



*The key to your profit*

# Low Pathogenic AI

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H&N Layer Academy 21<sup>st</sup>-25<sup>th</sup> October 2024

# Biography-Denis Yau

- BVM 1986 (NTU), MSc 1988 (RVC)
- Department head, Technical manager, CP China 1989-1992
- Technical & Marketing Director, BU head Intervet 1992-2009
- Private consultancy 2010-2014
- Technical & Marketing Director, MSD Global, APAC & sub-Saharan 2015-2019
- Poultry veterinarian, CityU Veterinary Faculty 2019 till now

## WHAT TO KNOW

- Avian influenza refers to disease in birds caused by infection with avian (bird) influenza (flu) Type A viruses.

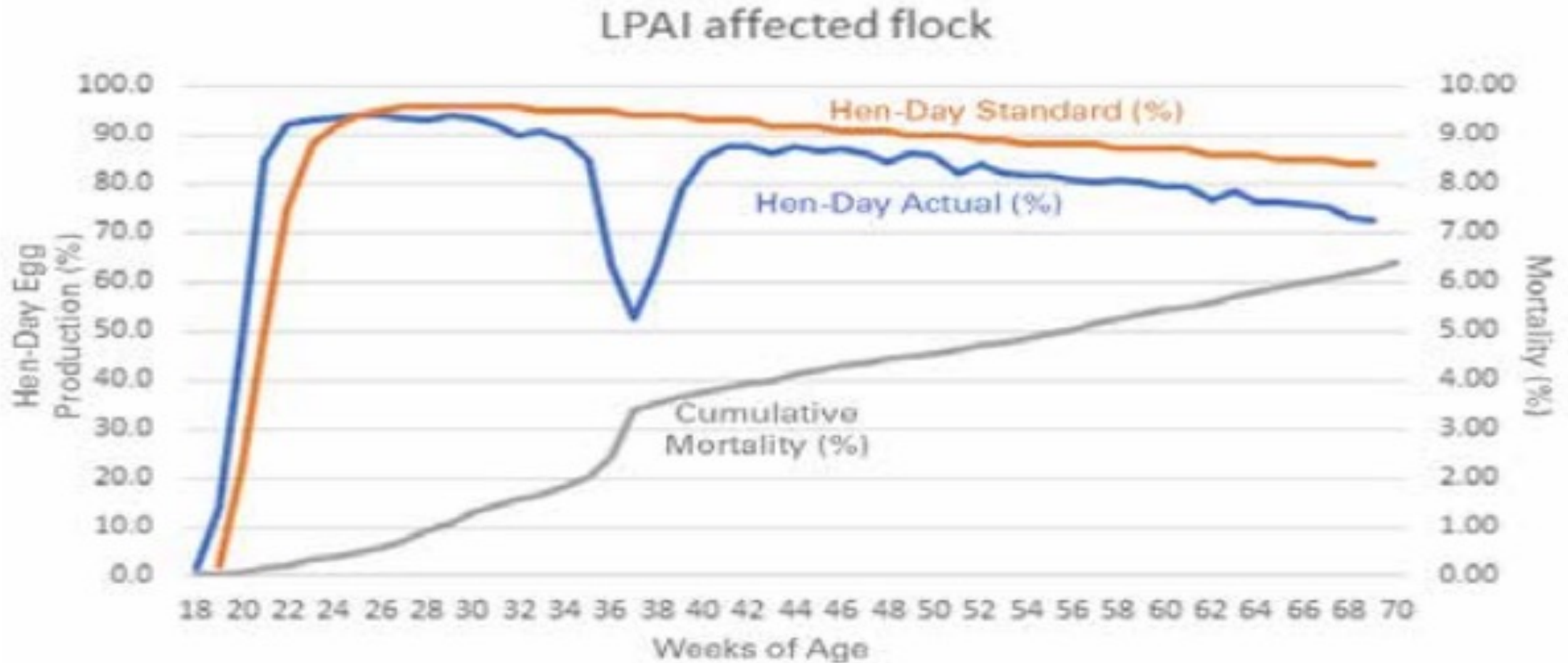
## Definition LPAI

[https://www.cdc.gov/bird-flu/virus-transmission/avian-in-birds.html#:~:text=Low%20Pathogenic%20Avian%20Influenza%20\(LPAI,disease%20in%20infected%20wild%20birds](https://www.cdc.gov/bird-flu/virus-transmission/avian-in-birds.html#:~:text=Low%20Pathogenic%20Avian%20Influenza%20(LPAI,disease%20in%20infected%20wild%20birds)

- **Low Pathogenic Avian Influenza (LPAI):** Low pathogenic avian influenza viruses cause either no signs of disease or mild disease in chickens/poultry (such as ruffled feathers and a drop in egg production). Most avian influenza A viruses are low pathogenic and cause few signs of disease in infected wild birds. In poultry, some low-pathogenic viruses can mutate into highly pathogenic avian influenza (HPAI) viruses.
- the potential for low pathogenic avian influenza A(H5) and A(H7) viruses to evolve into highly pathogenic avian influenza A(H5) and A(H7) viruses with major agricultural implications

# Egg drop caused by LPAI

<https://www.hyline.com/Upload/Resources/TU%20LPAI%20ENG.pdf>



# DDx major poultry respiratory diseases

	AI/ND	IB	ILT	MG	IC
Speed of spread	rapid	rapid	moderate	slow/persistent	rapid→chronic
Duration	2wks	2wks	2-4wks	wks-months	wks-months
Egg drop	0-100%	<50%	1-20%	1-20%	<50%
Mortality	0-100%	low	high	low	negligible
Morbidity	0-100%	high	0-50%	high	high
Clinical signs	Egg production stops within 3d, acute respiratory diseases with CNS and high mortality, egg quality	Acute epornitic respiratory diseases without CNS signs but sharp drop in egg production and quality	Severe dyspnoea with blood mucus high mortality	Chronic respiratory signs influenza by weather	Acute or chronic respiratory signs, facial oedema, copious nasal discharge

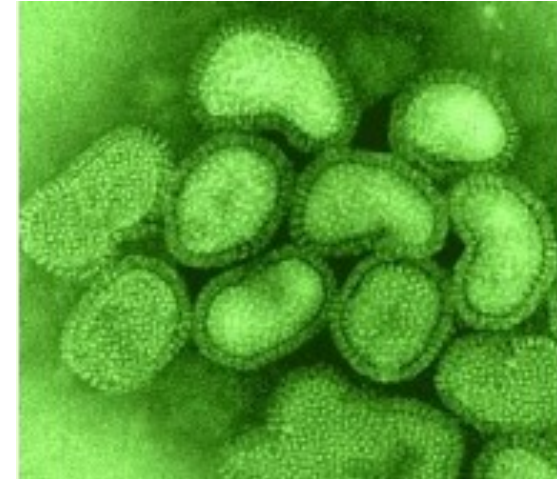
# Common respiratory diseases in Asia

- Newcastle disease (ND)
- Infectious bronchitis (IB)
- Avian metapneumovirus infection (aMVP)
- Infectious laryngotracheitis (ILT)
- Mycoplasmosis (M.gallisepticum/M.synoviae)
- Infectious Coryza (IC)
- Colibacilliosis (E.coli)
- Ornithobacterium rhinotracheale infection (ORT)

# Intervention

## Surveillance

- ✓ serum and choanal/cloacal swab
- ✓ Serology HI
  - Periodically @ sensitive season
  - Quarterly
  - DDx for respiratory diseases, production drop
    - Vaccine failure ND Genotype VII, IB variants, MG/MS E.coli co-infections
- ✓ PCR [https://cdn.who.int/media/docs/default-source/influenza/molecular-detection-of-influenza-viruses/protocols\\_influenza\\_virus\\_detection\\_feb\\_2021.pdf](https://cdn.who.int/media/docs/default-source/influenza/molecular-detection-of-influenza-viruses/protocols_influenza_virus_detection_feb_2021.pdf)



