



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

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- **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.
 - 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.
 - Project 2.1.2.** Co-infection of turkeys with respiratory pathogens including LPAIV, NDV, and Mycoplasma.

Co-infection of poultry with respiratory pathogens

- Haghghat *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
- 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
- Sid *et al.*, 2015. Co-infections with Mycoplasma (MG, MS), AIV, IBV, and aMPV found in flocks with high mortality in Algeria
- Hassan, *et al.*, 2016. Mixed viral infections, especially with **IBV** and H9N2 **LPAIV** viruses, are the predominant etiology of respiratory disease problems in broiler chickens in Egypt (47% flocks). Increased mortality observed in these flocks

Co-infection of chickens with IBV and LPAIV

- 4 week old SPF Leghorn chickens
- Intraocular and intranasal inoculation with:
 IBV: commercial Mass vaccine $10^{3.5}$ EID₅₀/dose
 LPAIV: **H9N2**, H5N2; 10^7 EID₅₀/dose



Single
 Simultaneous
 Sequential

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

LPAIV viruses

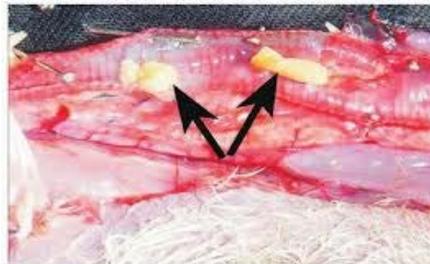
H5N2 LPAIV (A/chicken/Mexico-Coahuila/IA20/11/2011)

- Reported in Mexico since the early 1990s
- 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)

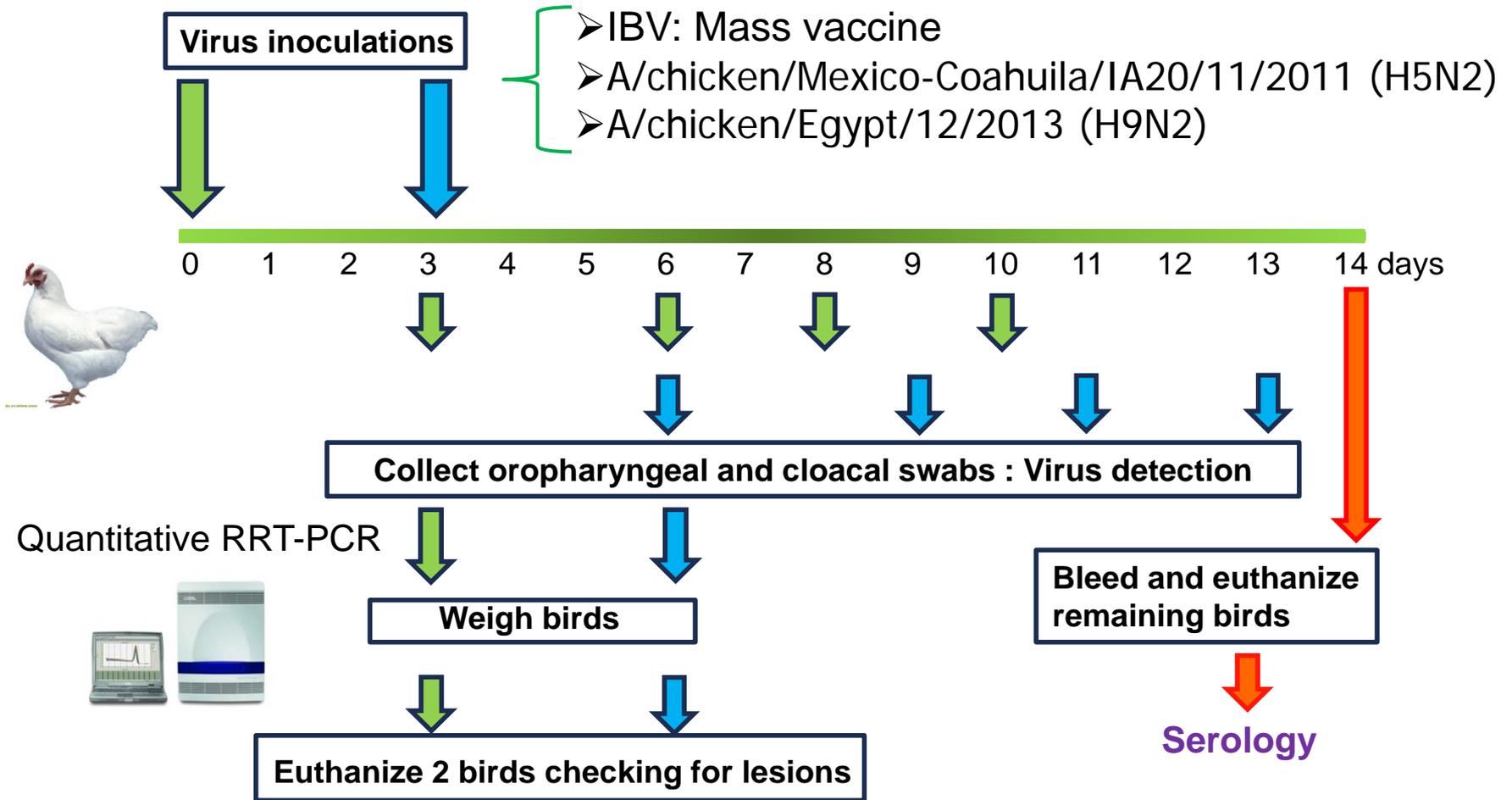
- Endemic in the Middle East and Asia and some countries in Africa
- Subclinical infections in experimental studies, but mortalities up to 60% reported in the field. Also controlled by vaccination

