



# Effect of Infectious Bronchitis Virus on the infectivity and transmissibility of H5N2 low pathogenicity avian influenza virus in chickens

Mary Pantin-Jackwood<sup>1</sup>, Diane Smith<sup>1</sup>, Christina Leyson<sup>1</sup>,  
Sungsu Youk<sup>1</sup>, Mark Jackwood<sup>2</sup>

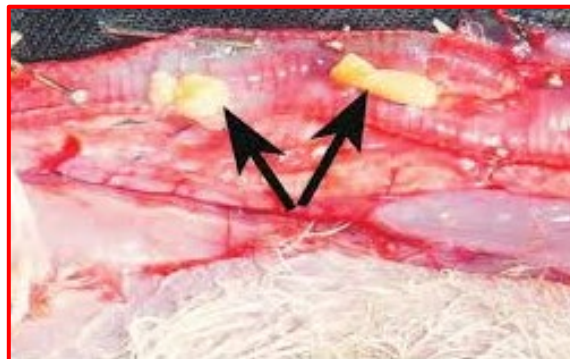
*<sup>1</sup>Exotic and Emerging Avian Viral Diseases Unit, Southeast Poultry Research Laboratory U.S. National Poultry Research Center, ARS-USDA, Athens, GA. <sup>2</sup>Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, Athens, GA*

- **Objective 2:** Investigate the multifactorial etiology involving poultry respiratory diseases
  - **Activity 2.1:** Study the interactions of relevant respiratory and immunosuppressive agents based on species (broiler, layer, and turkeys), their clinical outcomes, transmission patterns, and potential changes in vaccination and diagnostic strategies.

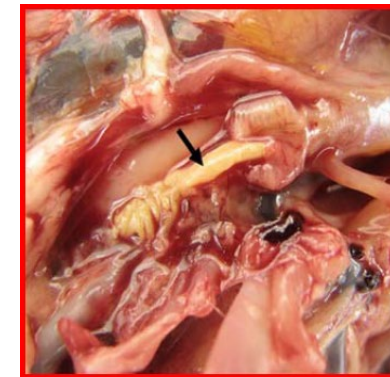


# Co-infection of chickens with respiratory pathogens

- Low pathogenicity avian influenza virus (LPAIV) causes a mild respiratory disease in chickens, but some strains are associated with moderate to severe clinical signs (*Umar et al., 2017; Smietanka et al., 2014*)
- Tracheal plugs are commonly reported with H5N2 and H9N2 LPAIV's but these lesions have not been experimentally reproduced with these strains alone
- Infectious bronchitis virus (IBV) live vaccines have been shown to increase the severity of H9N2 LPAIV infections in commercial chicken operations (*Karimi-Madab et al., 2010*)
- Experimental coinfection of chickens with IBV and H9N2 LPAIV induced severe clinical signs and more severe histological lesions than the viruses given alone (*Haghighat-Jahromi et al., 2008; Hassan et al., 2017; Huang et al., 2017*)



From: Varsa Vahini Dr. K.S.Prajapati



From: Haghighat-Jahromi et al. 2008

Fig. 1. Cast formation in the tracheal bifurcation (arrow) extending to the lower bronchi of a dead chicken from the AIV + IBLV-inoculated group.

# Co-infection of SPF chickens with IBV and LPAIV

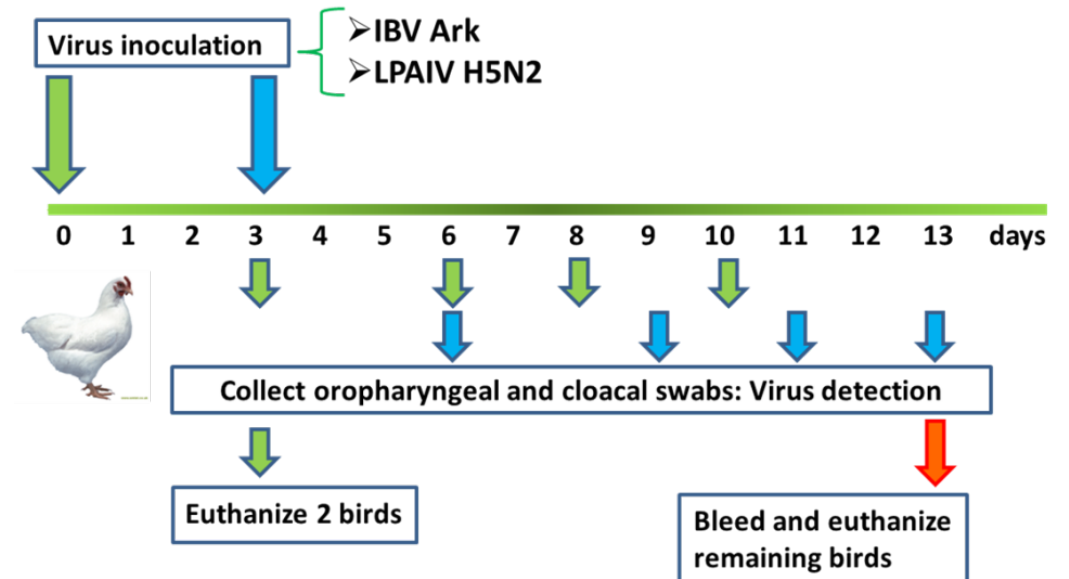
## Study 1

*Can we reproduce the severe tracheal lesions with LPAIV and IBV?*

- 3 week old SPF Leghorn chickens
- Intraocular and intranasal inoculation with:
  - IBV: Ark99;  $10^4$  EID<sub>50</sub>/dose
  - LPAIV: A/chicken/Mexico/IA20/11/2011 H5N2;  $10^7$  EID<sub>50</sub>/dose

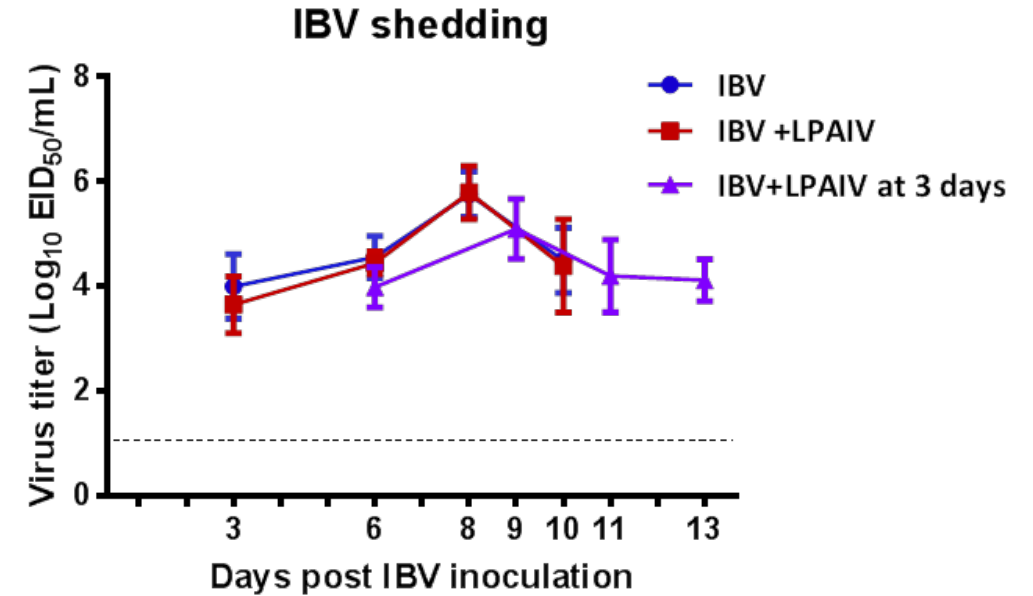
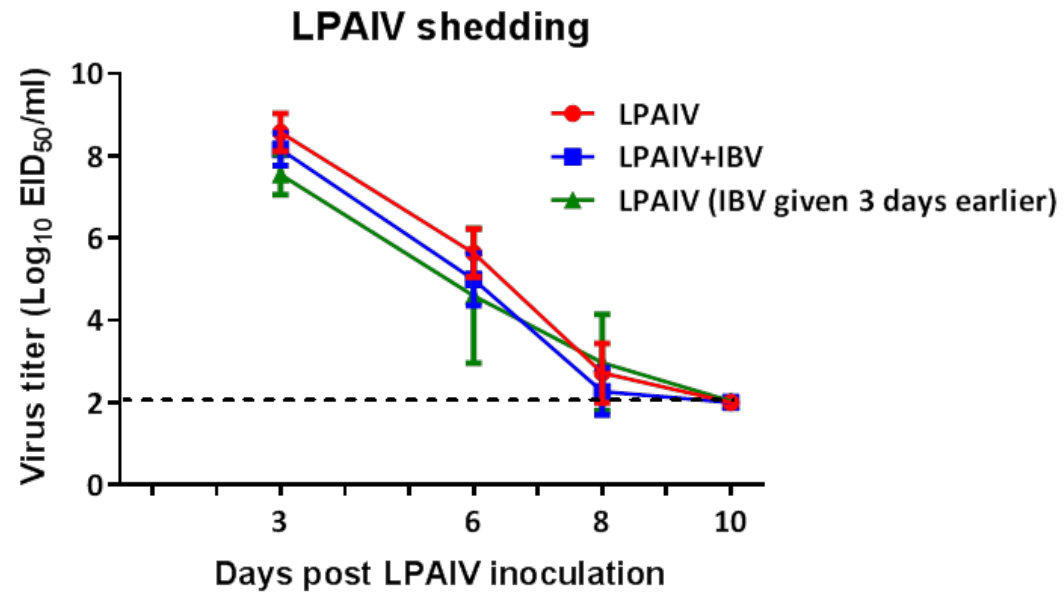