# LPAI H9N2

## Correlation of avian influenza-H9N2 with high mortality in broiler flocks in the southwest of Tripoli, Libya

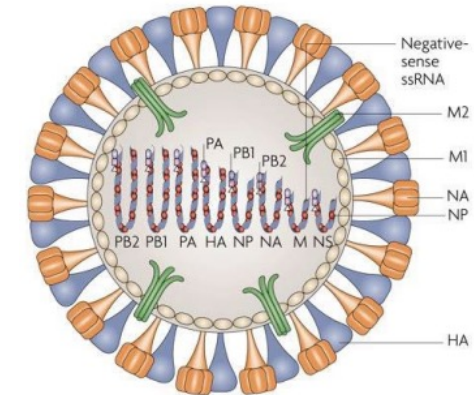
[Ahmed Shaban Kassem Agha](#)<sup>1</sup>, [Imad Benlashehr](#)<sup>2,\*</sup>, [Khalid Mohammed Naffati](#)<sup>1</sup>, [Salah Abdulhadi Bshina](#)<sup>3</sup>,  
[Ahmed Abdulmajed Khashkhosha](#)<sup>1</sup> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10399647/>

## The evolution, characterization and phylogeography of avian influenza H9N2 viruses from India

[Deeksha S. Tare](#)<sup>a</sup>, [Shailesh D. Pawar](#)<sup>a</sup> ✉, [Sachin S. Keng](#)<sup>a</sup>, [Sadhana S. Kode](#)<sup>a</sup>,  
[Atul M. Walimbe](#)<sup>b</sup>, [Vinayak V. Limaye](#)<sup>c</sup>, [Jayati Mullick](#)<sup>a</sup>

<https://www.sciencedirect.com/science/article/pii/S0042682222002173>





**The Low Pathogenic Avian Influenza (LPAI) H9N2 virus is among the most prevalent AIV subtypes in poultry and is also known to cause human infections characterized by mild respiratory illness. As on February 2022, 97 human cases of LPAI H9N2 had been reported worldwide, characterized by flu-like symptoms (WHO, 2022, 2021). In India, H9N2 has been enzootic in poultry for more than two decades. Seroprevalence of antibodies (6.2%) against H9N2 among poultry workers, and the first human case of H9N2 in India have been reported (Pawar et al., 2012c; Potdar et al., 2019). In addition, the LPAI H9N2 virus contributes towards the genesis of zoonotic AIVs such as HPAI H5Nx (clade 2.3.2 and 2.3.4), HPAI H7N9 and H10N8 which cause morbidity and mortality in humans (Peacock et al., 2019).**





# A global perspective on H9N2 AIV

<https://doi.org/10.3390/v11070620>

by T. (Thomas) P. Peacock <sup>1,2</sup>, Joe James <sup>1,2,†</sup> , Joshua E. Sealy <sup>1,3</sup>  and Munir Iqbal <sup>1,\*</sup>  

**Figure 1.** Phylogeographic range of poultry-adapted H9N2 lineages. Countries where only BJ94 lineage viruses are found shown in red, where only G1-W viruses found shown in blue, where mixtures of BJ94 and G1-E sub-lineage viruses are found shown in orange, where mixtures of BJ94 and G1-W sub-lineage viruses are found shown in purple, where only poultry-adapted Y439-lineage viruses are found shown in light pink. H9N2-positive countries where H9N2 lineage hasn't been determined shown in grey. Figure made using [mapchart.net](http://mapchart.net).



# LPAI H9N2

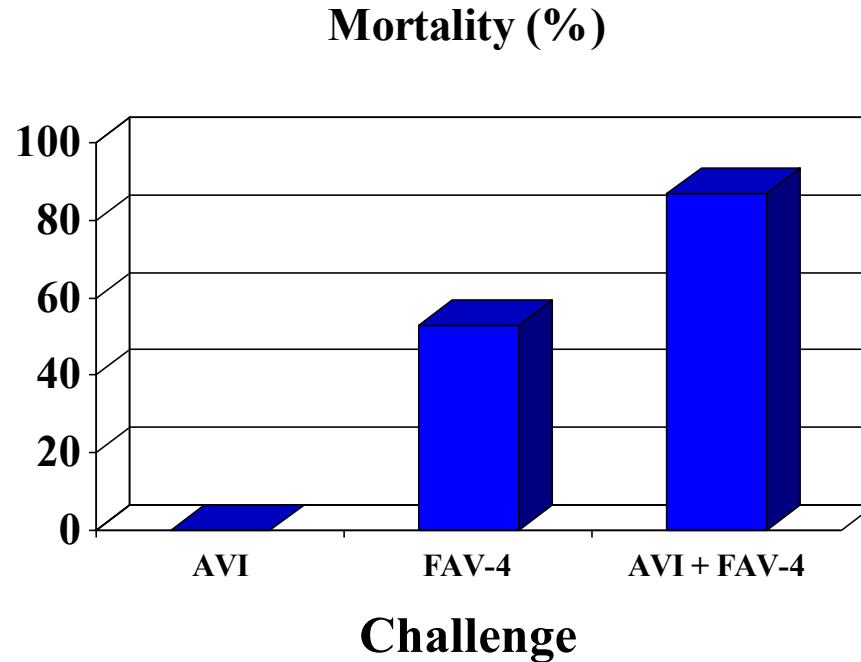
- H9N2 isolates from clinical conditions in South-East Asian and Middle Eastern poultry industries
- IVPI = 0.0
- No multiple basic amino acids at the hemagglutinin cleavage site

# Viral infections that could play a role in disease problems caused by AVI H9N2

- Fowl Adenovirus (FAV)
- Reovirus (REO)
- Infectious Bronchitis Virus (IBV)
- Newcastle Disease Virus (NDV)