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



Varsa Vahini Dr. K.S.Prajapati

Co-infection of chickens with IBV and LPAIV's

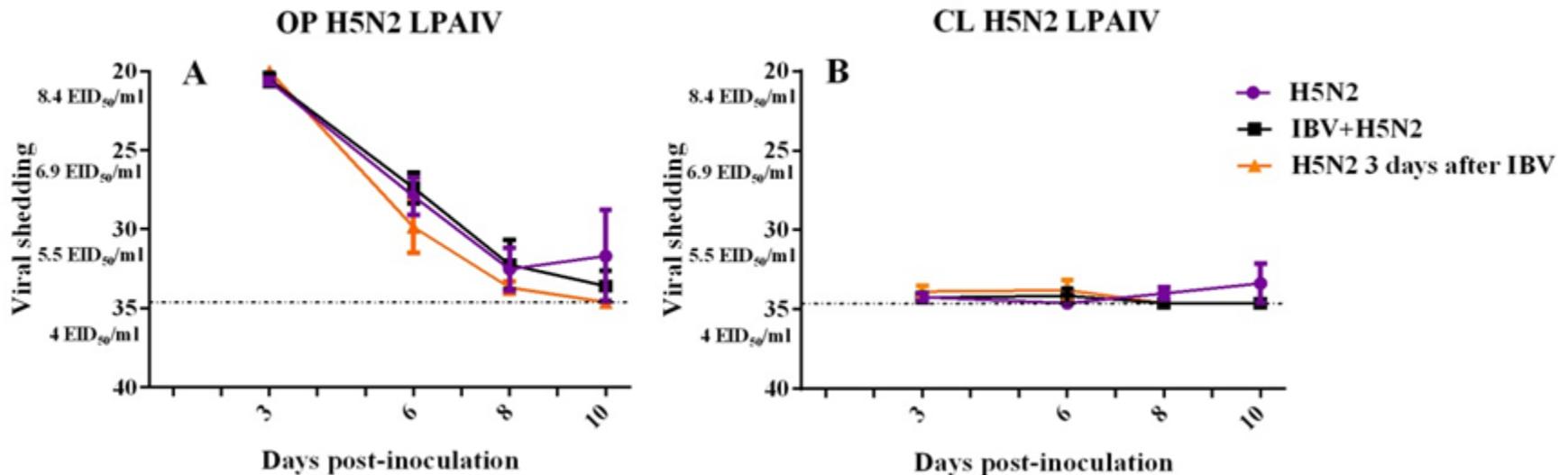


No clinical signs and no significant differences in weights at 3 and 6 dpi

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.