Groups	Day of inoculation	
	Day 0	Day 3
1 (controls)	-	-
2	LPAIV	-
3	IBV	-
5	LPAIV + IBV	-
7	IBV	LPAIV



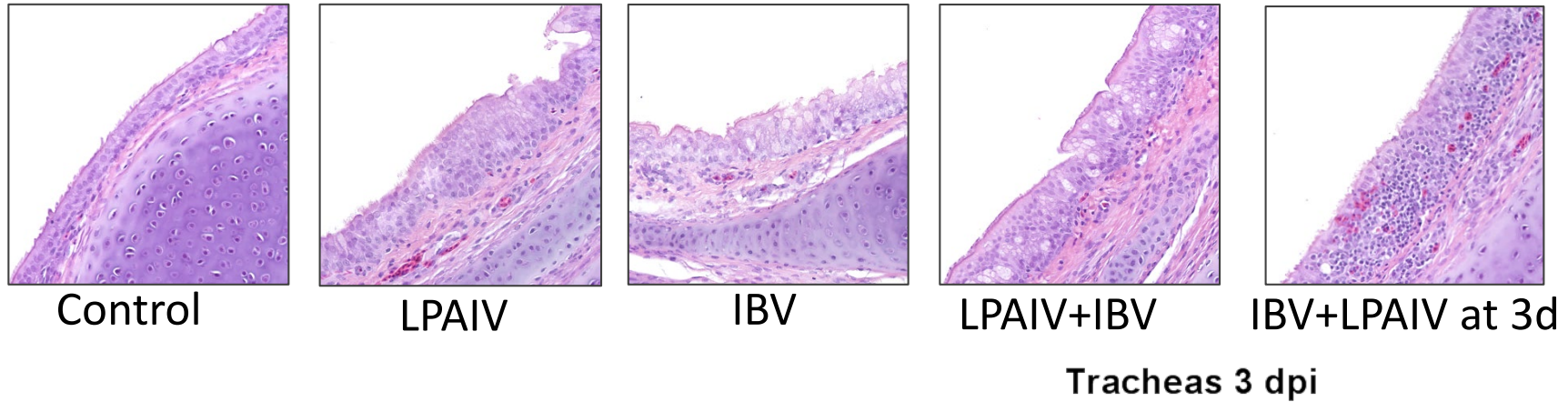
# Study 1 -Results

Clinical signs: Mild to moderate conjunctivitis

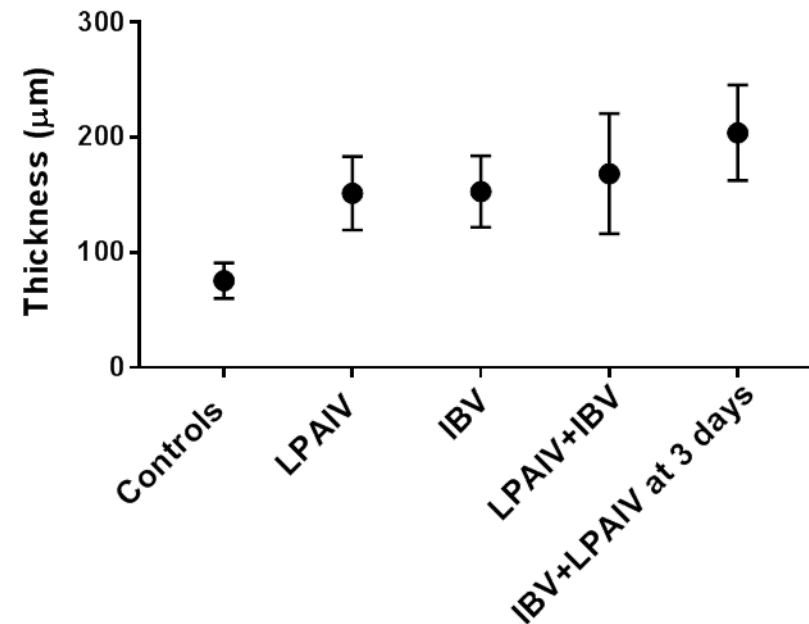


Groups	% Serology positive	
	AIV (HI)	IBV (ELISA)
LPAIV	100%	-
IBV	-	40%
LPAIV + IBV	100%	60%
IBV + LPAIV at 3 days	100%	50%

## Study 1: Tracheal lesions at 3 dpi



- Grossly, no or mild lesions were observed in the trachea
- **No bronchial casts or tracheal plugs were present**



Measurements taken from tracheas from 2 birds at 3 dpi. Mean thickness in micrometers  $\pm$  SD

# Co-infection of chickens with Mycoplasmas

- Involvement of mycoplasmas in multiple respiratory infections has been well documented (*Jordan, 1975; Bradbury, 1984;* )
- Experimental coinfection with LPAIV significantly enhanced the pathogenicity of *Mycoplasma gallisepticum* (MG) (*Stipkovits et al., 2012*)
- Interactions with IBV are known to increase the severity of MG and *M. synoviae* (MS) infections (*Omuro et al., 1971; Timms, 1972; Hopkins and Yoder, 1982, 1984; King et al., 1973; Kleven et al., 1978; Springer et al., 1974*)

