

Co-infection studies in chickens and turkeys with different respiratory pathogens

- Our projects address 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 studies in chickens and turkeys with different respiratory pathogens

- **Project 2.1.1.** Co-infection of broiler chickens with LPAIV, IBV and Mycoplasma to reproduce bronchial casts and mortality reported with LPAIV infections in the field. Inoculate SPF broilers with LPAIV and IBV and/or Mycoplasma. Initially we will use an H5N2 LPAI Mexican isolate, an IBV vaccine strain and a *M. synoviae* (MS) isolate. Later, other strains will be examined, and similar experiments will be conducted in commercial broilers to account for other variables.
- **Project 2.1.2.** Co-infection of turkeys with respiratory pathogens including LPAIV, NDV, and Mycoplasma. Inoculate SPF turkeys of different ages with LPAIV, NDV and Mycoplasma in different combinations. Several subtypes of LPAI viruses can infect turkeys, including strains of swine origin, causing diverse clinical signs. Initially we will use a poultry origin LPAI isolate, a NDV vaccine strain and a *M. meleagridis* (MM) isolate. Later, other strains will also be examined,
- **Year 1: Co-infection of SPF and commercial broiler chickens with H5N2 LPAIV, IBV (vaccine strain) and MS.**

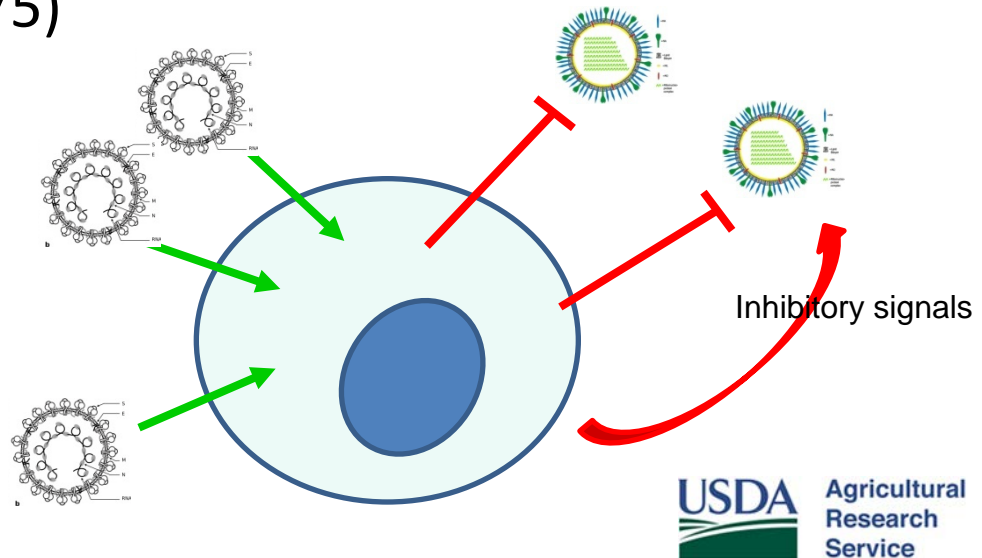
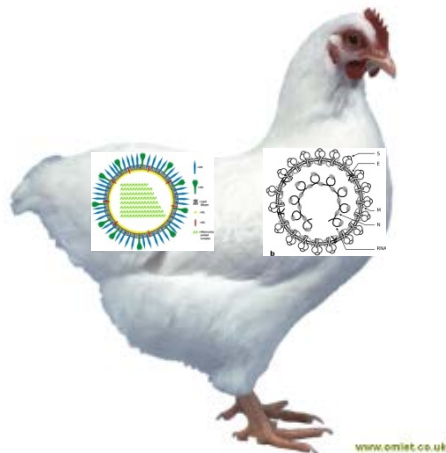
Co-infection of poultry with respiratory viruses

Possible outcomes:

- Replication of co-infecting viruses is not affected: similar clinical outcomes
- Replication of one virus is affected by the other: increase or decrease in viral replication and clinical signs

Viral Interference:

- Infection of a host with one virus may affect infection by a second virus (Dianzani, 1975)

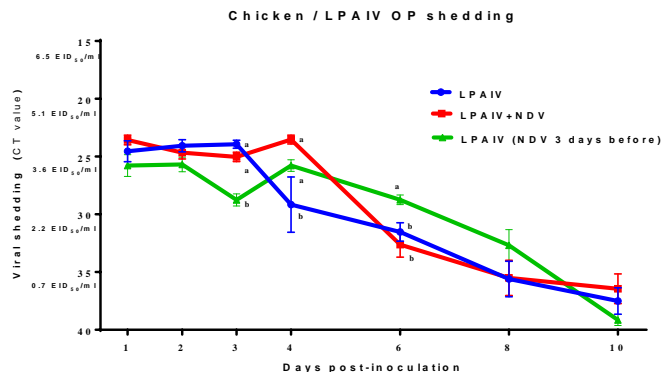


Co-infection of poultry with respiratory viruses

Gelb, *et al.*, 2007. No replication interference between infectious bronchitis virus (IBV) and NDV vaccine strains when combined; however IBV-Ark affected the immune response to NDV-C2

Vagnozzi *et al.*, 2010. NDV and IBV vaccines affected tracheal replication of a TCO infectious laryngotracheitis virus vaccine. The CEO vaccine was not affected; in fact it interfered with the NDV and IBV vaccines

Costa-Hurtado *et al.*, 2014. Co-infection of chickens and turkeys with LPAIV and La Sota NDV affected viral replication patterns but had no effect on clinical signs



Costa-Hurtado et al. Veterinary Research 2014, 45:1
<http://www.veterinaryresearch.org/content/45/1/1>



RESEARCH

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Virus interference between H7N2 low pathogenic avian influenza virus and lentogenic Newcastle disease virus in experimental co-infections in chickens and turkeys