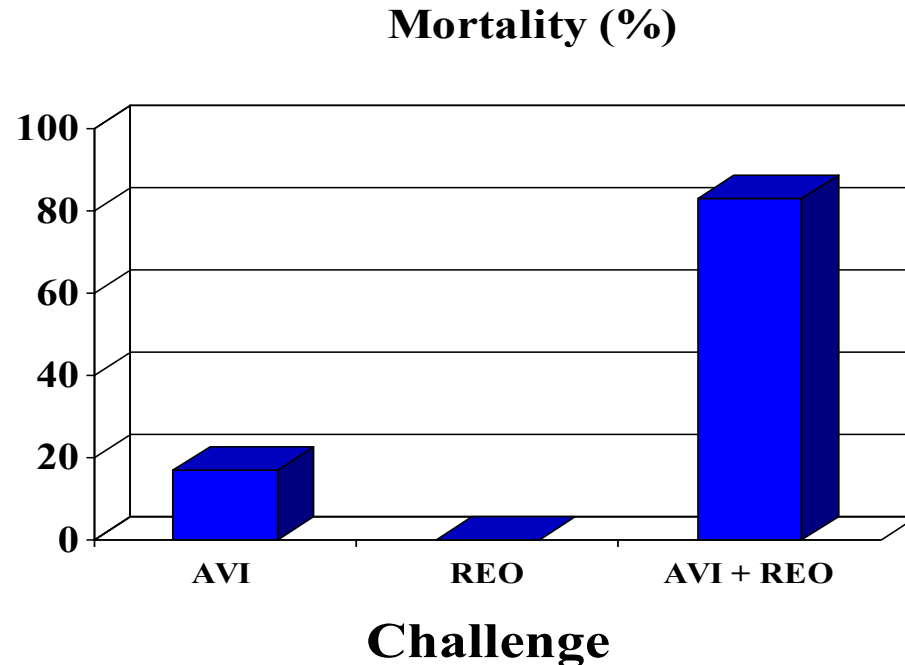
# AVI H9N2 and/or Fowl Adenovirus FAV-4

4 WOA SPF chickens were challenged with AVI H9N2 (IN) or FAV-4 (IM) or both and observed for 2 weeks



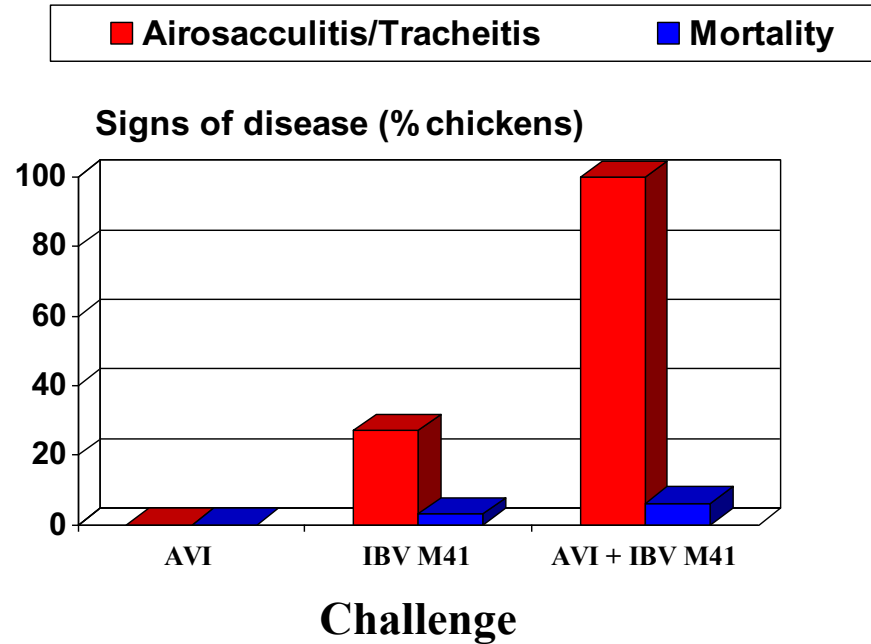
# AVI H9N2 and REO -ERS

SPF chickens were vaccinated with REO (live vaccine, strain 2177) when DO. At 3WOA, the chickens were challenged with AVI H9N2 (aerosol, IN and IV route) or REO-ERS (footpad route) or both and observed for 2 weeks



# AVI (H9N2) and IBV M41

Day-old SPF chickens were challenged with AVI H9N2 (IN) or IBV M41 (IO) or both and observed for 3 weeks

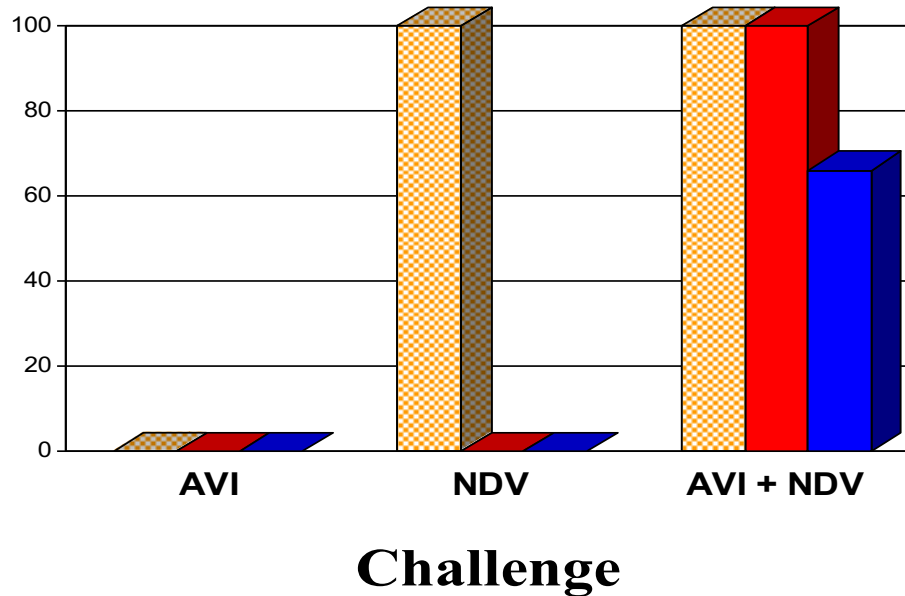


# AVI H9N2 and/or NDV (strain Beaudette)

Three weeks old SPF chickens were challenged with AVI H9N2 (IN) or NDV Beaudette (IO) or both and observed for 2 weeks



**Signs of disease (% chickens)**



# Conclusion

- Low pathogenic AVI H9N2 can enhance or cause disease in chickens infected by FAV, REO, IBV or NDV



# H9N2 Vaccination

- Can vaccination against the concurrent virus infections prevent the pathogenic influence of AVI H9N2 infection ?
  
- Need to vaccinate against AVI H9N2 ?

# Experiments to assess the influence of NDV and/or AVI vaccination on synergistic effects of AVI and NDV challenge

## Vaccination

- Day-old SPF chickens

## Vaccines

- Live NDV (strain Clone 30) vaccine (ocular)
- Live NDV (strain C2, mild) vaccine (ocular)
- Inactivated AVI (H9N2) vaccine (intramuscular)

# Experiments to access the influence of NDV and/or AVI vaccination on synergistic effects of AVI and NDV challenge

## Challenge

- Five weeks post vaccination
- Two weeks observation post challenge

## Challenge viruses

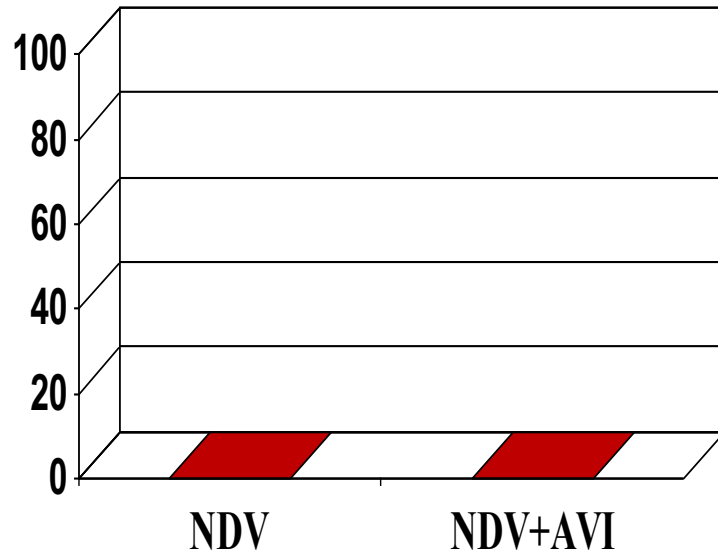
- NDV, strain Herts (ocular)
- NDV, strain Beaudette (ocular)
- AVI H9N2 (ocular, intranasal)