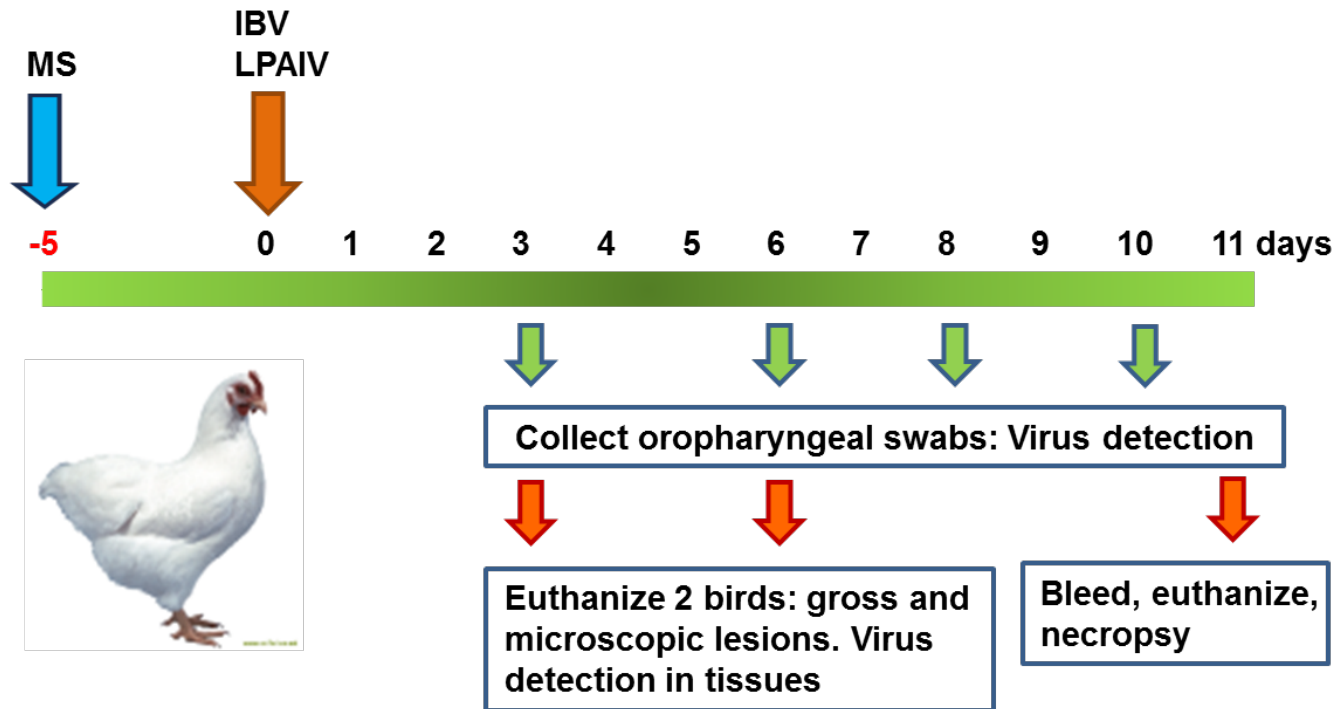
**Objective of Study 2: Determine the effect of co-infection with LPAIV, IBV and MS in chickens**



# Study 2

## Co-infection of SPF chickens with IBV, LPAIV and MS

- 3-week-old SPF Leghorn chickens, 12 birds per group
- Intraocular and intranasal inoculation with:
  - IBV**: Ark99;  $10^4$  EID<sub>50</sub>/dose
  - LPAIV**: A/chicken/Mexico/IA20/11/2011 H5N2;  $10^7$  EID<sub>50</sub>/dose
  - Mycoplasma Synoviae (MS)**: strain K6677,  $10^6$  CCU/dose



Groups	Day of inoculation	
	Day -5	Day 0
1 (controls)	-	-
2	-	LPAIV
3	-	IBV
4	-	LPAIV+IBV
5	MS	-
6	MS	LPAIV
7	MS	IBV
8	MS	LPAIV+IBV

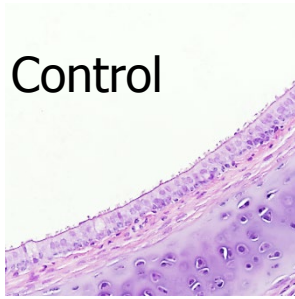


# Study 2 - Results

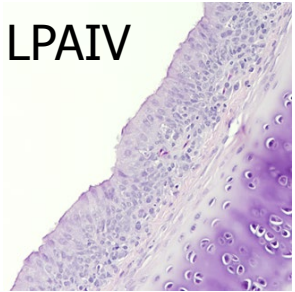


Day of inoculation		Clinical signs and gross lesions
Day -5	Day 0	
-	LPAIV	Mild conjunctivitis
-	IBV	Mild to moderate conjunctivitis
-	LPAIV+IBV	Mild to moderate conjunctivitis
MS	-	Mild to moderate conjunctivitis, pinpoint hemorrhages in trachea, congested organs
MS	LPAIV	Mild to moderate conjunctivitis, pinpoint hemorrhages in trachea, congested organs
MS	IBV	Moderate to severe conjunctivitis, nasal secretions, head shaking, rales, swollen head, cloudy airsac, mucous and hemorrhages in trachea, congested organs
MS	LPAIV+IBV	Moderate to severe conjunctivitis, nasal secretions, head shaking, rales, swollen head, cloudy airsac, mucous and hemorrhages in trachea, congested organs

Control



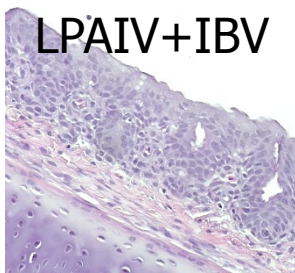
LPAIV



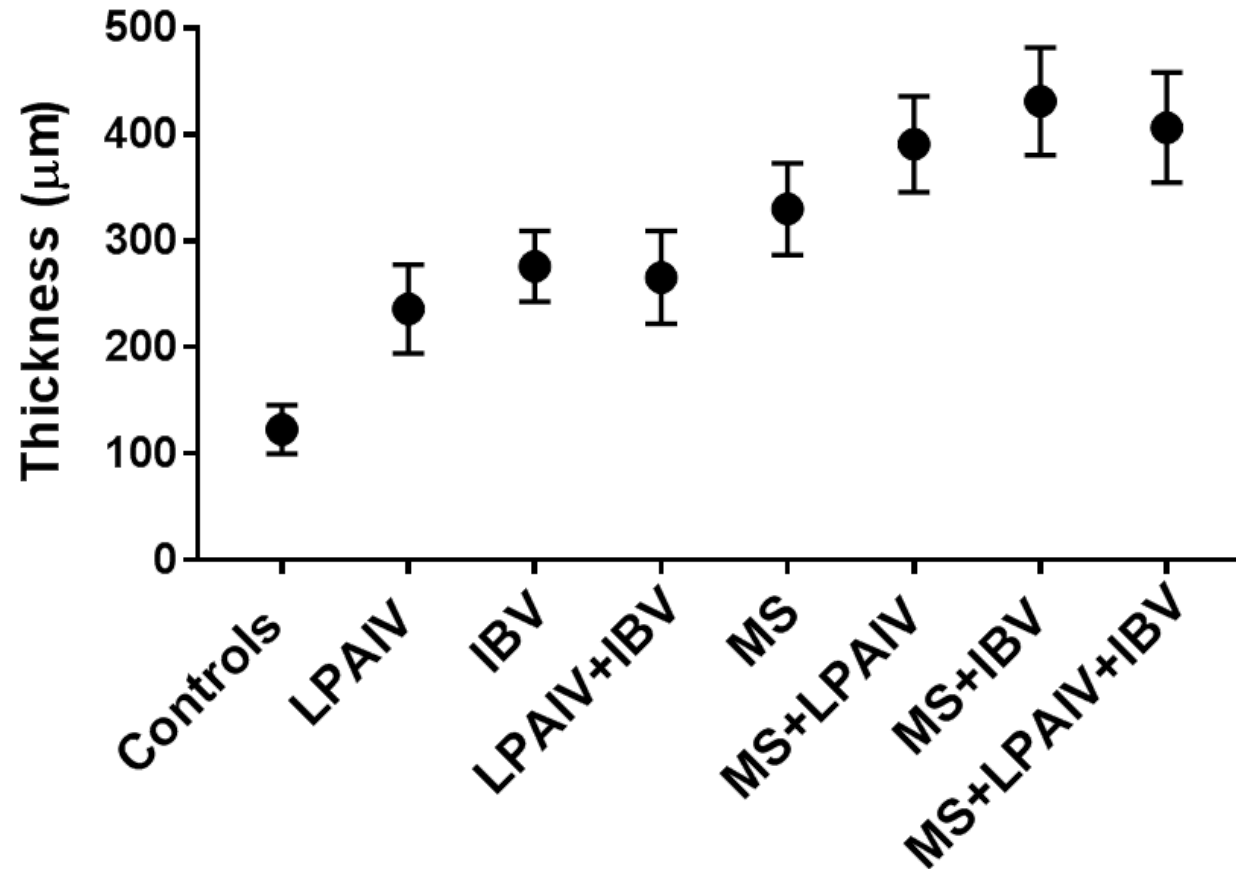
IBV



LPAIV+IBV

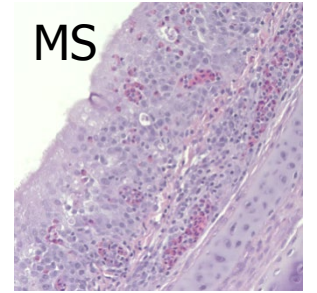


## Tracheas - 6 dpi

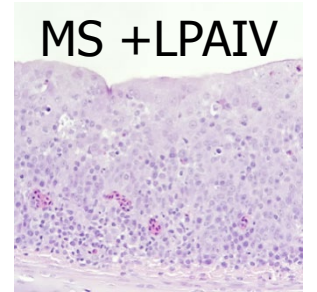


Measurements taken from tracheas from 2 birds at 6 dpi. Mean thickness in micrometers  $\pm$  SD