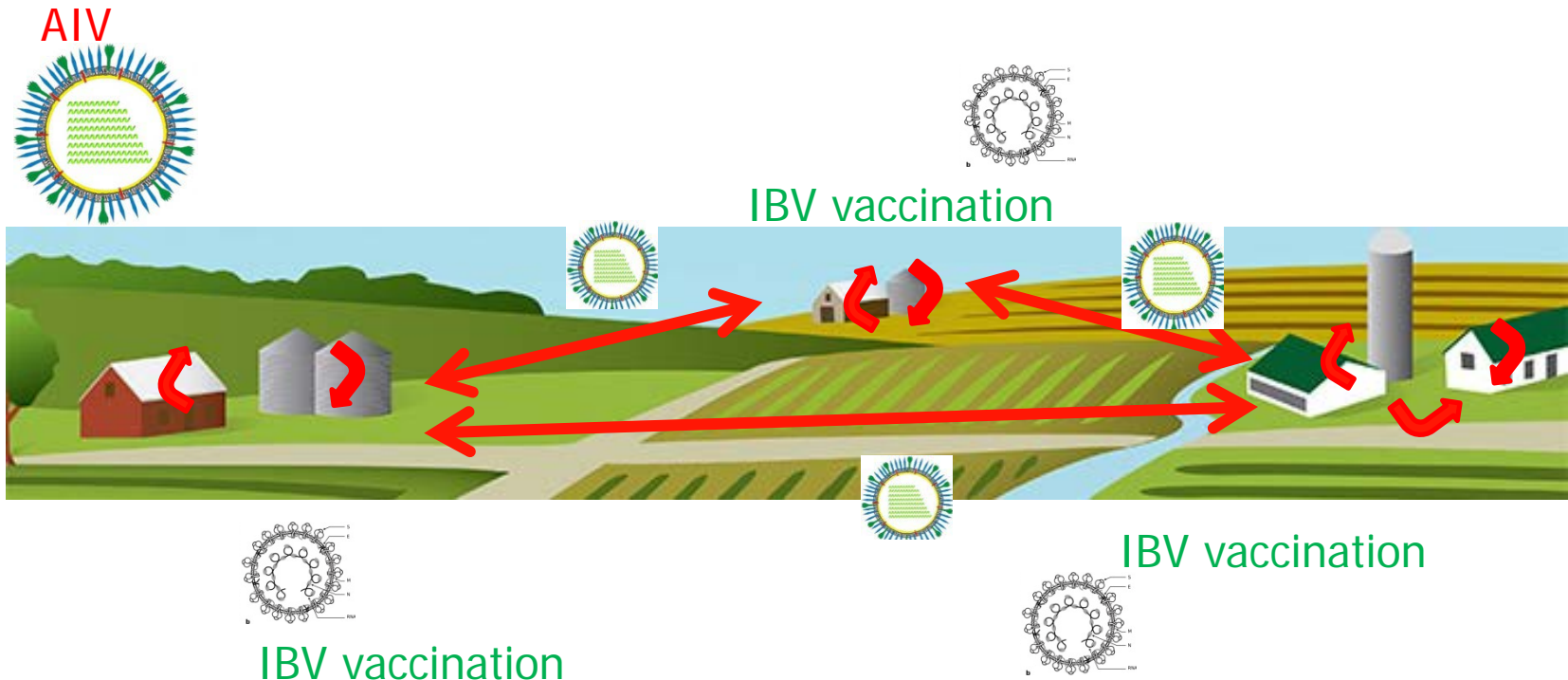
Mar Costa-Hurtado, Claudio L Afonso, Patti J Miller, Erica Spackman, Darrell R Kapczynski, David E Swayne, Eric Shepherd, Diane Smith, Aniko Zsak and Mary Pantin-Jackwood

Co-infection of poultry with LPAIV and IBV

- Haghighat *et al.*, 2008. Broilers co-infected with H9N2 **LPAIV** and **IBV** live vaccine (H120) showed increased clinical signs when compared to AIV-infected controls
- Karimi-Madab *et al.*, 2010. **IBV** live vaccine enhanced the virulence of H9N2 **LPAIV** in field conditions
- Chi-Sheng Chang *et al.*, 2011. Interference of H6N1 **LPAIV** infection with **IBV** vaccination

Objective

Study the effect of IBV infection on the replication of different LPAIV's in chickens



Co-infection of chickens with IBV and LPAIV's

- 4 week old SPF Leghorn chickens
- Intraocular and intranasal inoculation with:
IBV: commercial Mass vaccine
LPAIV: H9N2, H7N9, H5N2; dose 10^7 EID₅₀/ml



Single

Simultaneous

Sequential

Groups	Day of inoculation	
	Day 0	Day 3
1 (control)		
2	IBV	-
3	H9N2	-
4	H7N9	-
5	H5N2	-
6	IBV + H9N2	-
7	IBV + H7N9	-
8	IBV + H5N2	-
9	IBV	H9N2
10	IBV	H7N9
11	IBV	H5N2

LPAIV viruses used

H5N2 LPAIV (A/chicken/Mexico-Coahuila/IA20/11/2011)

- Reported in Mexico since the early 1990s. The HPAI form eradicated
- H5N2 LPAIV remains endemic and is controlled by vaccination
- High morbidity and moderate mortality associated with H5N2 infections

H9N2 LPAIV (A/chicken/Egypt/12/2013)