**Vaccination: NDV (Clone 30) / AVI H9N2**

**Challenge: NDV (Herts) / AVI H9N2**

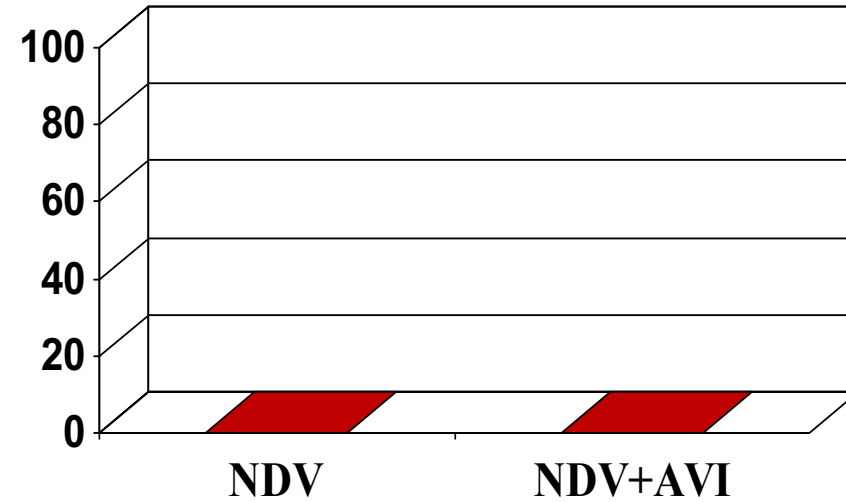
Signs of disease (% chickens) : No

**NDV challenge**



**Vaccination**

**NDV + AVI challenge**

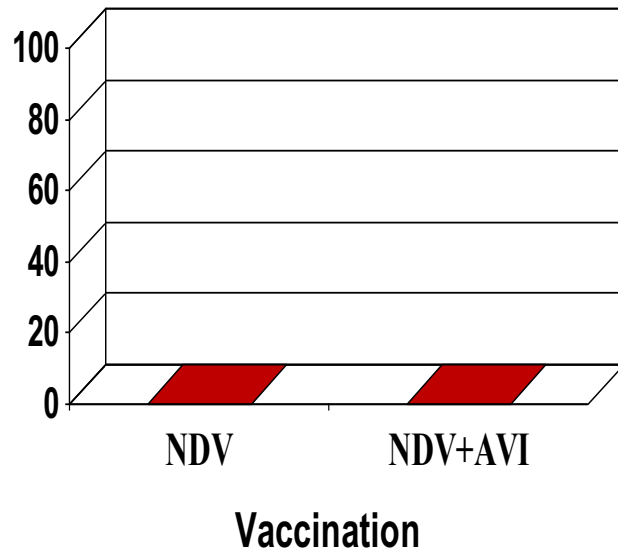


**Vaccination**

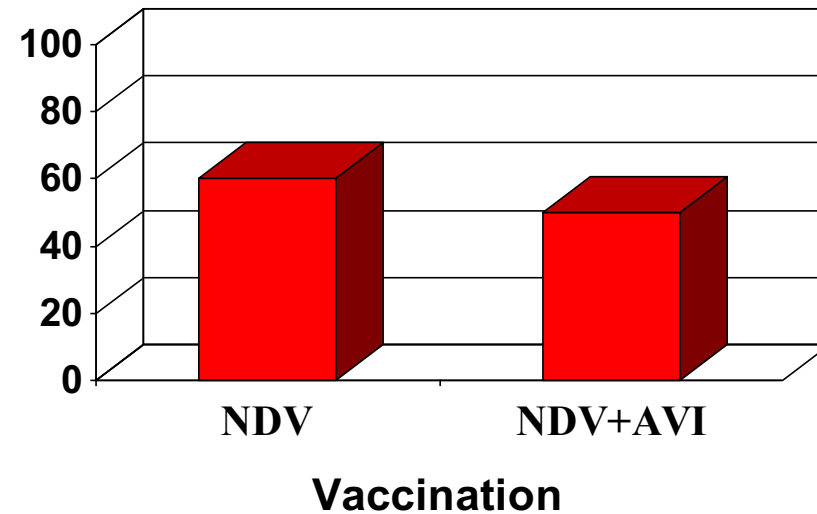
**Vaccination: NDV (C2, mild) / AVI H9N2**  
**Challenge: NDV (Herts) / AVI H9N2**

Signs of disease (% chickens) : Tracheitis

**NDV challenge**



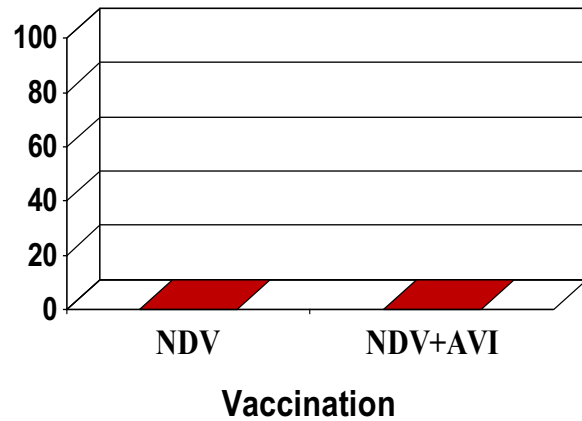
**NDV + AVI challenge**



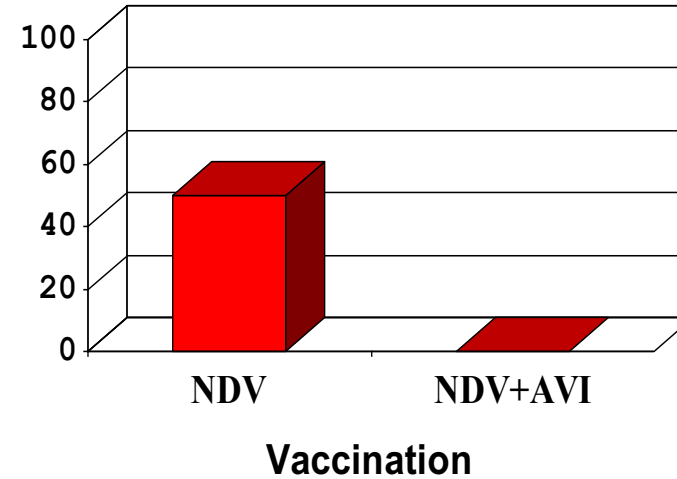
**Vaccination: NDV (C2, mild) / AVI H9N2**  
**Challenge: NDV (Beaudette) / AVI H9N2**

Signs of disease (% chickens) : Tracheitis

**NDV challenge**



**NDV + AVI challenge**



# Conclusion

- No H9N2 vaccination needed
- Review your current vaccination programme, particularly on those common poultry diseases in your region
- Apply proper management
- Enforce biosecurity

A black-outlined speech bubble with a tail pointing towards the bottom left. Inside the bubble, the text 'Yes, BUT...' is written in a bold, sans-serif font. 'Yes,' is in black, 'BUT' is in red, and there are three black dots following it.

**Yes, BUT...**

# Pathogenic potential of LPAI H9N2

- H9N2 regarded to cause disease and mortality in chickens in the field
- IVPI = 0
- Severe disease and mortality observed in the field usually not reproducible in chickens experimentally inoculated with single H9N2 without co-factor - except with A/Ck/Beijing/1/94 given orally



# Development of a challenge model

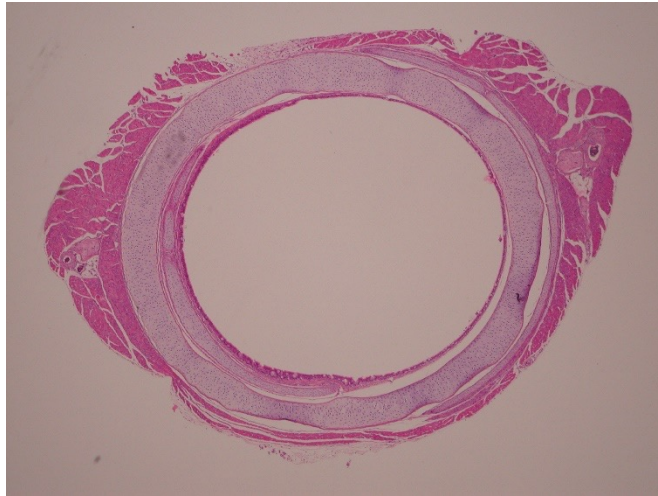
- H9N2 affects the respiratory tract
- Inoculation of H9N2 via respiratory route
- Assessment of H9N2 potential to cause ciliostasis and/or lung lesions