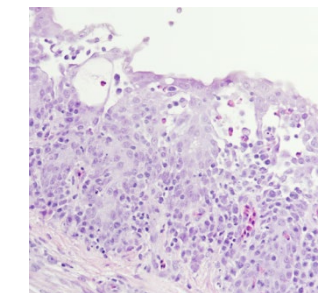
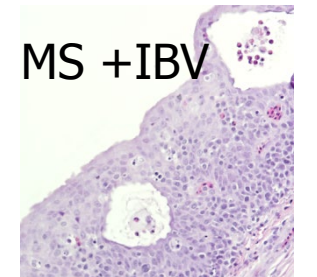
MS



MS +LPAIV



MS +IBV



MS +LPAIV+IBV

## Study 2 - Trachea lesions scores day 11

Day of inoculation		Trachea scoring at the end of study
Day -5	Day 0	
-	LPAIV	8 birds -
-	IBV	8 birds -
-	LPAIV+IBV	8 birds -
MS	-	8 birds +
MS	LPAIV	2 birds +; 4 birds ++ 2 birds +++
MS	IBV	1 bird +; 4 birds ++; 2 birds +++; 1 bird ++++
MS	LPAIV+IBV	6 birds +++; 2 birds ++++

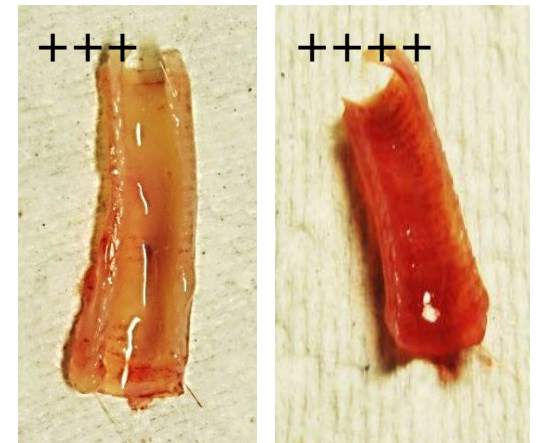
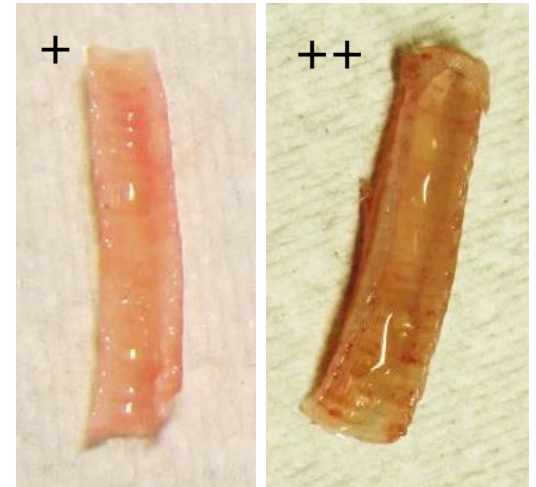
- = no gross lesions