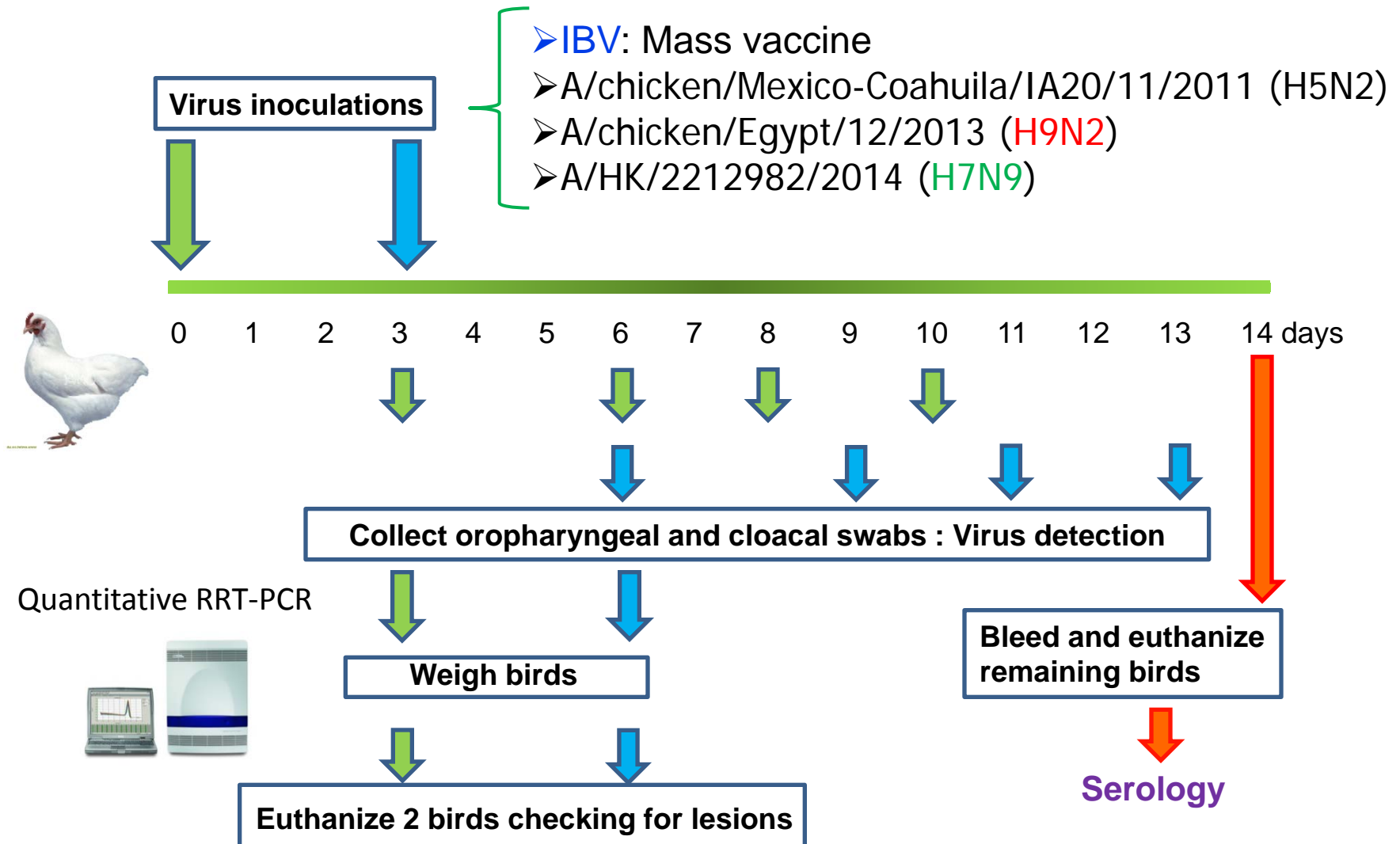
- Endemic in the Middle East and Asia and some countries in Africa
- Subclinical infections in experimental studies, but mortalities up to 60% reported in the field. Also controlled by vaccination

Bronchial cast formation a common finding in H5N2 and H9N2 infected flocks

H7N9 LPAIV (A/HK/2212982/2014)

- Zoonotic, reported in humans since 2012
- Poultry source of infection for humans (live bird markets)
- Birds show no clinical signs of infection

