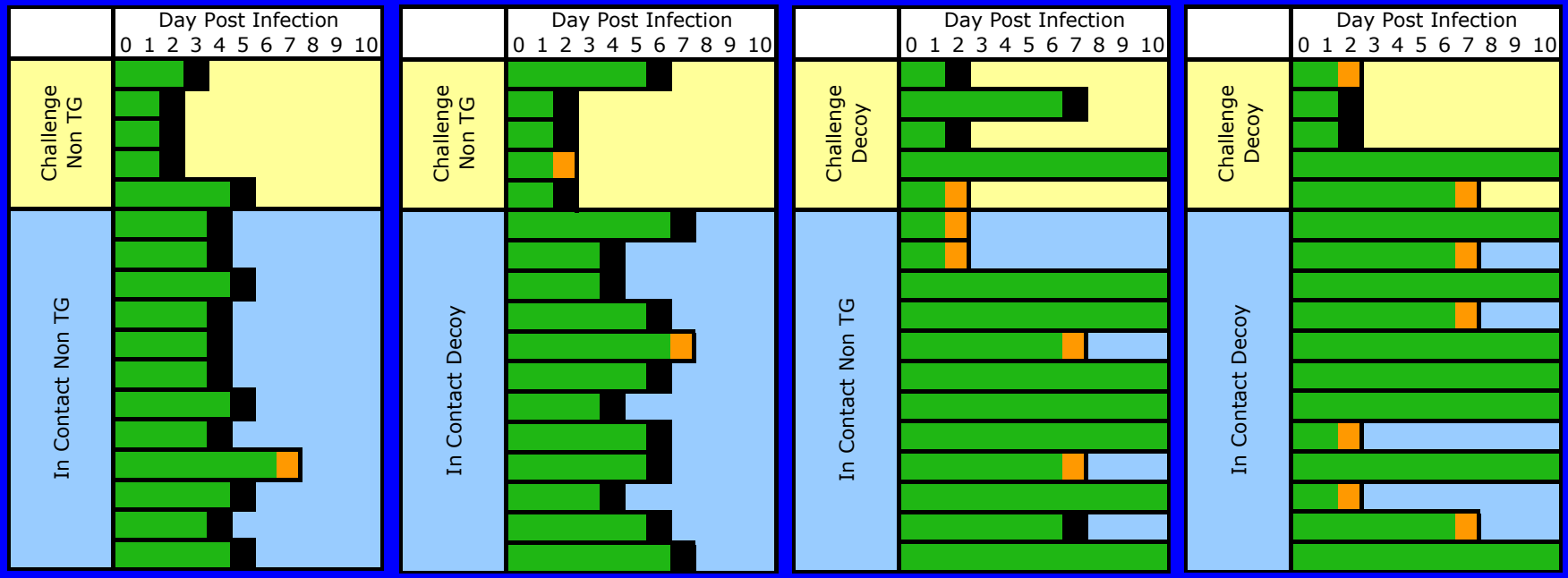
Chickens challenged with 10^4 EID50 dose of Highly Pathogenic H5N1 Influenza

Uninfected chickens co-housed with infected chickens on day 0 post infection

Day of death / euthanasia recorded

Day healthy birds killed for tissue sampling recorded

Experiment ended on day 10 post infection



Decoy prevents onward viral transmission from transgenic birds

Flu driven inhibitors

- Use flu virus to directly express inhibitory gene.