+= mucous

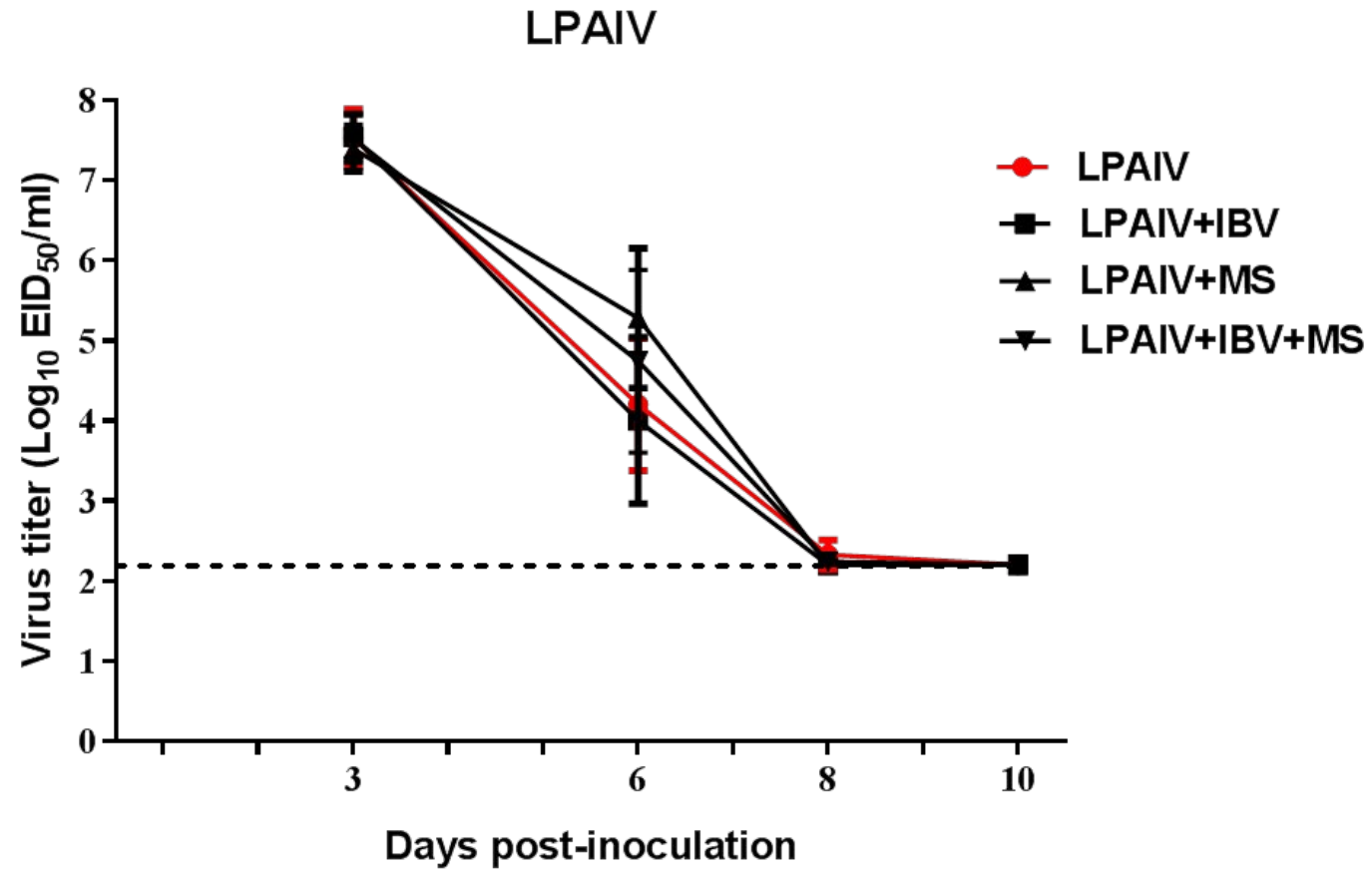
++=mucous and pinpoint hemorrhages

+++ = mucous and hemorrhage

++++ = severe hemorrhage



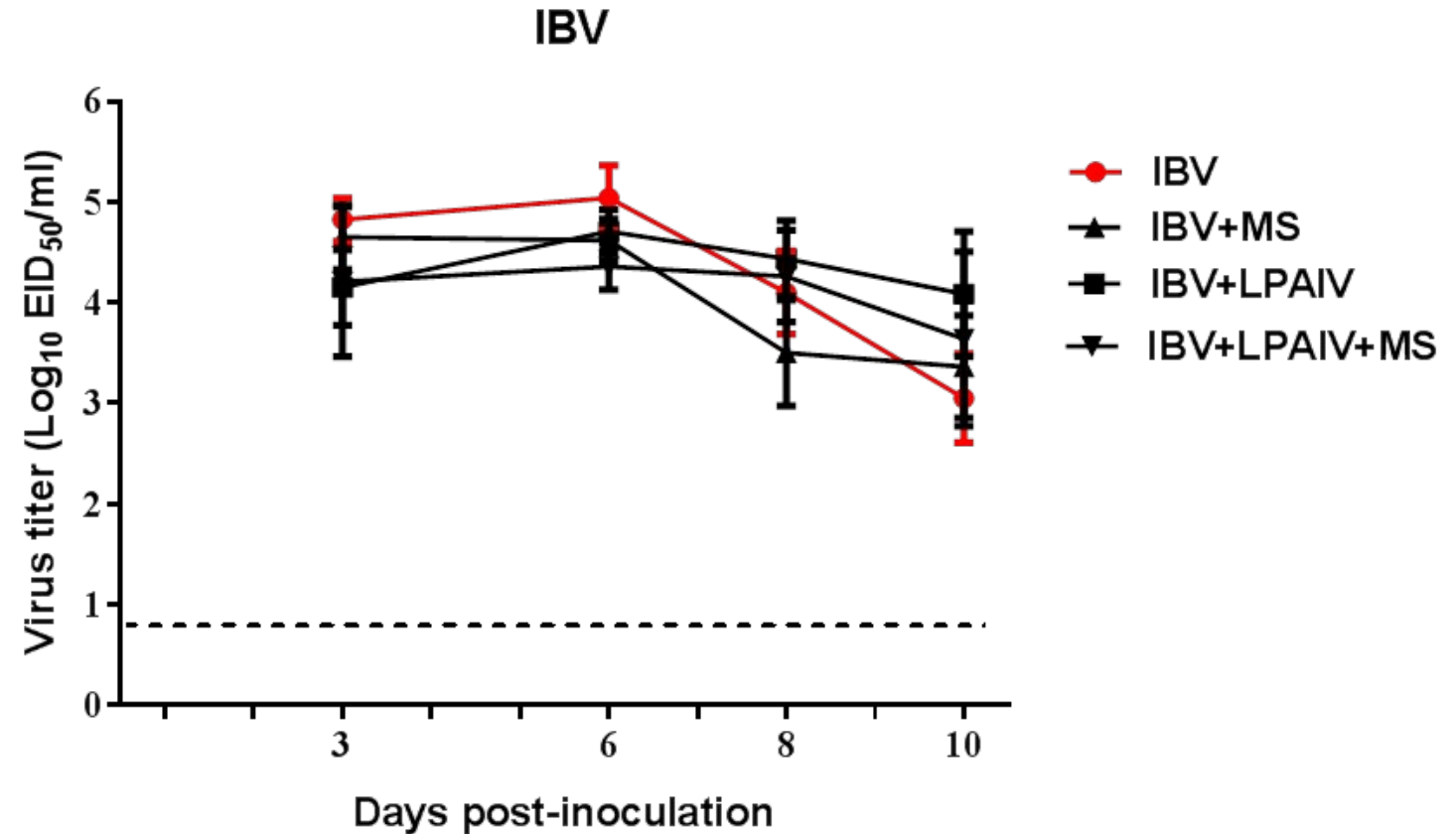
# LPAIV shedding



Significantly different at:

6 dpi: LPAIV+IBV (4.01) vs. **LPAIV+MS (5.28)** (\* $P=0.02$ )

# IBV shedding

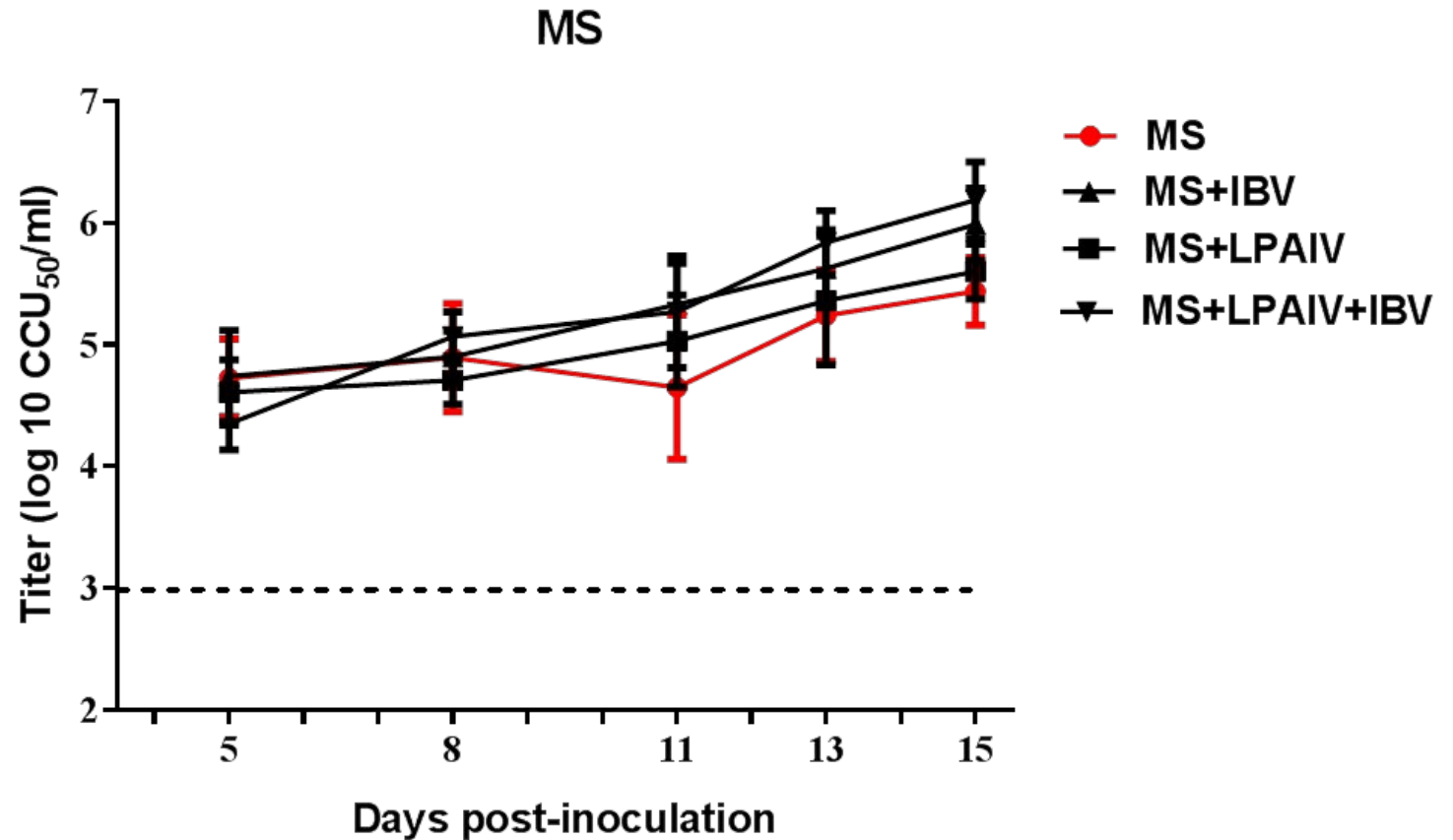


## Significantly different at:

- 3 dpi: **IBV (4.8)** vs. IBV+LPAIV (4.15) (\*\*P=0.004)  
**IBV (4.8)** vs. IBV+LPAIV+MS (4.14) (\*P=0.01)  
IBV+LPAIV (4.15) vs. **IBV+MS (4.65)** (\*P=0.04)
- 6 dpi: **IBV (5.04)** vs. IBV+LPAIV (4.71) (\*P=0.02)  
**IBV (5.04)** vs. IBV+MS (4.62) (\*\*P=0.003)  
**IBV (5.04)** vs. IBV+LPAIV+MS (4.36) (\*\*\*\*P<0.00001)