Co-infection of chickens with IBV and LPAIV's

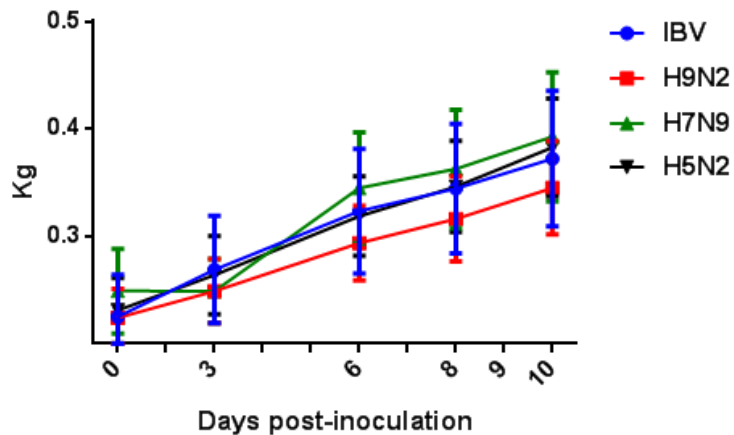


Results

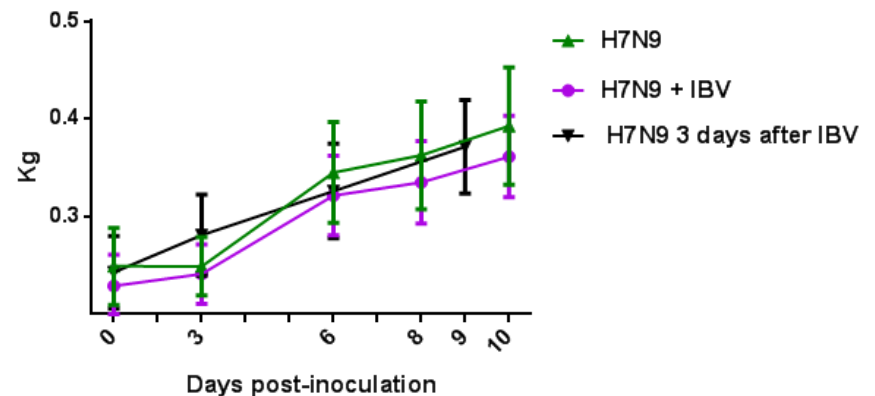
No clinical signs

No statistically significant differences in weights at 3 and 6 dpi

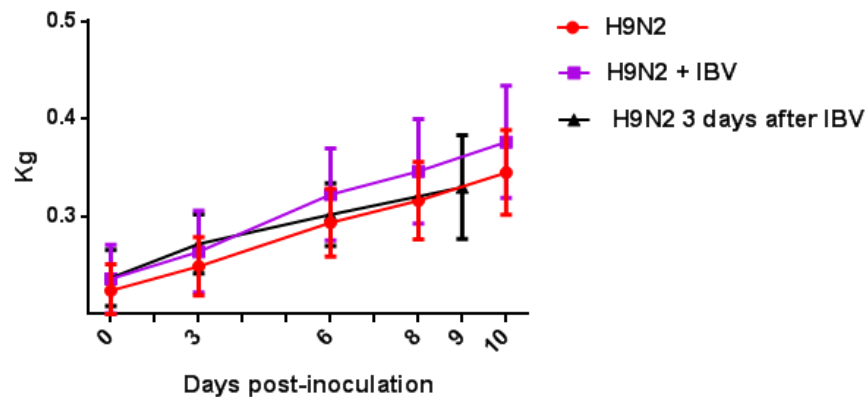
Weights single infected



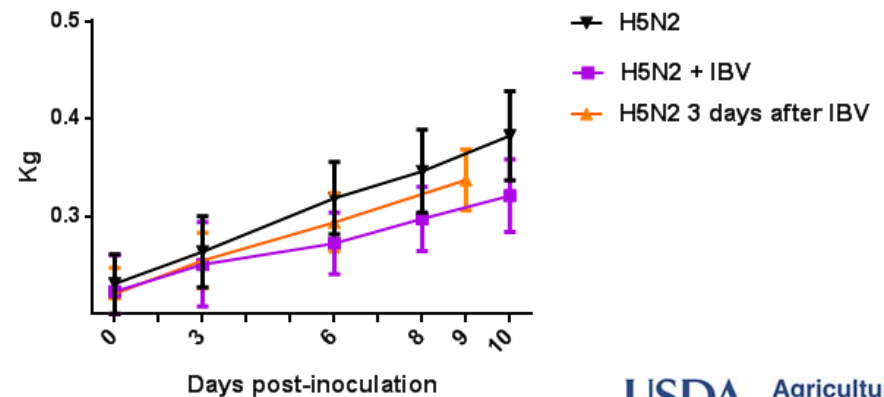
H7N9



H9N2



H5N2

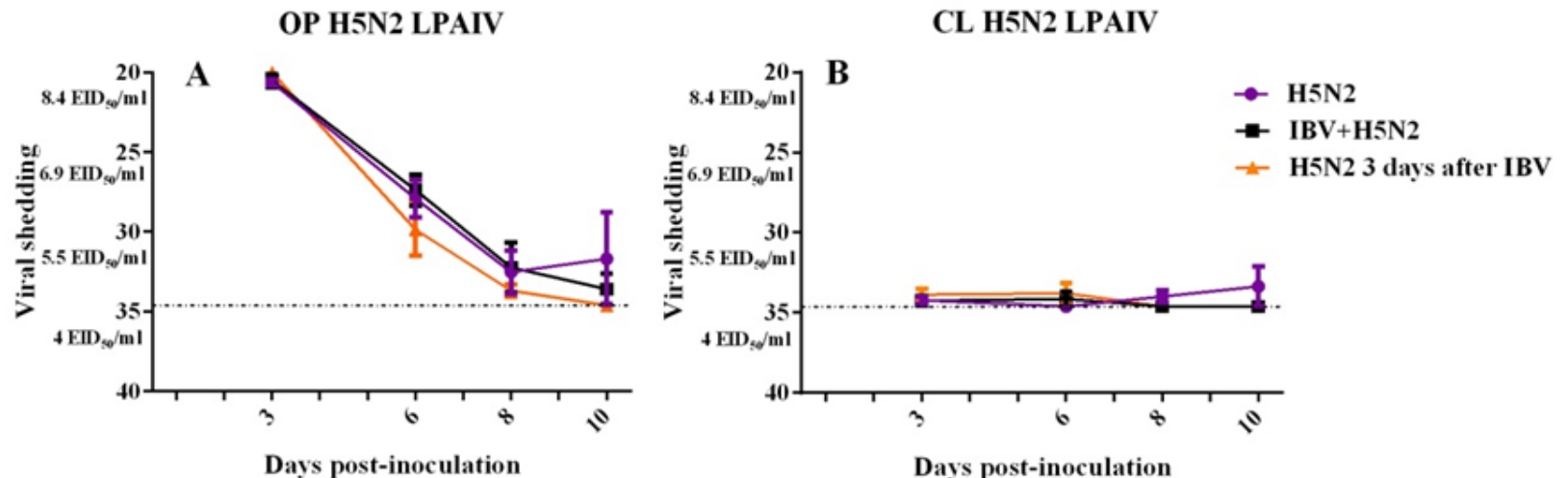


One-way ANOVA with Bonferroni multiple comparison analysis was used to evaluate virus titers in swabs

H5N2 LPAIV

No effect on **H5N2** LPAIV shedding was observed in co-infected birds when compared to single infected birds, this virus being shed in high titers from all inoculated birds. Serology similar among groups