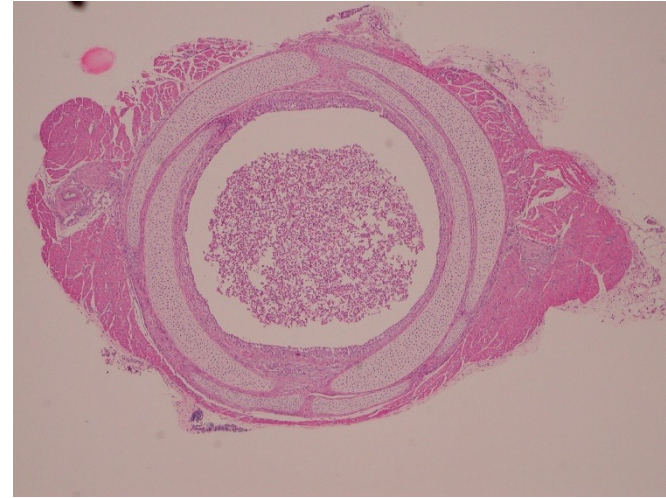
# Tracheae



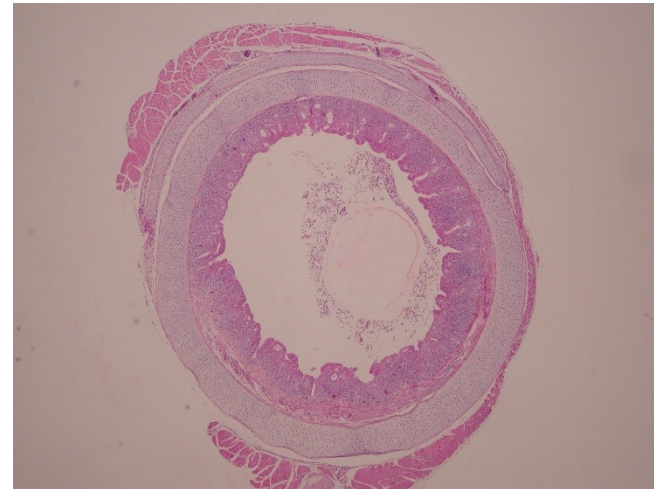
# Tracheitis



Normal trachea (40x)



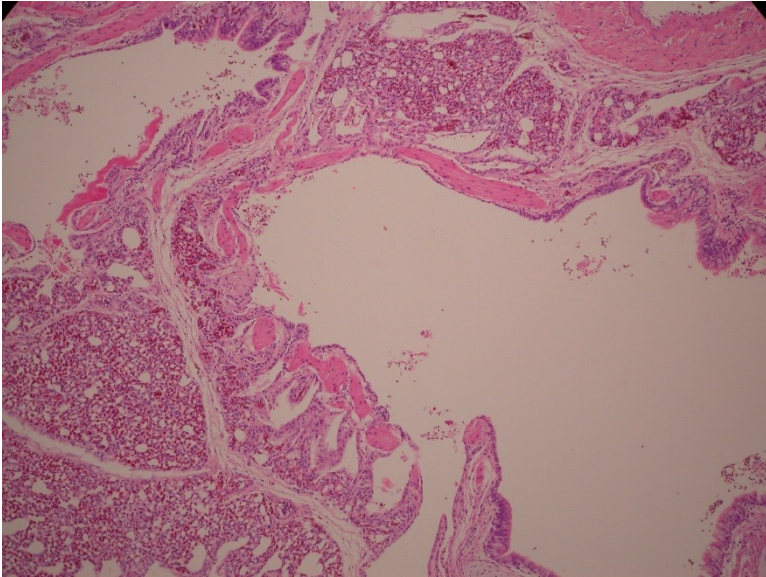
Luminal obstruction (40x)



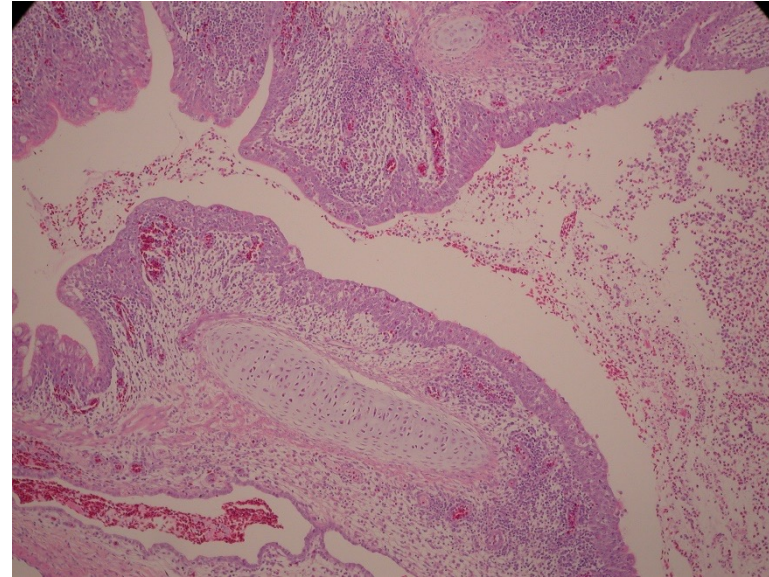
Epithelial hyperplasia (40x)



# Bronchitis

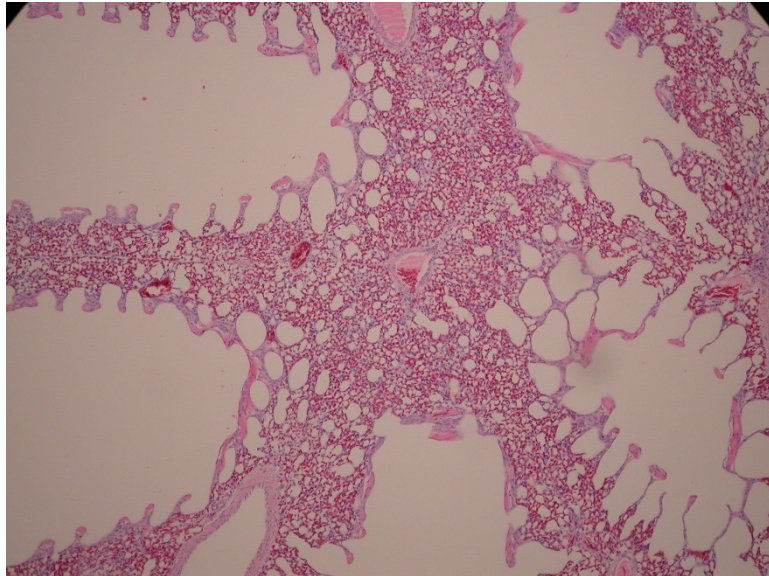


Normal secondary  
Bronchus (100x)