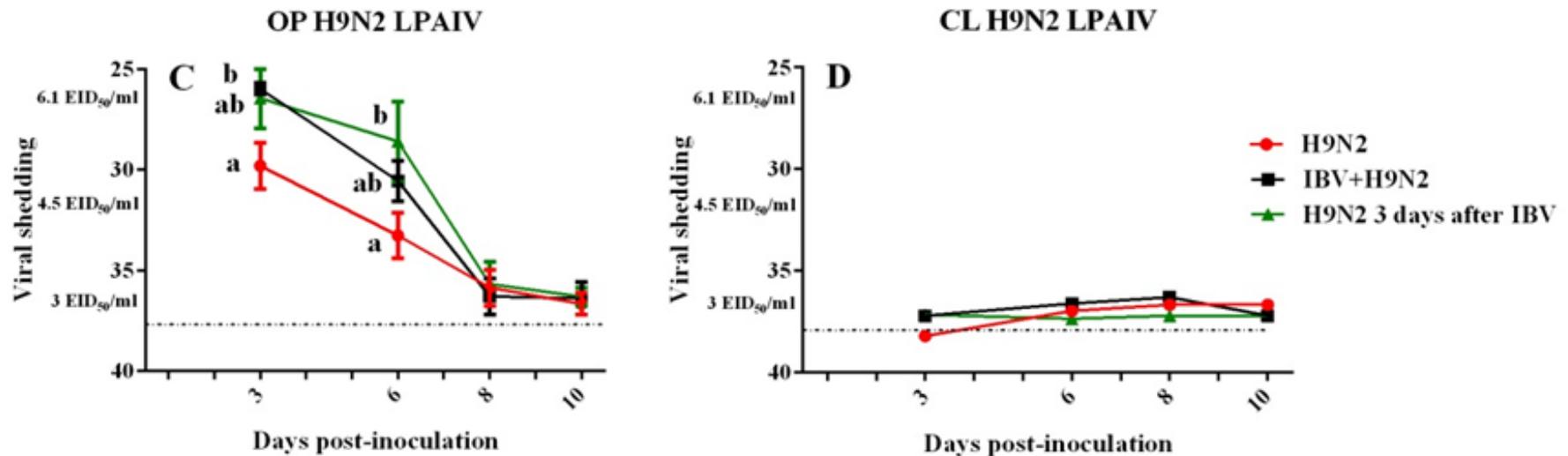
Virus shedding



H9N2 LPAIV

Chickens co-infected with IBV shed higher titers of the H9N2 LPAIV at 3 and 6 dpi.

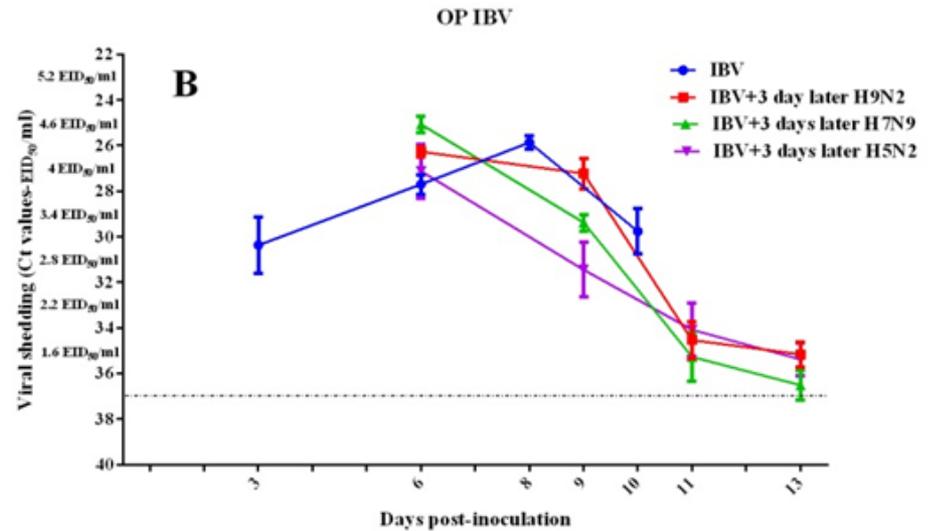
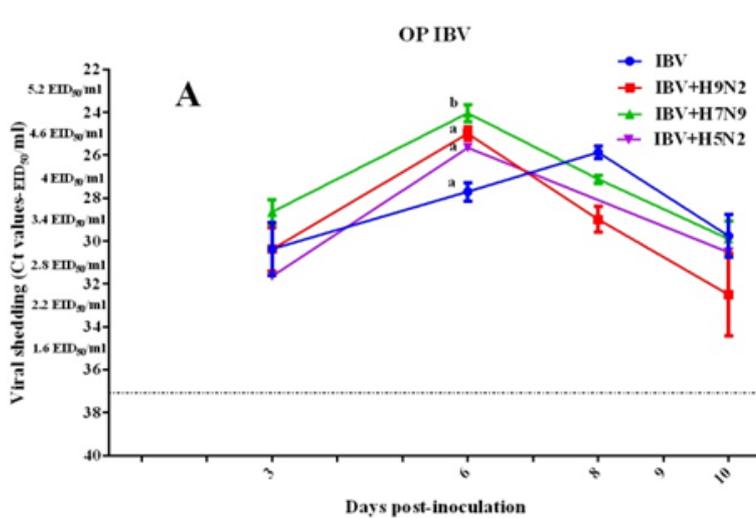
Virus shedding