Virus shedding

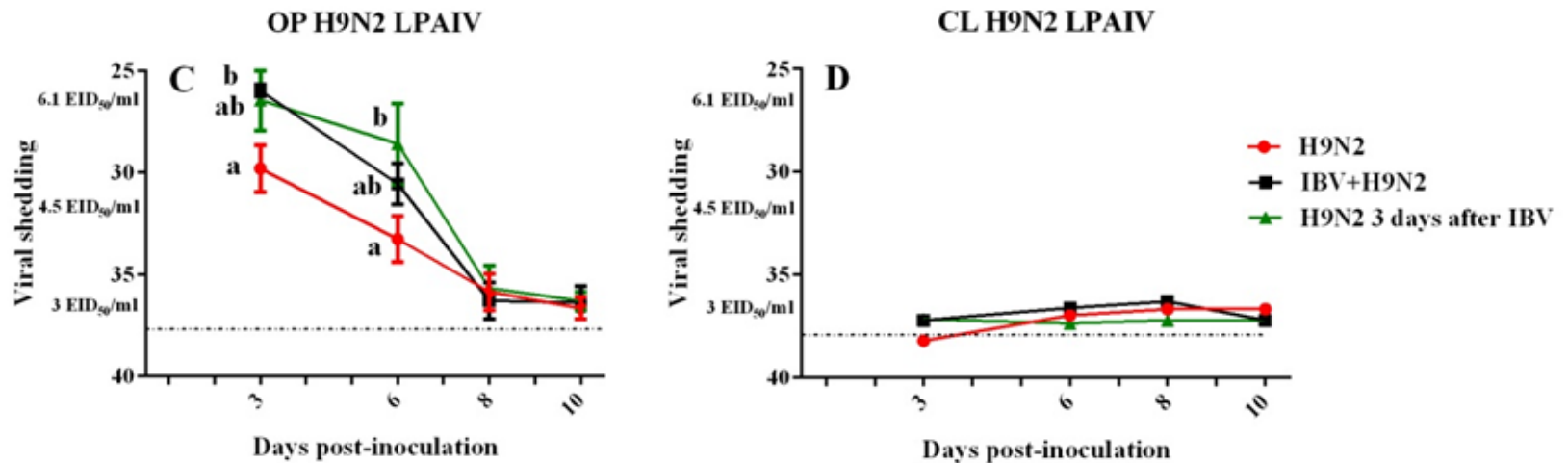


- **H5N2** LPAIV is well adapted to chickens since has been circulating in poultry since the early 1990s.
- The presence of another virus does not affect its replication

H9N2 LPAIV

Chickens co-infected with IBV shed higher titers of the **H9N2** LPAIV at 3 and 6 dpi. No differences in serology

Virus shedding



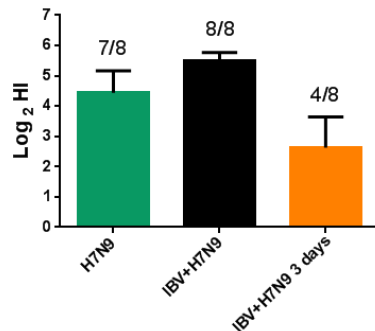
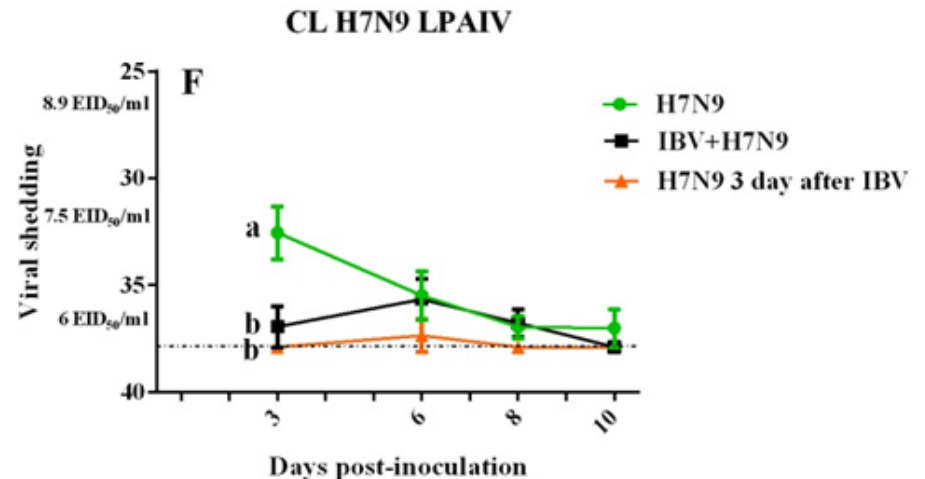
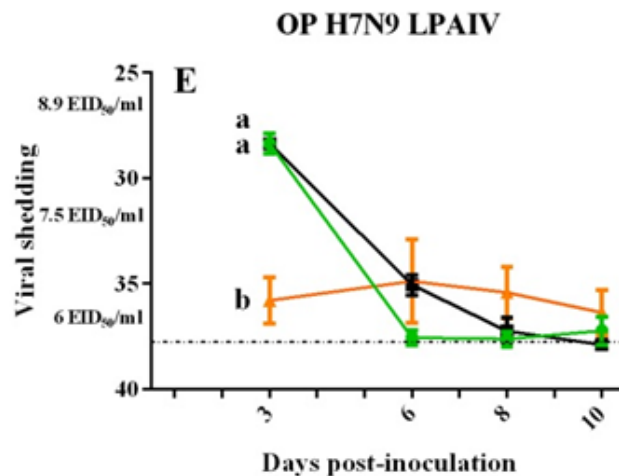
One-way ANOVA with Bonferroni multiple comparison analysis was used to evaluate virus titers in swabs

- Haghighat *et al.* Broilers co-infected with H9N2 **LPAIV** and **IBV** live vaccine (H120) showed increased clinical signs when compared to AIV-infected controls.
- Karimi-Madab *et al.* **IBV** live vaccine enhanced the virulence of H9N2 **LPAIV** in field conditions.

H7N9 LPAIV

Lower titers of the H7N9 LPAIV were shed by birds previously infected with IBV and serology titers were also lower in this group