**IBV replication  
negatively affected  
by coinfection with  
LPAIV and MS**

# MS shedding



## Significantly different at:

11 dpi: MS (4.6) vs. MS+IBV (5.3) (\*\*P=0.009)

MS (4.6) vs. MS+LPAIV+IBV (5.27) (\*P=0.02)

13 dpi: MS (4.2) vs. MS+IBV (5.6) (\*P=0.02)

15 dpi: MS (5.4) vs. MS+IBV (5.9) (\*\*P=0.002)

MS (5.4) vs. MS+LPAIV+IBV (6.1) (\*\*\*\*P<0.0001)

MS+LPAIV (5.6) vs. MS+IBV (5.9) (\*P=0.04)

MS+LPAIV (5.6) vs. MS+LPAIV+IBV (6.1) (\*\*P<0.001)

**MS growth positively  
affected by coinfection  
with LPAIV and IBV**

# Summary

- Co-infection of chickens with H5N2 LPAIV and IBV Ark did not cause clinical signs more severe than the viruses alone. Tracheal microscopic lesions were not severe enough to result in gross lesions
- Co-infection of chickens with MS, IBV and/or LPAIV increases tracheal lesions which can be severe enough to result in tracheal plugs
- Use of commercial chickens and different strains of LPAIV, IBV and MS could give different results



- Will coinfection with IBV affect the infectivity and transmissibility of LPAIV in chickens?
  - Mean bird infectious dose ( $BID_{50}$ )
  - Transmission to direct contacts

AVIAN DISEASES 52:455–460, 2008

Using Mean Infectious Dose of High- and Low-Pathogenicity Avian Influenza Viruses  
Originating from Wild Duck and Poultry as One Measure of Infectivity and  
Adaptation to Poultry

David E. Swayne<sup>AC</sup> and Richard D. Slemons<sup>B</sup>

- For other poultry origin LPAIV's:
  - H7N9 Tennessee 2017 LPAIV  $BID_{50} = 5.6 \log_{10} EID_{50}$
  - H7N8 Indiana 2016 LPAIV  $BID_{50} = 4 \log_{10} EID_{50}$
  - No transmission to contacts

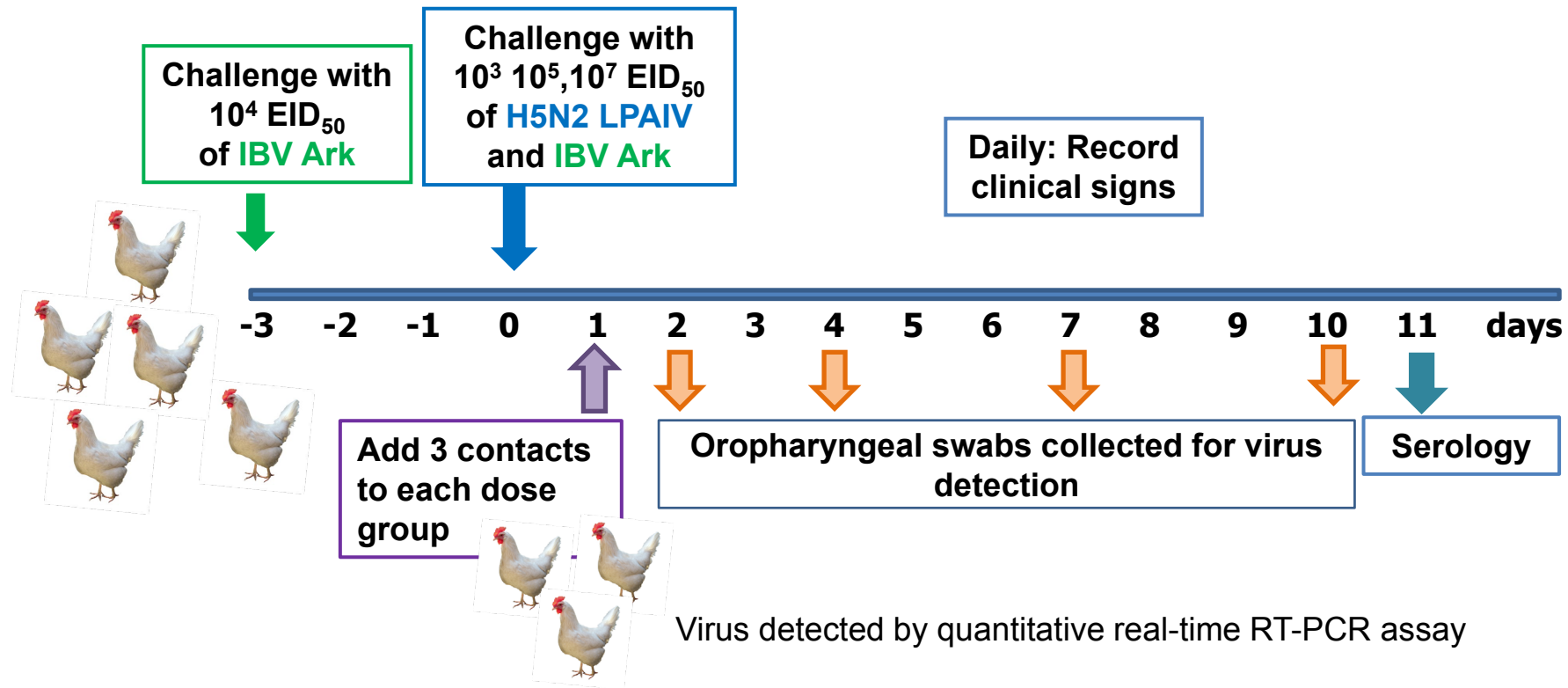
# Effect of IBV on the infectivity and transmissibility of H5N2 LPAIV in chickens