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

IBV

Virus shedding



- The peak of viral replication occurred earlier in simultaneously co-infected groups

Co-infection of chickens with IBV and LPAIV

- 2 similar studies
- 3 week old SPF Leghorn chickens
- Intraocular and intranasal inoculation with:
IBV: **ArkDPI**; dose 10^4 EID₅₀/dose
LPAIV: H9N2, H5N2; 10^7 EID₅₀/dose

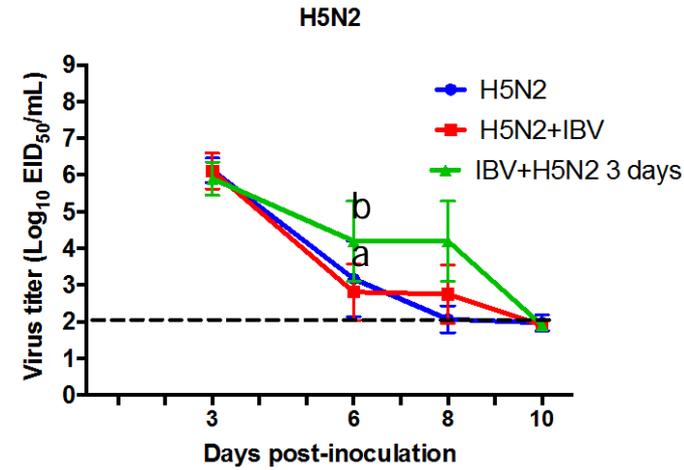
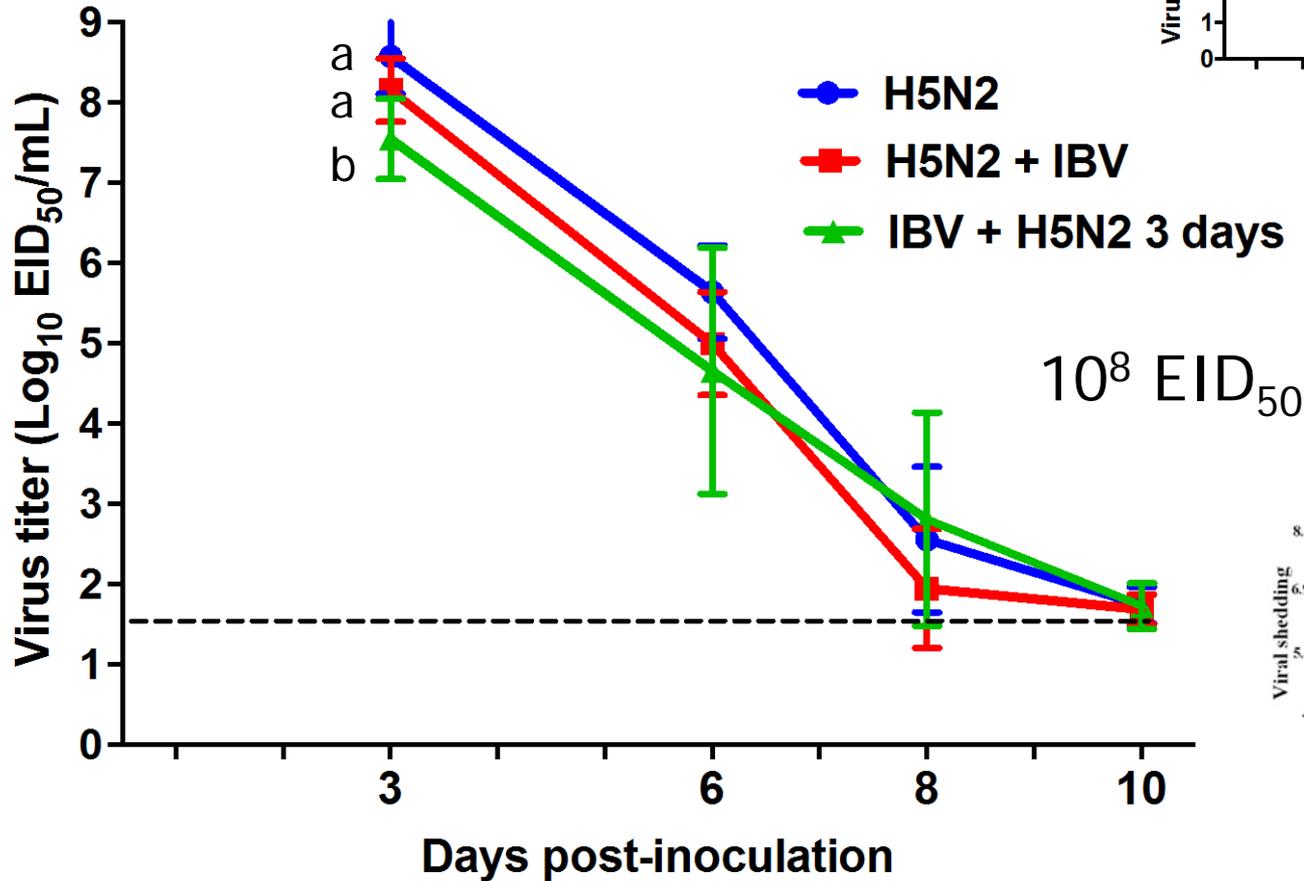