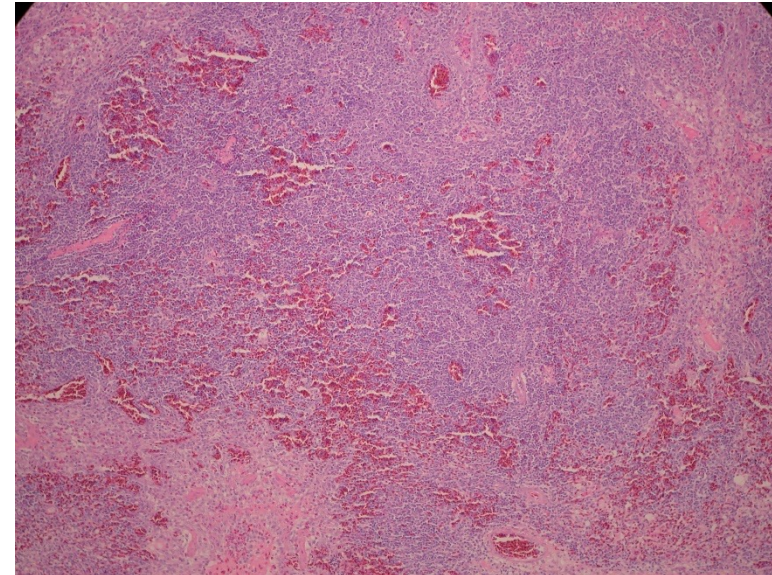


Obstructive proliferative  
Bronchitis (100x)

# Pneumonia



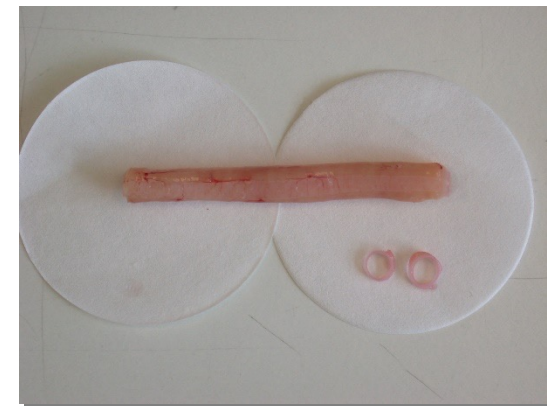
Normal lung parenchyma  
(100x)



Pneumonia (100x)

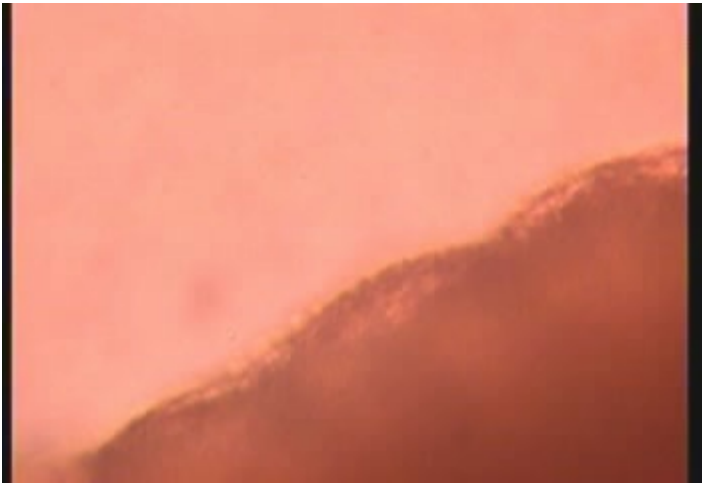
# Ciliary activity of tracheal explants

- ❑ Transverse sections of the upper part, middle part and lower part of the trachea prepared. One section of each part was examined by microscopy for ciliary activity.
- ❑ Each section was scored on a scale from 0 (complete ciliostasis), 25, 50, 75 or 100 % (100 % vigorous ciliary movement).
- ❑ Per group of chickens the mean ciliary activity (%) was calculated.

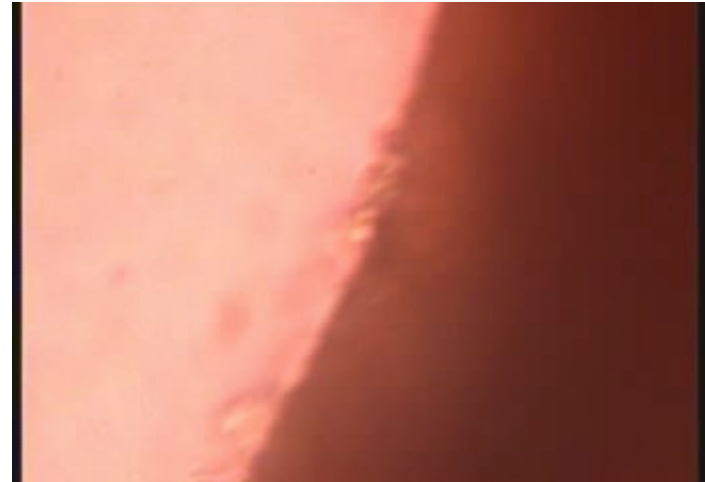




# Ciliary activity



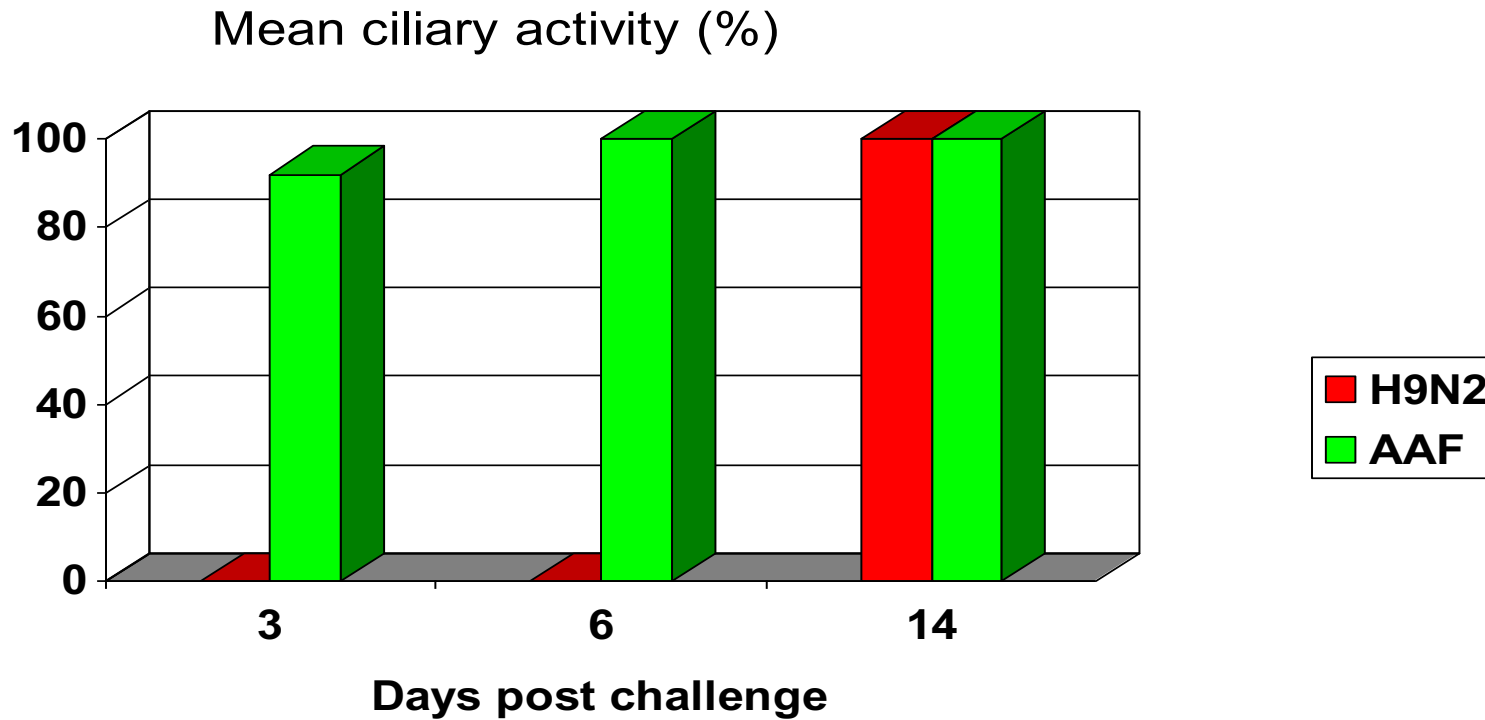
**100 % activity**



**0 % activity**

# Ciliostasis in SPF chicken after spray inoculation

H9N2. allantoic fluid (AAF)