Green Fluorescent Protein

Chicken Interferon-alpha

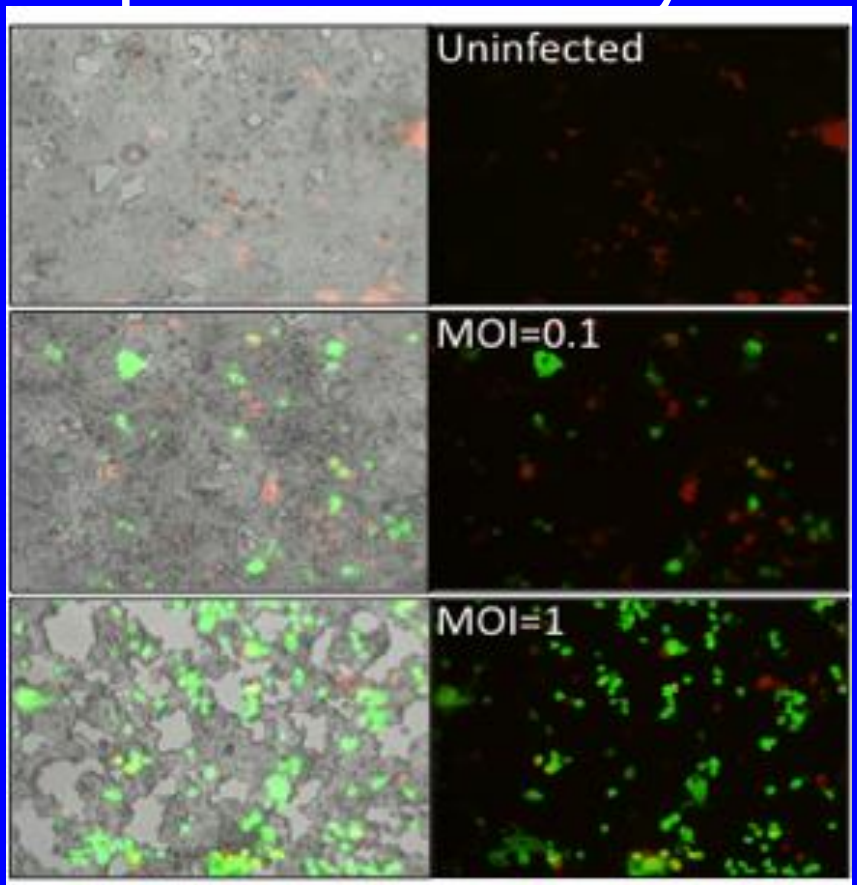
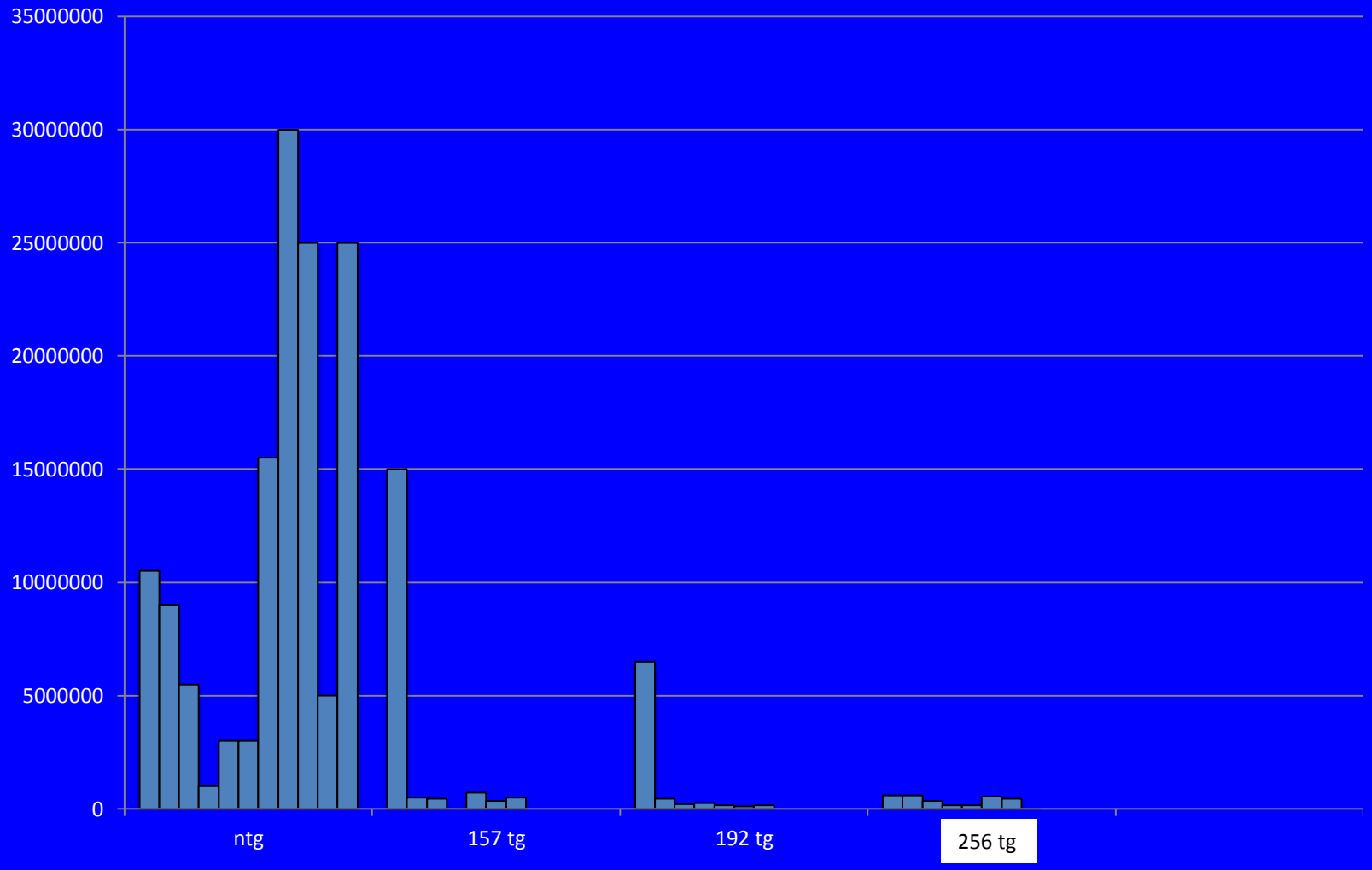


Figure 2. Induction of GFP expression by A/WSN/33 infection of 293T cells co-transfected with 500ng GFP mini-replicon vector and 50ng DS-Red control plasmid.