Virus shedding

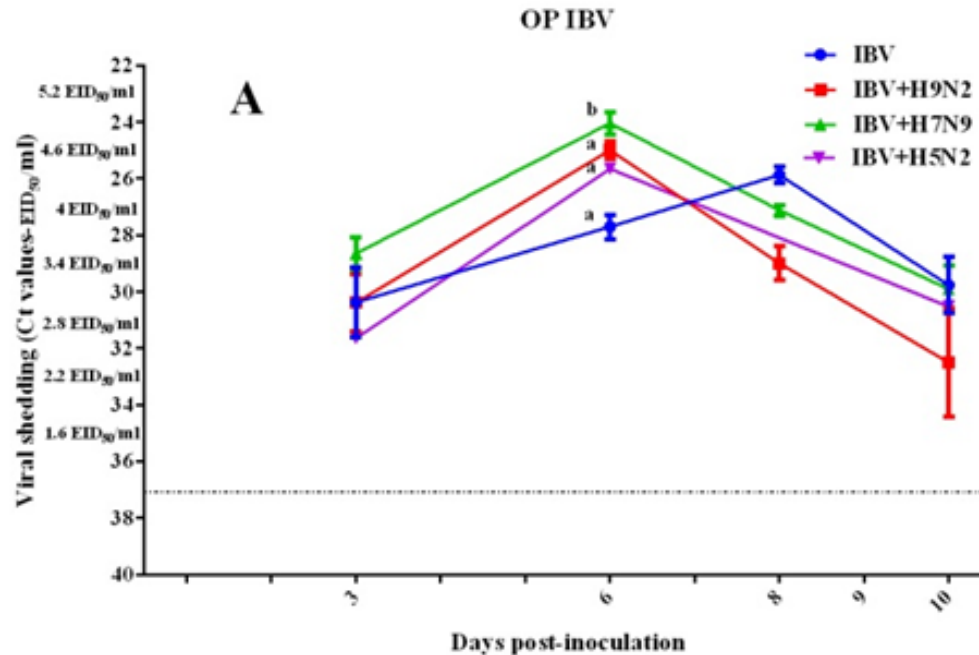


One-way ANOVA with Bonferroni multiple comparison analysis was used to evaluate virus titers in swabs
Fisher's exact test (two-tailed) for +/- virus shed compared to single-infected

- H7N9 is not well adapted to poultry
- More easily affected by the presence of another virus

IBV

Virus shedding in simultaneously co-infected birds

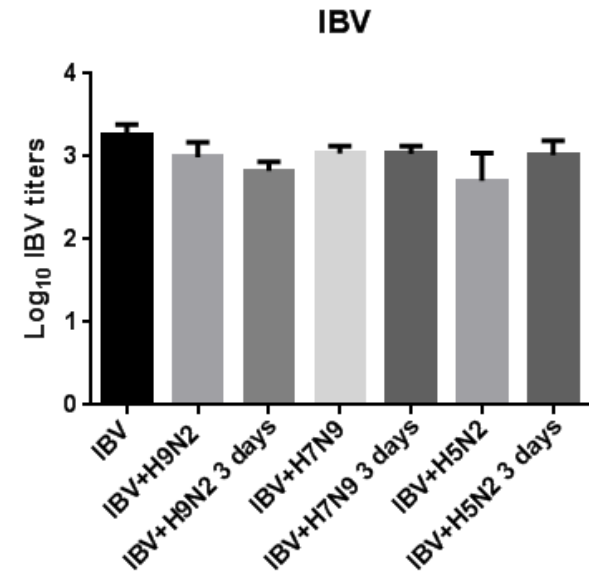
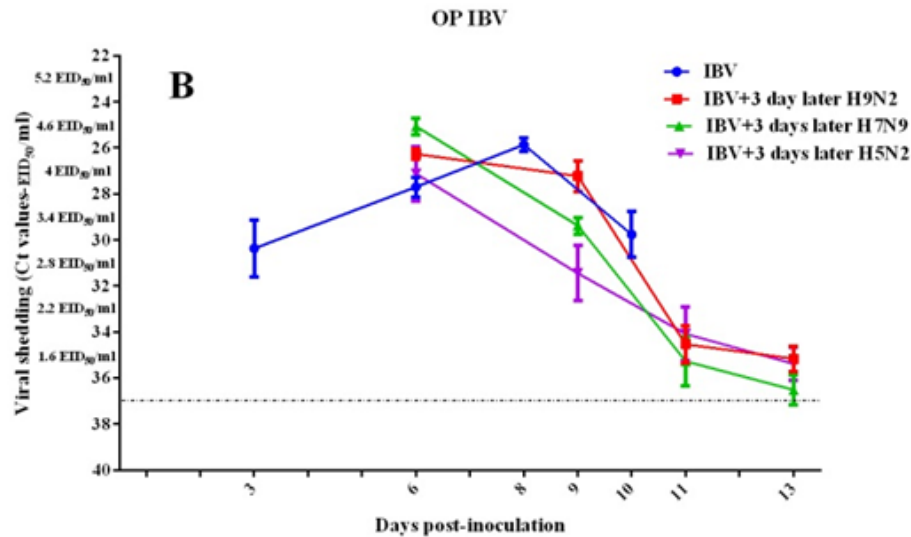


- The peak of viral replication occurred earlier in simultaneously co-infected groups
- Significant difference in IBV titers at 3 dpi in group co-infected with H7N9

One-way ANOVA with Bonferroni multiple comparison analysis was used to evaluate virus titers in swabs