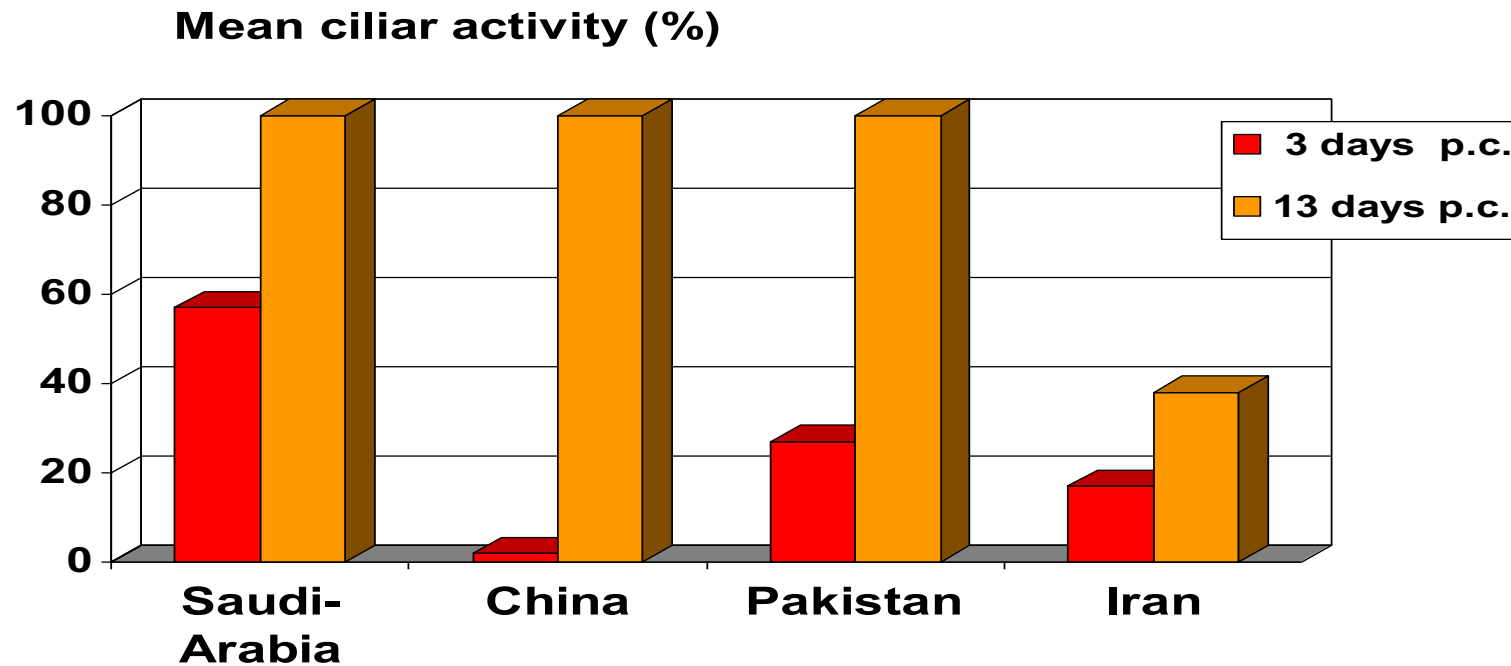


# Ciliary activity after challenge with H9N2 isolates from four different countries

Spray challenge of DO chickens .

Determination of mean ciliary activity at 3 and 13 days post challenge.

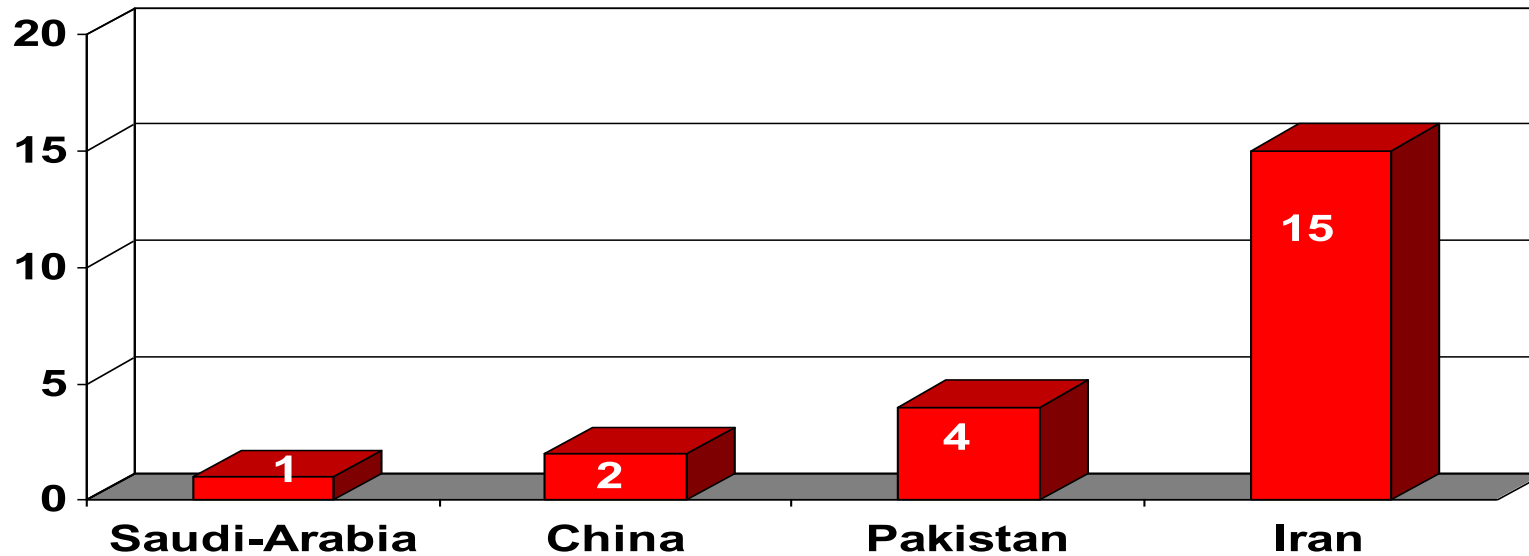
Per isolate were 5 chickens examined



# Mortality after challenge with H9N2 isolates from four different countries

Spray challenge. Observation period two weeks post challenge.  
Per isolate were 20 DO chickens challenged