	Groups	Day of inoculation	
		Day 0	Day 3
Single	1 (controls)	-	-
	2	IBV	-
	3	H5N2	-
	4	H9N2	-
Simultaneous	5	IBV + H5N2	-
	6	IBV + H9N2	-
Sequential	7	IBV	H5N2
	8	IBV	H9N2

Minimal clinical signs seen in birds infected with ArkDPI

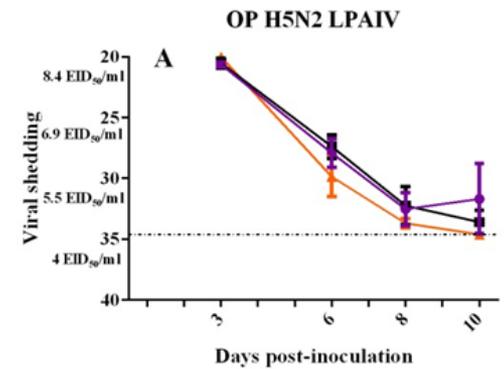
H5N2

Viral shedding H5N2



10⁷ EID₅₀

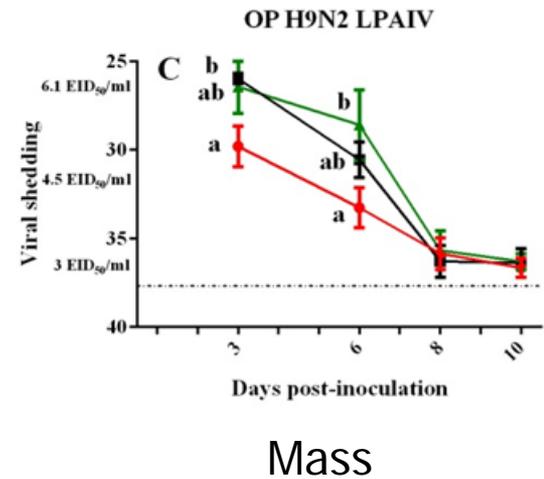
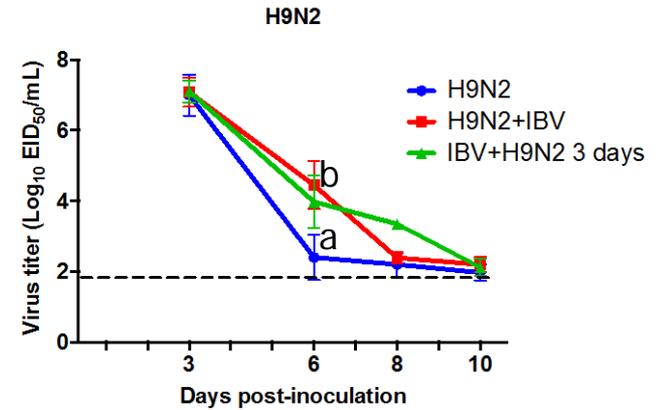
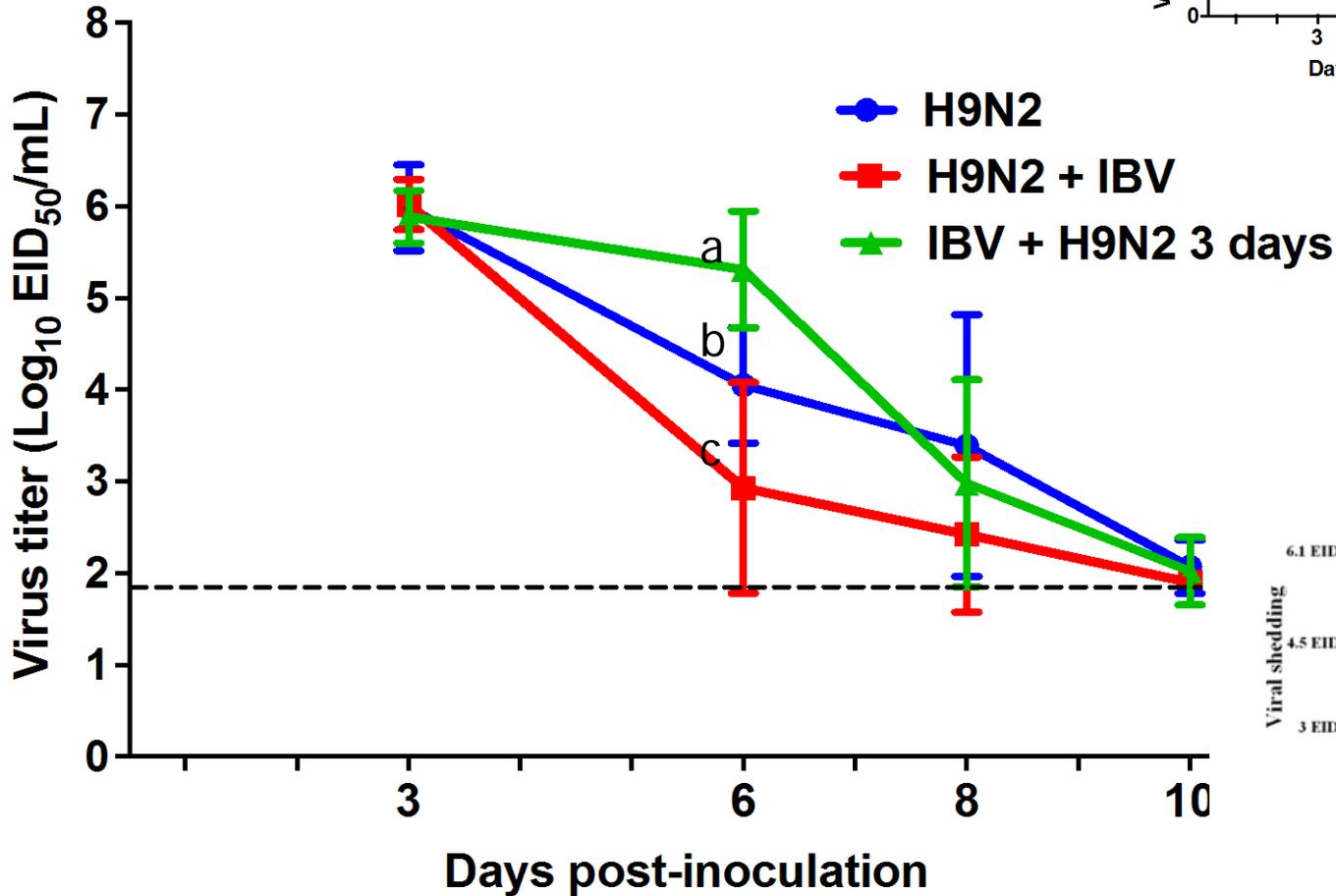
10⁸ EID₅₀