IBV

Virus shedding in sequentially co-infected birds



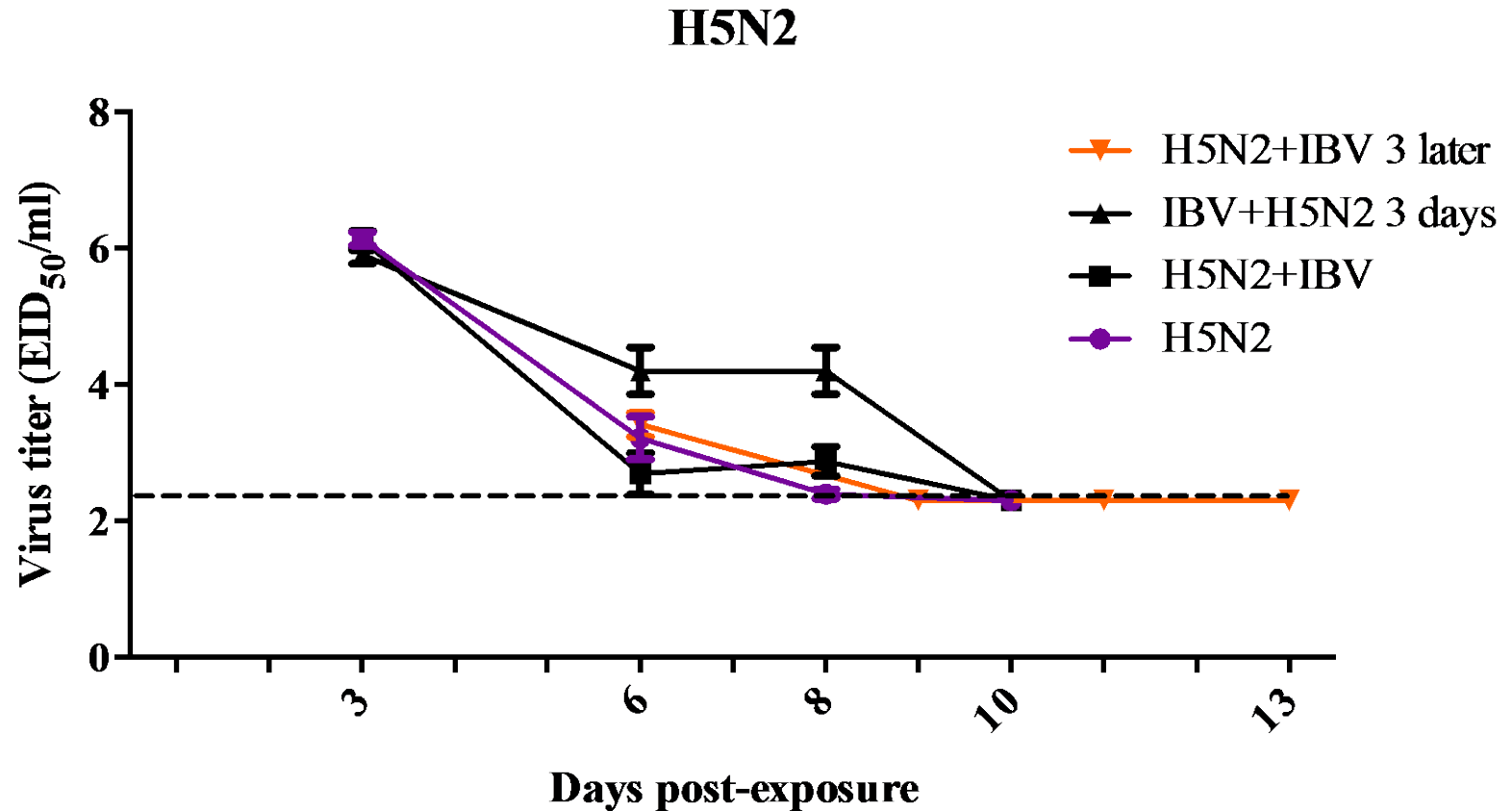
Co-infection of chickens with IBV and LPAIV's

- 3 week old SPF Leghorn chickens
- Intraocular and intranasal inoculation with:
IBV: Ark dpi; dose 10^4 EID₅₀/ml
LPAIV: H9N2, H5N2; dose 10^7 EID₅₀/ml

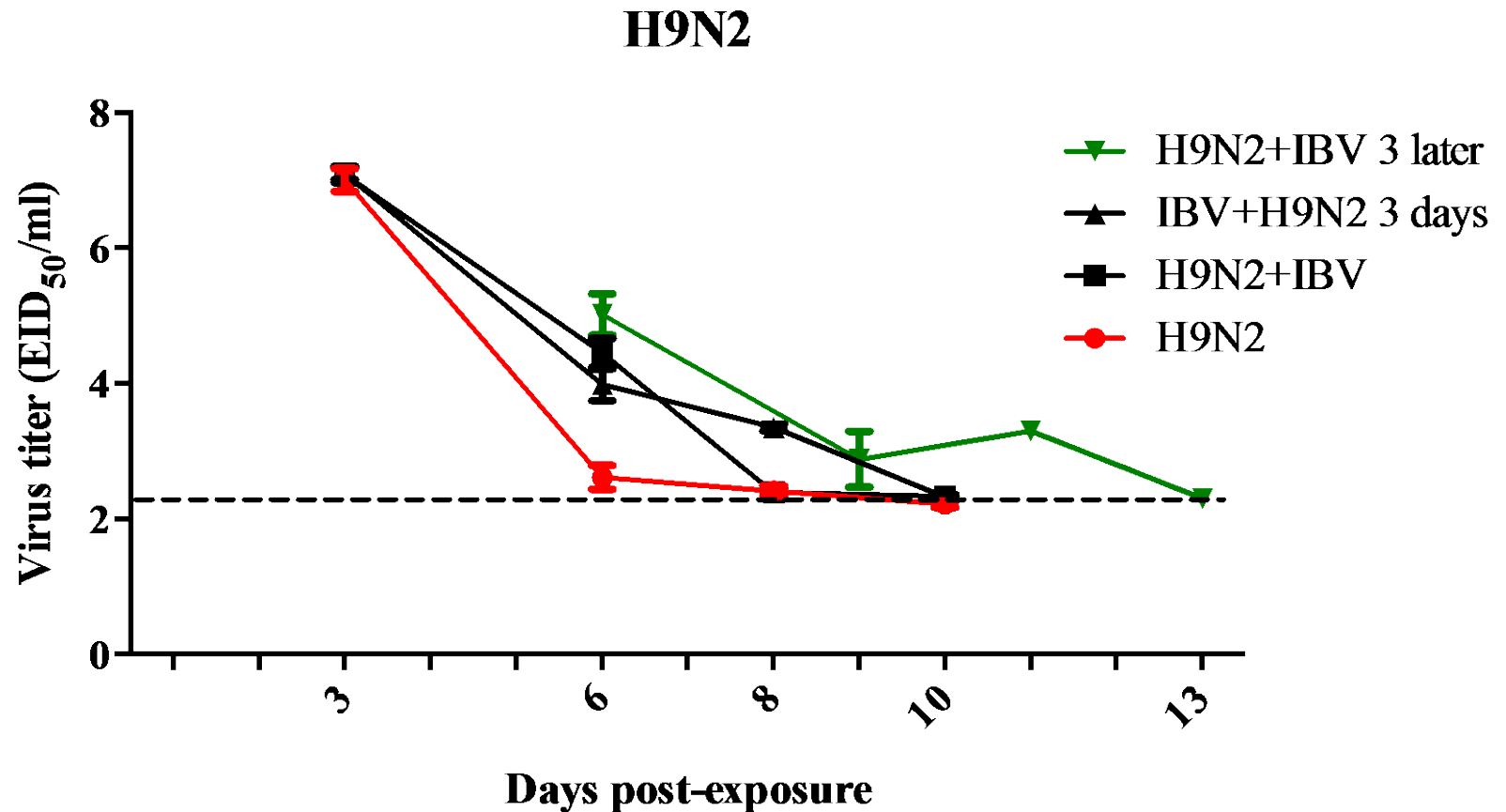


	Groups	Day of inoculation	
		Day 0	Day 3
Single	1 (controls)	-	-
	2	IBV	-
	3	H5N2	-
	4	H9N2	-
Simultaneous	5	IBV + H9N2	-
	6	IBV + H5N2	-
Sequential	7	IBV	H5N2
	8	IBV	H9N2
	9	H5N2	IBV
	10	H5N9	IBV