**Number dead chickens**



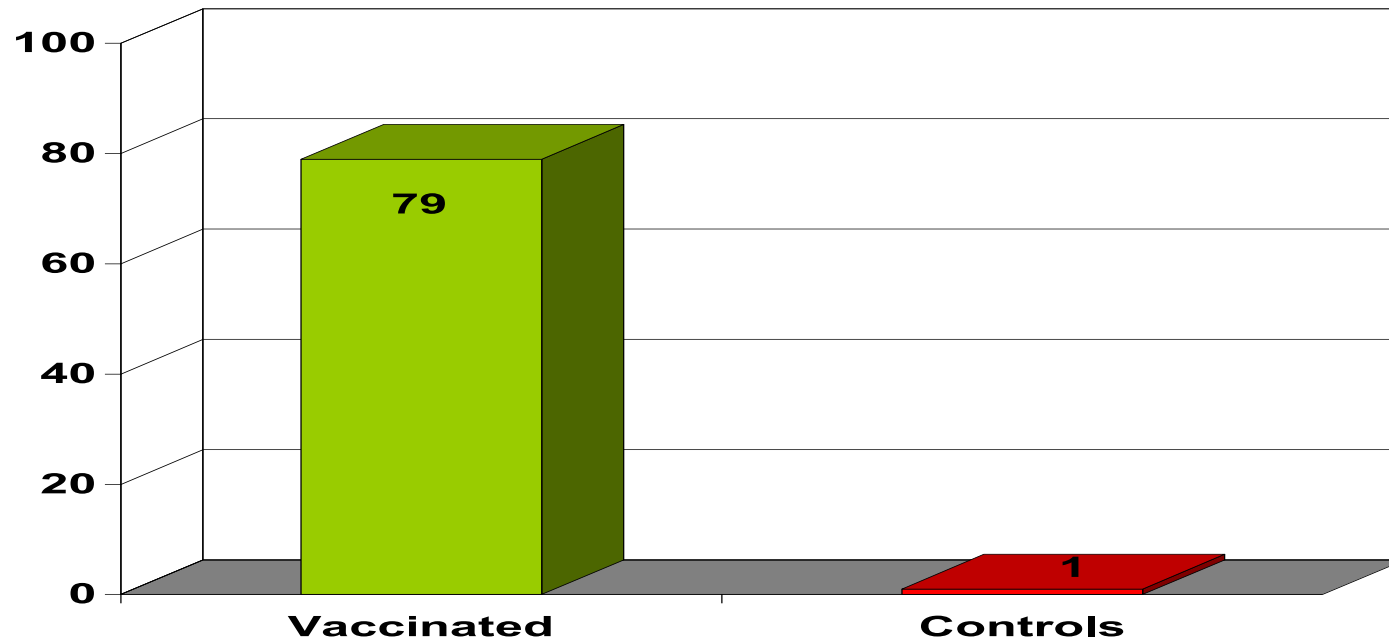
# Vaccination and challenge

- Vaccination of DO chickens via SC route with inact vaccine
- Challenge at 3WOA via spray with H9N2
- Examination of ciliary activity
- Observation for clinical signs

# Ciliary activity 3 days post challenge in vaccinated & control chickens

Challenge H9N2 (United Arab Emirates) via spray.  
10 vaccinated and 7 control chickens examined

**Mean ciliary activity (%)**

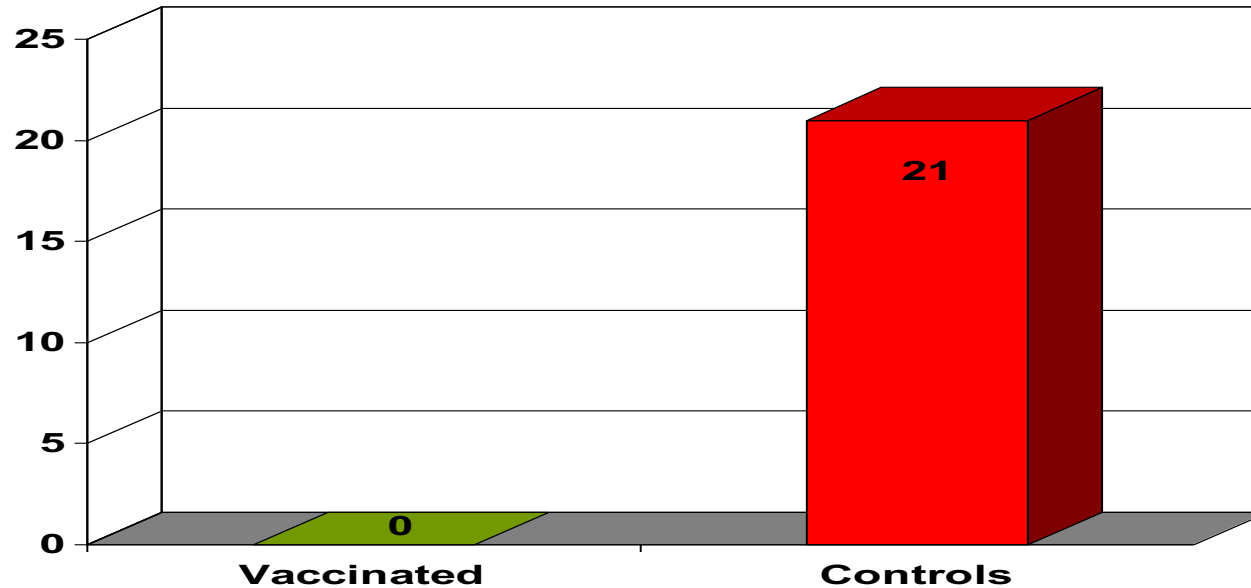


# Mortality in after challenge in vaccinated & control chickens

Challenge H9N2 (United Arab Emirates) AE) via spray.

Mortality within 11 days post challenge. 23 vaccinated and 25 control chickens.

**Number dead chickens**



# Summary

- Application of H9N2 via respiratory tract can cause disease and mortality in chickens without co-factor
- Model to measure efficacy of vaccination
- Inactivated vaccine protects against H9N2 disease induced by local respiratory challenge

# Conclusions

- Low pathogenic AVI H9N2 viruses can enhance or cause disease in chickens infected with other viruses
- Protection can be achieved by efficient vaccination against concurrent virus infections
- H9N2 vaccination can be necessary to complement standard vaccination programmes

# Common diseases vaccination programme

- ND: live and inact vaccines
- IB: protectotype approach
- ILT: rHVT vector vaccination
- aMPV: live (chicken origin) + inact
- MG/MS: spray vaccination
- IC: ABC trivalent inac
- IBD: rHVT vector and breeder vaccination
- CAV: breeder vaccination
- Reo: breeder vaccination



# Prevention of Poultry Diseases



Vaccination  
Monitoring

Record  
Keeping

Biosecurity

Balance  
Nutrition  
Management

**Biosecurity is no joke**

# Key issues-Biosecurity

- ✓ **Perimeter biosecurity**-hard outer shell
- ✓ **Bedding** management
- ✓ Building **maintenance**/design
- ✓ **Location** -near coast / wetlands migration routes /lakes
- ✓ Management and **the controlling mind-don't rely on your SOPs**
- ✓ **Record keeping** and in an accessible format
- ✓ Pointless D without C (**C&D**)
- ✓ **Staff/PPE discipline**
- ✓ **Virus survival** is for weeks
- ✓ **Flooding**
- ✓ **Ponds** on site/in the ranges **vehicles**-restrict access
- ✓ Separate **premises under same management** in close proximity

# Bring home message

- Management ventilation/stocking density/balance feed
- Monitoring
- Prevention of other common respiratory diseases ND/IB/aMPV/MG
- Prevention of immunosuppressive diseases IBD/CAV/Reo
- Biosecurity cleaning and disinfection/quarantine
- Vaccination storage/application/vaccination programme

# References

- Avian influenza in birds: Causes and how it spreads  
[https://www.cdc.gov/bird-flu/virus-transmission/avian-in-birds.html#:~:text=Low%20Pathogenic%20Avian%20Influenza%20\(LPAI,disease%20in%20infected%20wild%20birds](https://www.cdc.gov/bird-flu/virus-transmission/avian-in-birds.html#:~:text=Low%20Pathogenic%20Avian%20Influenza%20(LPAI,disease%20in%20infected%20wild%20birds)
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<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10399647/>
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<https://www.sciencedirect.com/science/article/pii/S0042682222002173>
- A Global Perspective on H9N2 Avian Influenza Virus  
<https://doi.org/10.3390/v11070620>
- Avian Respiratory Coinfection and Impact on Avian Influenza Pathogenicity in Domestic Poultry: Field and Experimental Findings  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5876583/pdf/vetsci-05-00023.pdf>



**Thanks For  
Listening**