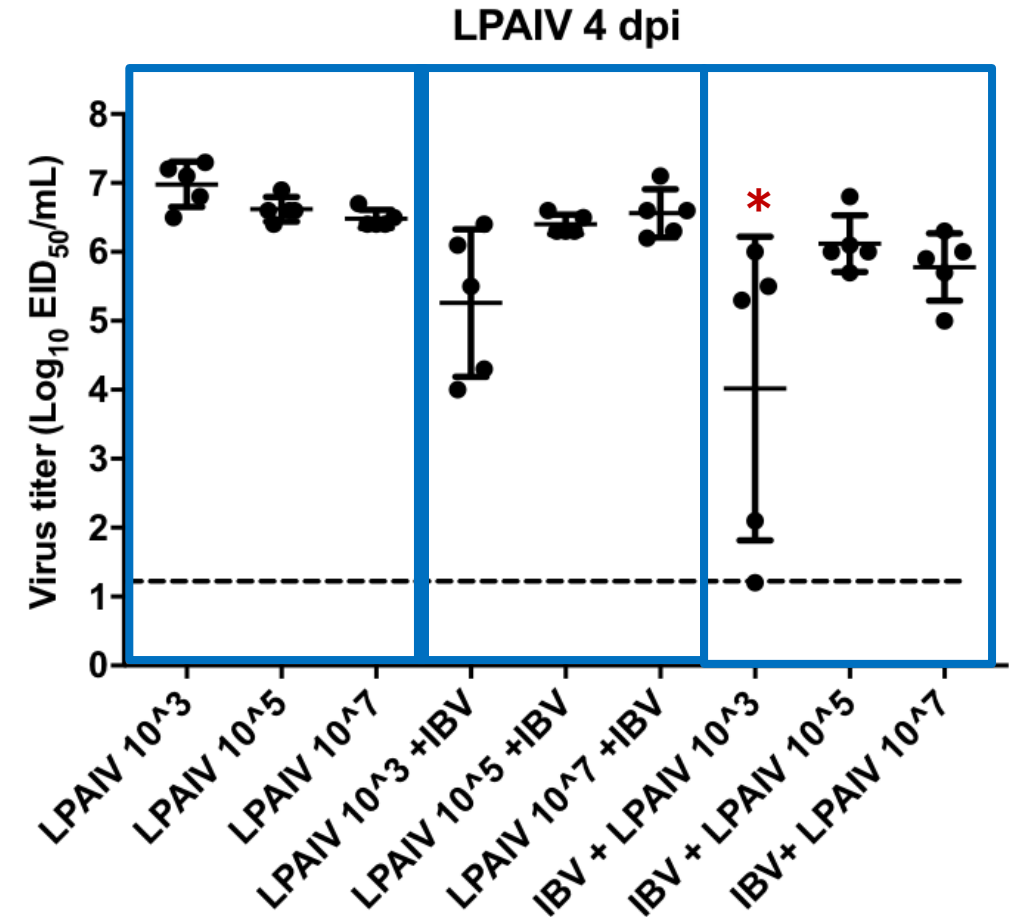
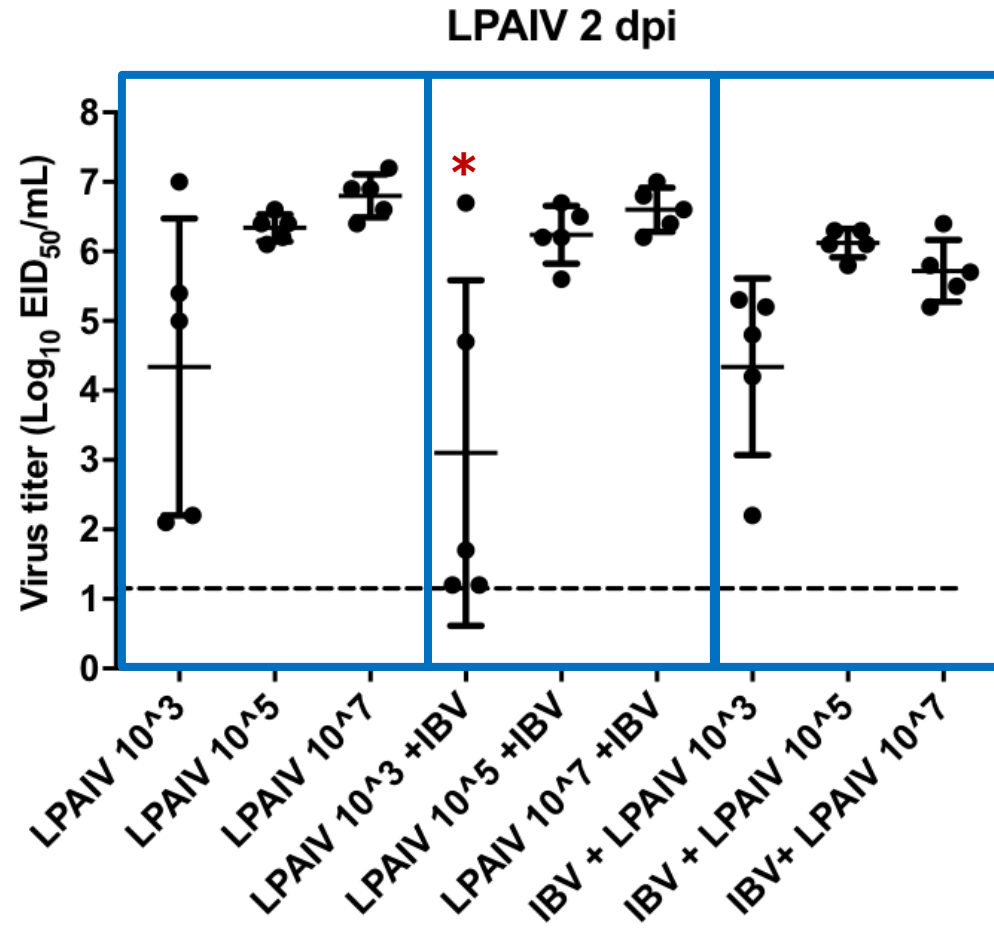
- 3 week old SPF Leghorn chickens
- 5 birds per group + 3 direct contacts
- Intraocular and intranasal inoculation with:
  - **IBV**: Ark99;  $10^4$  EID<sub>50</sub>/dose
  - **LPAIV**: A/chicken/Mexico/IA20/11/2011 H5N2;  $10^{3-7}$  EID<sub>50</sub>/dose

Groups	Day of inoculation	
	Day -3	Day 0
1	-	$10^3$ LPAIV
2	-	$10^5$ LPAIV
3	-	$10^7$ LPAIV
4	-	$10^3$ LPAIV + IBV
5	-	$10^5$ LPAIV + IBV
6	-	$10^7$ LPAIV + IBV
7	IBV	$10^3$ LPAIV
8	IBV	$10^5$ LPAIV
9	IBV	$10^7$ LPAIV
10	-	-