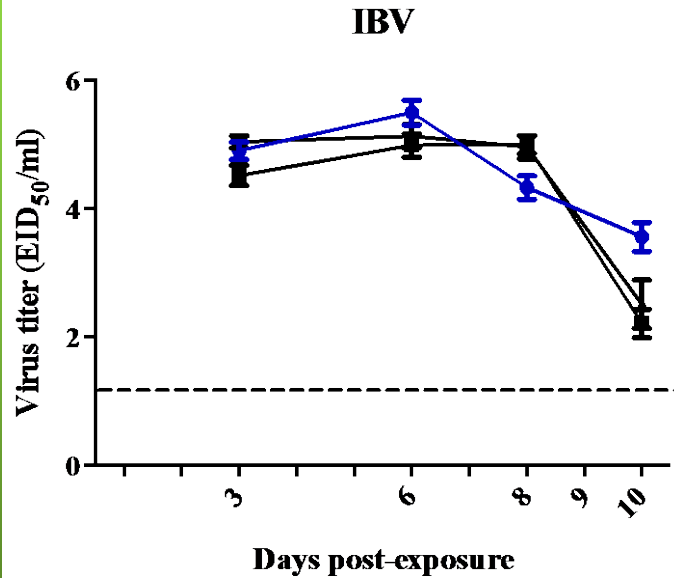
H5N2 virus shedding



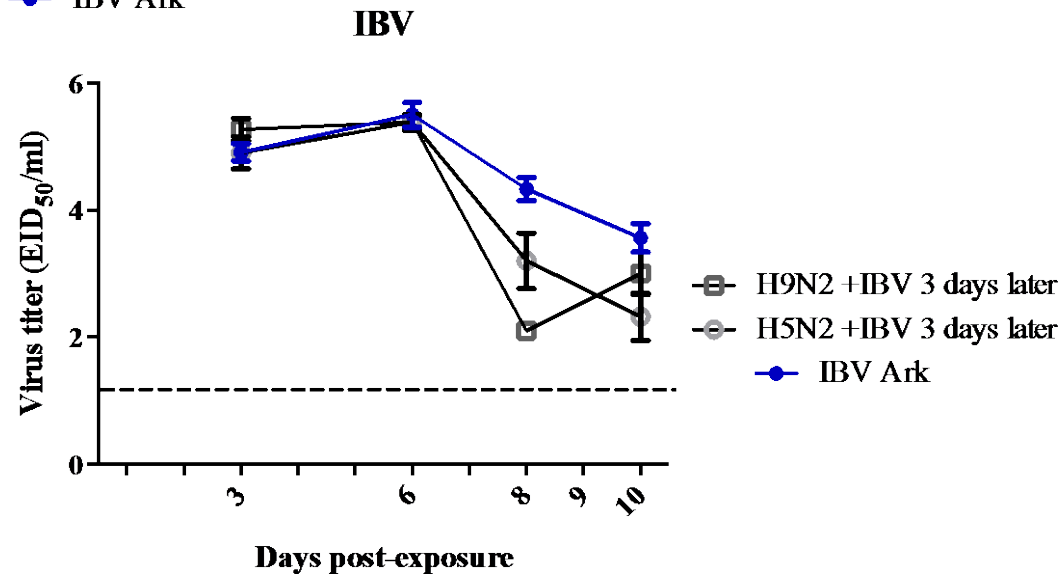
H9N2 virus shedding



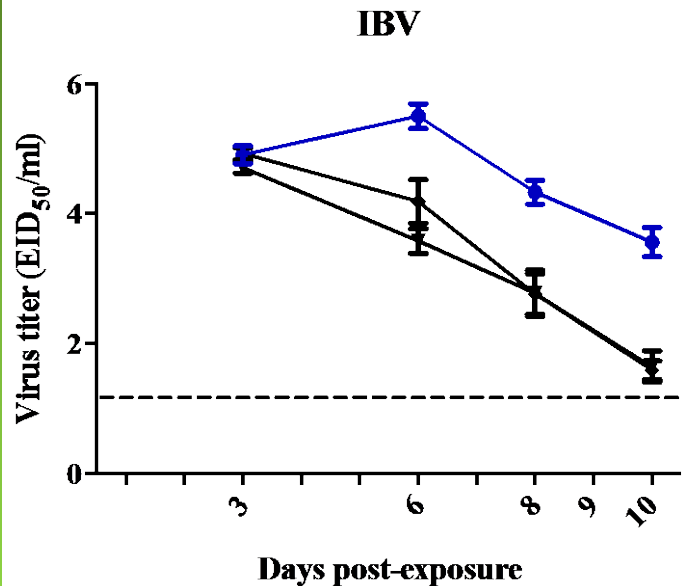
IBV virus shedding



- IBV+H9N2
- IBV+H5N2
- IBV Ark



- H9N2 +IBV 3 days later
- H5N2 +IBV 3 days later
- IBV Ark



- IBV+H9N2 3 days later
- IBV+H5N2 3 days later
- IBV Ark

Summary

- In conclusion, the effect of co-infection in chickens with IBV and LPAIV varies depending on the virus strains and the timing of co-infection, with exacerbation, reduction, or no effect on virus shedding