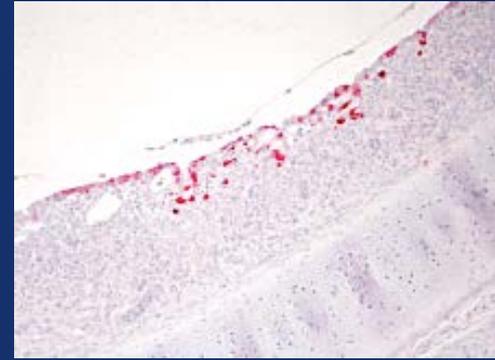
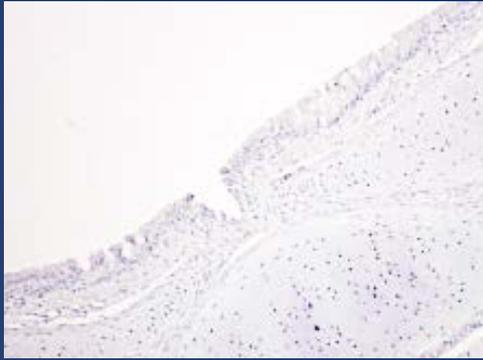
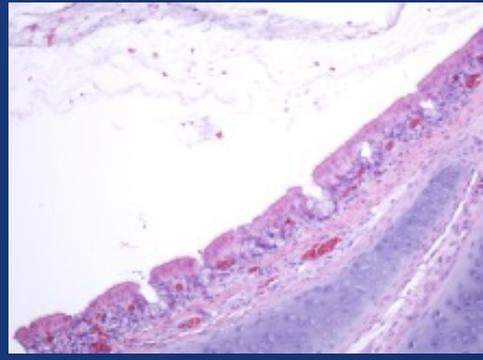
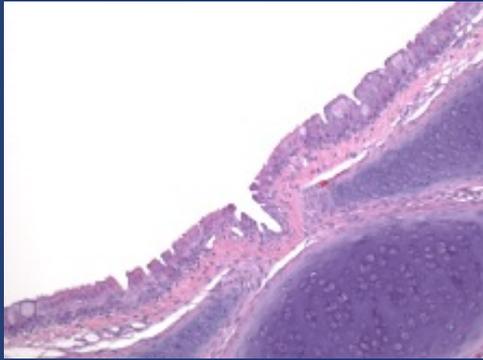
Mass