# Effect of IBV on the infectivity and transmissibility of H5N2 LPAIV in chickens



# LPAIV shedding

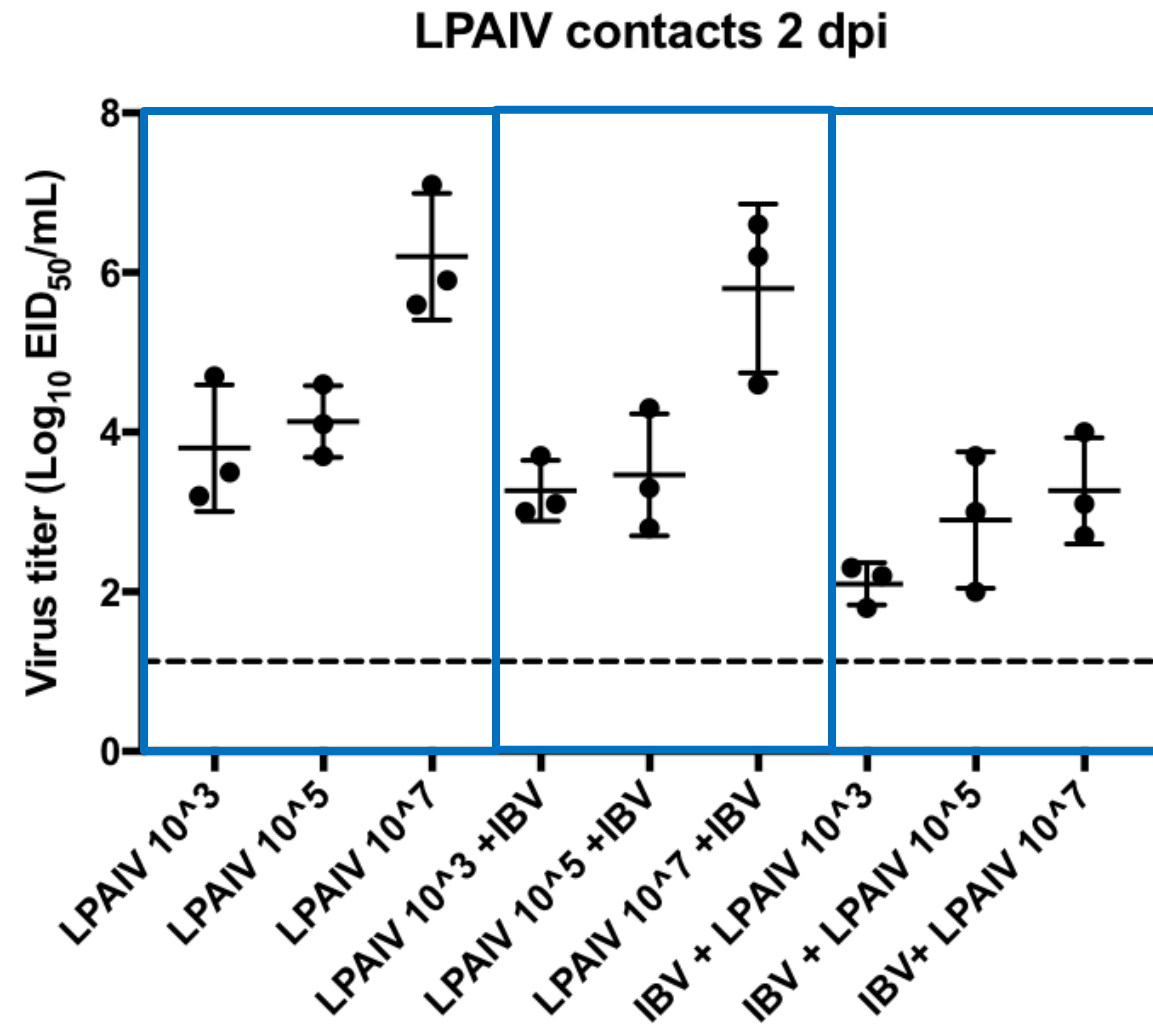


$$BID_{50} = < 3 \log_{10} EID_{50}$$

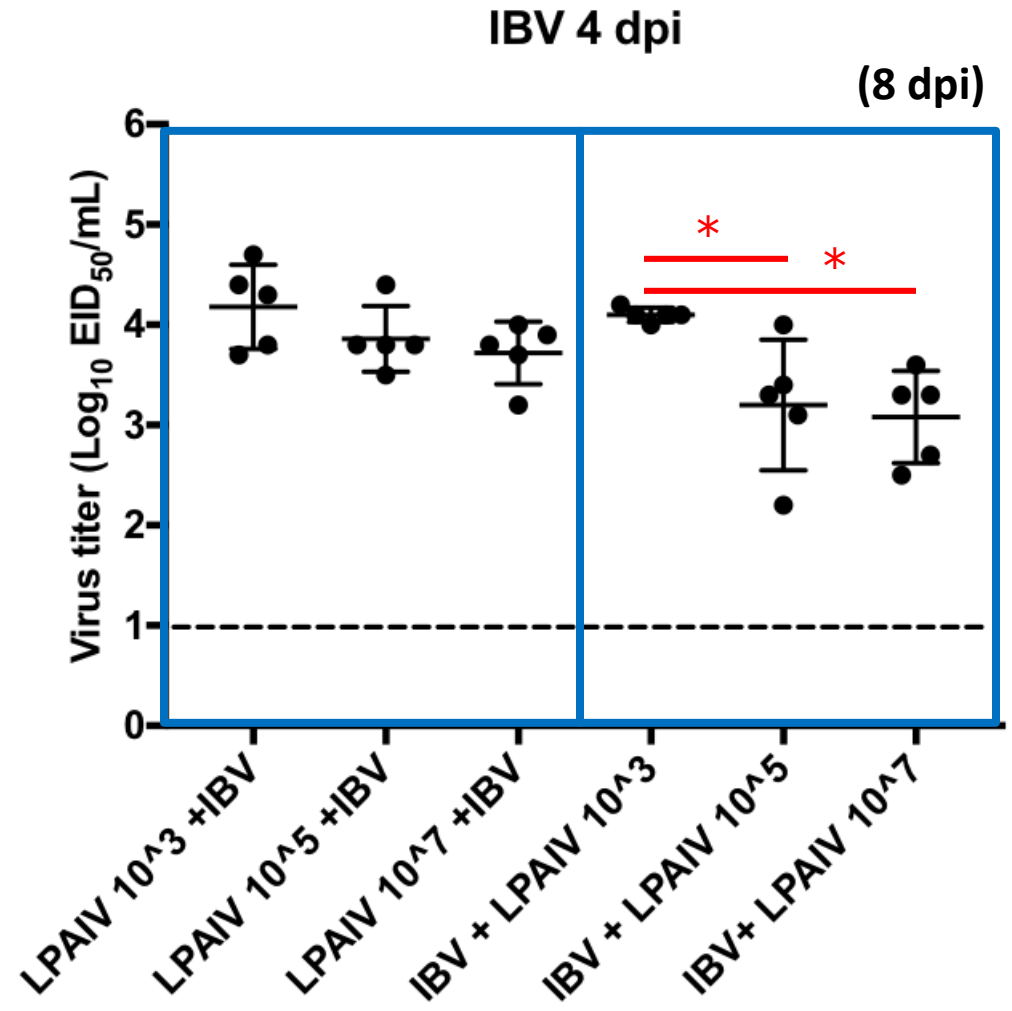
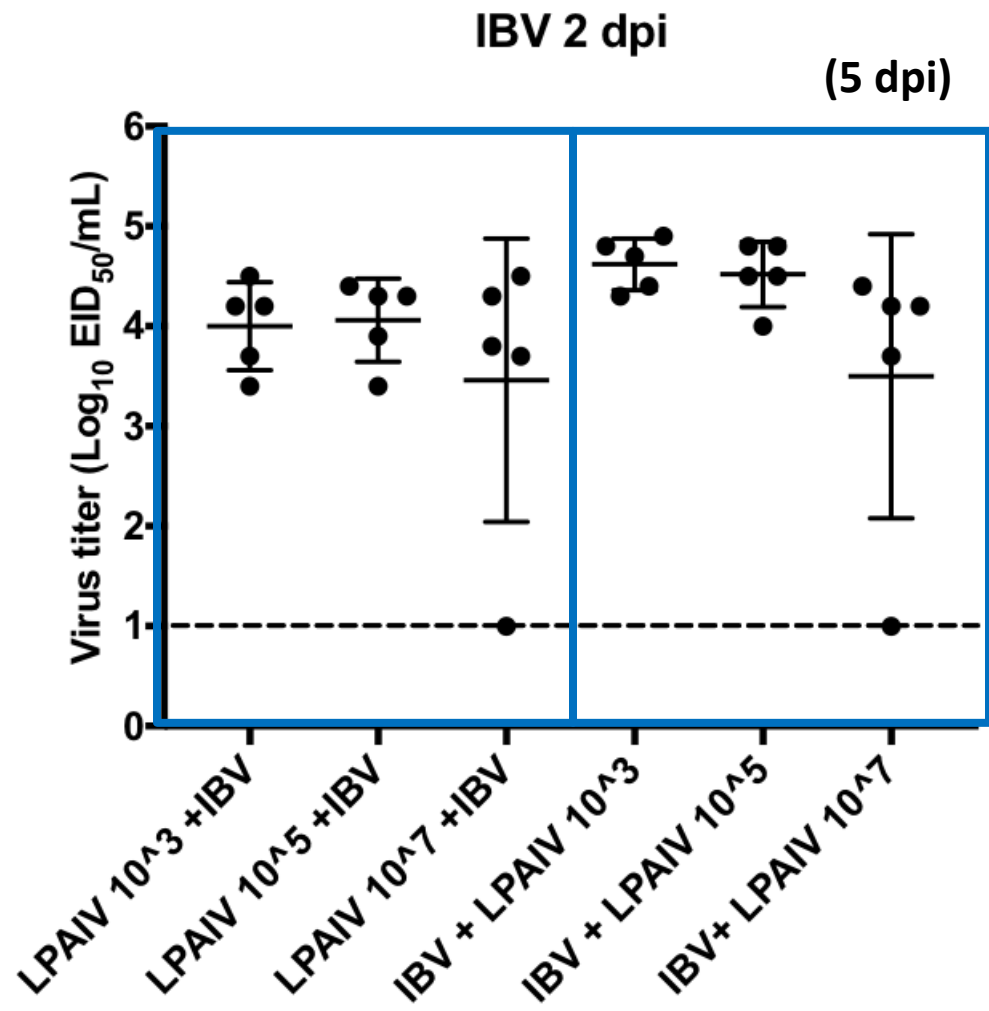
\* Significant by ANOVA

# LPAIV - Transmission to contacts

By 2 dpi all direct contact chickens were positive for H5N2 LPAIV



# IBV shedding and transmission



**Virus transmitted to all contacts; similar titers for all groups**

\* Significant by ANOVA

# Summary

- The Mexican lineage H5N2 LPAIV is very adapted to chickens.
- As seen in our previous studies, virus interference was observed between IBV and LPAIV, but coinfection with IBV did not affect negatively the infectivity and transmissibility of the LPAIV.
- **Next step:** Examine the effect of IBV infection on the infectivity and transmissibility of other less chicken adapted LPAIV viruses (H7N9, H7N8).



# Acknowledgements

We thank Nikolai Lee,  
Gerald Damron and Roger  
Brock for technical  
assistance.