Viral yield from interferon chicken cell lines



In vivo challenge of interferon GM chickens

H5N1 infected Donor birds

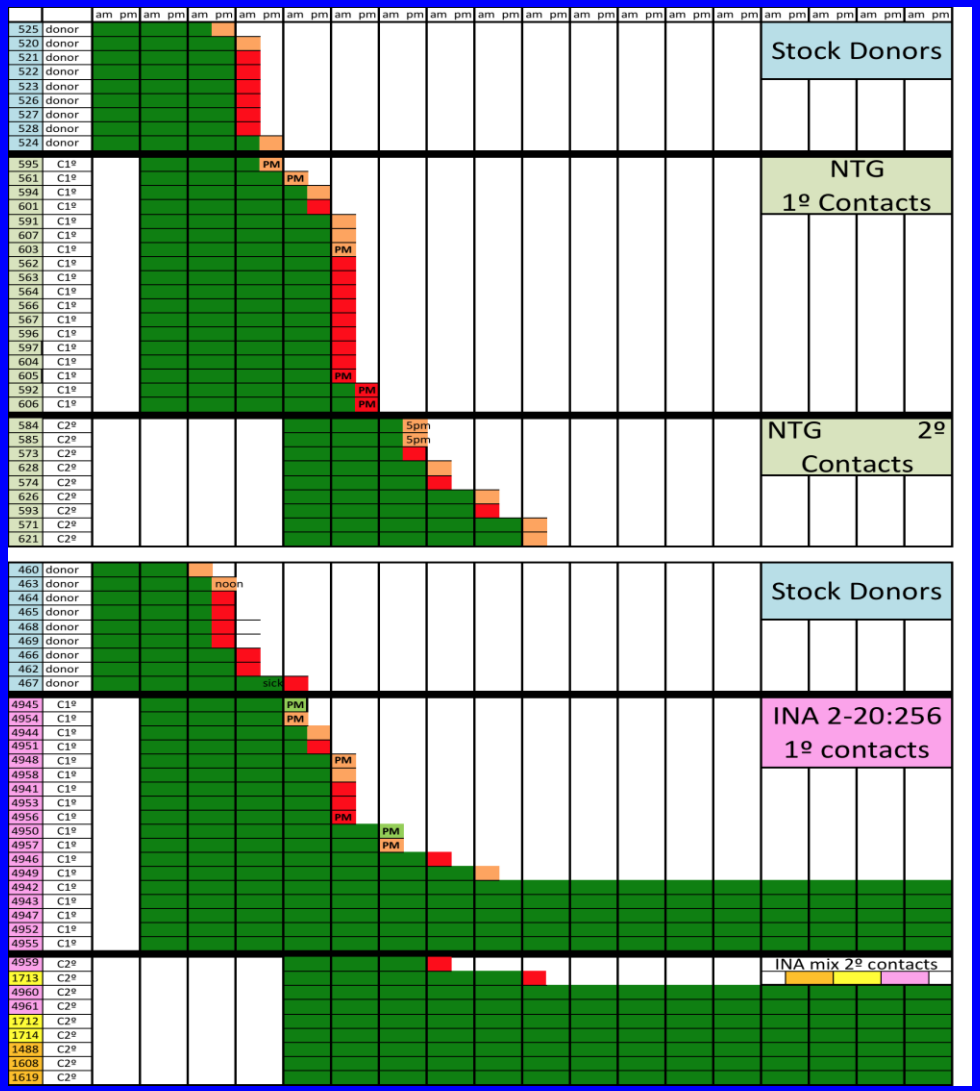
Non GM Primary Contact Birds

Non GM Secondary Contact Birds

H5N1 infected Donor birds

GM Primary Contact Birds

GM Secondary Contact Birds



Virus driven interferon expression increases survival in infected birds and decreases onward transmission

Prospects

- Individually, these inhibitors reduce susceptibility to the virus, and subsequent transmission, and so would be expected to prevent virus spreading through a flock of birds
- Combinations of inhibitors within the same bird can be achieved by cross breeding with the potential to provide complete resistance to the virus
- These inhibitors are active against all subtypes of influenza virus
- The technology works, but are people ready to accept GM animals?

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