H9N2

Viral shedding H9N2



Histo/AI-IHC



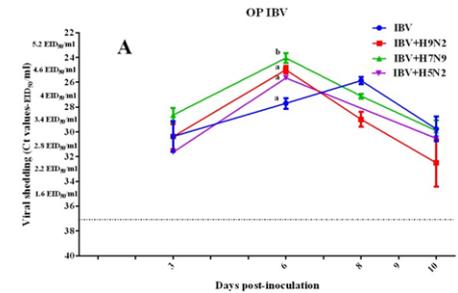
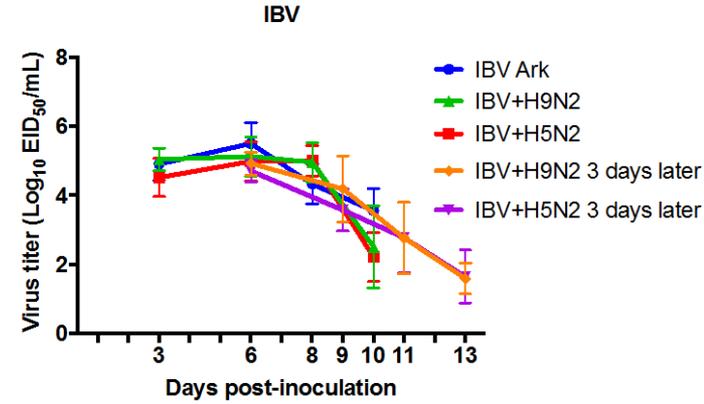
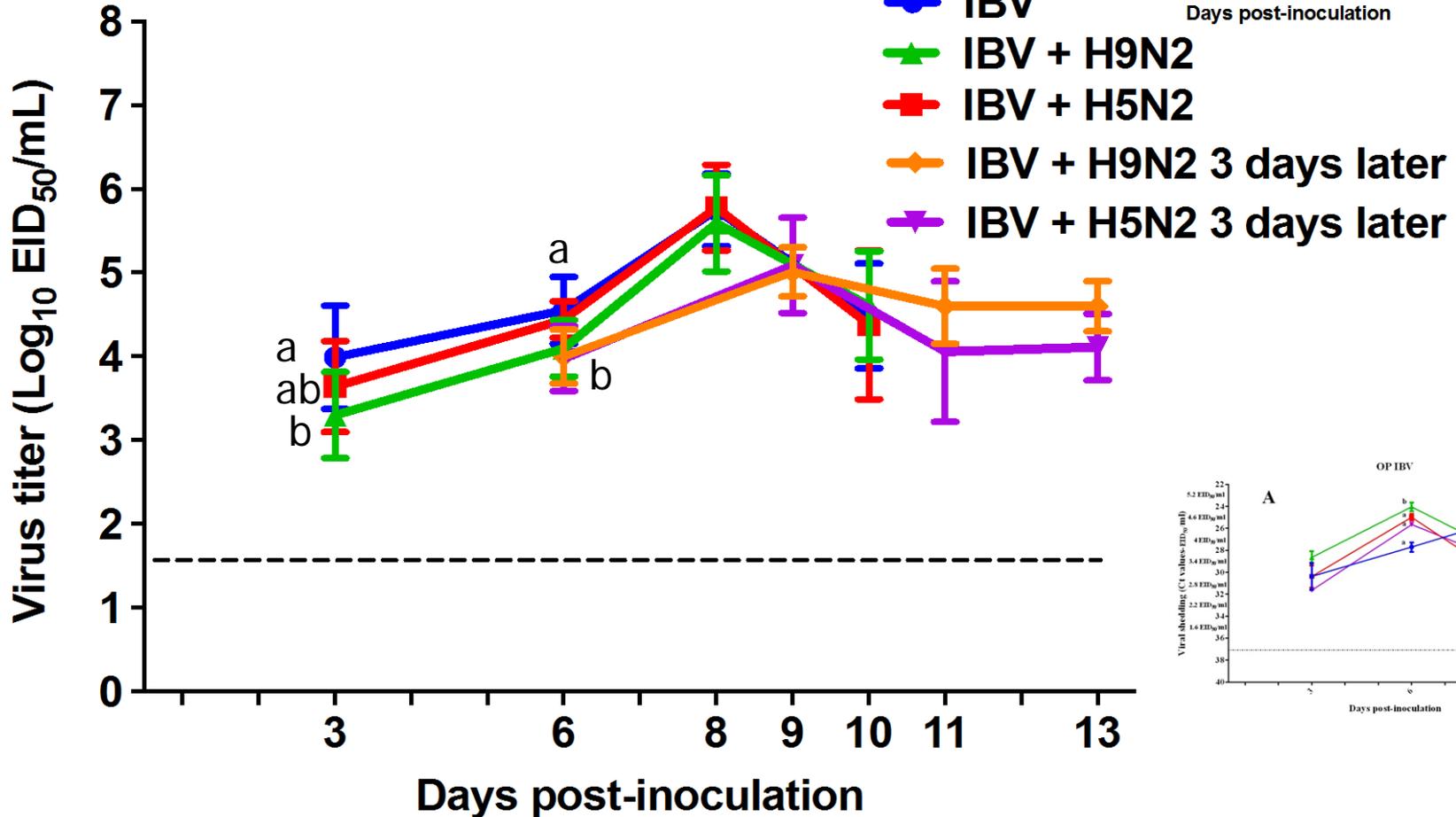
H9N2

Ark+H9N2

Ark + H9N2
3 days later

IBV

Viral shedding IBV



Summary

- The effect of co-infection with IBV and LPAIV in chickens varies depending on the virus strain, titer, and the timing of co-infection, with exacerbation, reduction, or no effect on virus shedding
- Groups that received IBV 3 days before challenge with H9N2 LPAIV shed significantly higher LPAIV titers than groups only challenged with LPAIV
- Grossly, no or mild lesions were observed in the trachea, but microscopically typical lesions of IBV infection were present in all birds infected with IBV. AIV virus staining was more frequent in birds challenged with IBV 3 days before challenge with H9N2, indicating that previous damage to the trachea increases LPAIV replication
- No bronchial casts or tracheal plugs were observed, corroborating that other pathogens are necessary to reproduce these lesions reported in the field

Challenges:

- Problems getting IBC and IACUC approvals for using *Mycoplasma* sp. The approvals have finally been obtained so the experiments will be conducted in the next months

Next:

- Follow up study in which we will co-infect chickens with LPAIV, IBV and *Mycoplasma synoviae* (MS)
- Co-infection study in turkeys using LPAIV, NDV, and *Mycoplasma meleagridis* (MG)

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