

## **PATHOGENICITY**

Investigate the multifactorial etiology involving poultry respiratory diseases

## **Objective 2.2.2: Characterization of innate immunity in chickens administered a live attenuated infectious laryngotracheitis virus (ILTV) chicken embryo origin (CEO) vaccine alone or combined with select viral respiratory vaccines**

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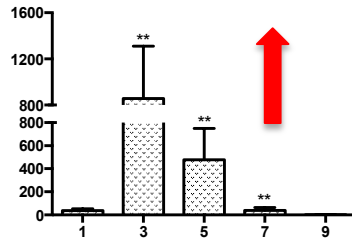
- **1st Year:** Innate Immune response post-CEO vaccination, IBV, and Mycoplasmas.
- **2nd Year:** Immune response post-challenge in ILTV vaccinated chickens.
- **3rd Year:** Immune response post co-administration of viral respiratory vaccines.
- **4rd Year:** Immune response post-challenge of ILTV and IBV vaccinated chickens.
- **5Th Year:** Immune response post-challenge of ILTV and NDV vaccinated chickens.

- The overall objective of this project is to identify the **key innate immune pathways** associated with **ILTV CEO** vaccination and how this innate pathways are altered by vaccination with infectious bronchitis virus (IBV) or infection with Mycoplasma or low pathogenic avian influenza.

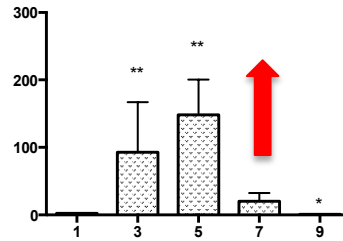
- Why innate immune responses? essential to establish protection against ILTV, IBV, LPAI, Mycoplasma?

# Trachea

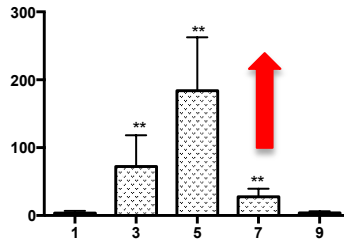
a. IL-8



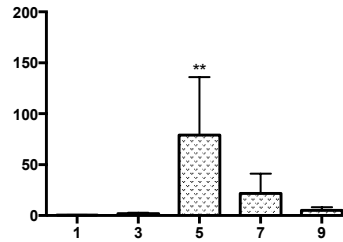
b. IL-1 $\beta$



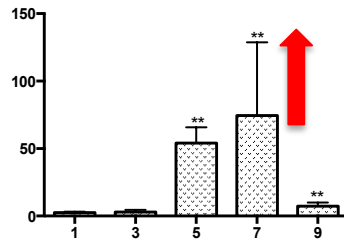
c. IL-10



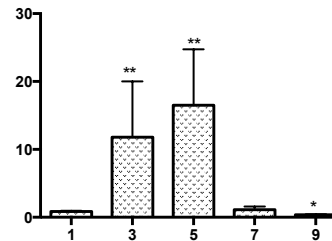
d. IL-13



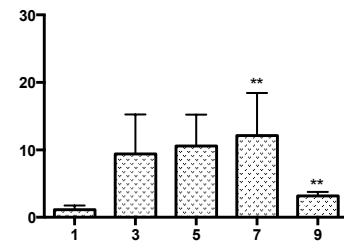
e. IFN- $\gamma$



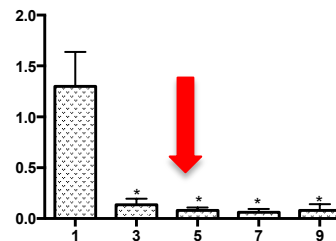
f. IFN- $\alpha$



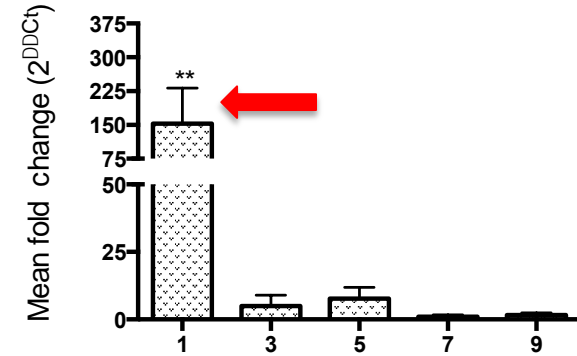
g. IL-2



h. iNOS



a. Harderian gland IFN- $\gamma$



Virulent Strain 63140

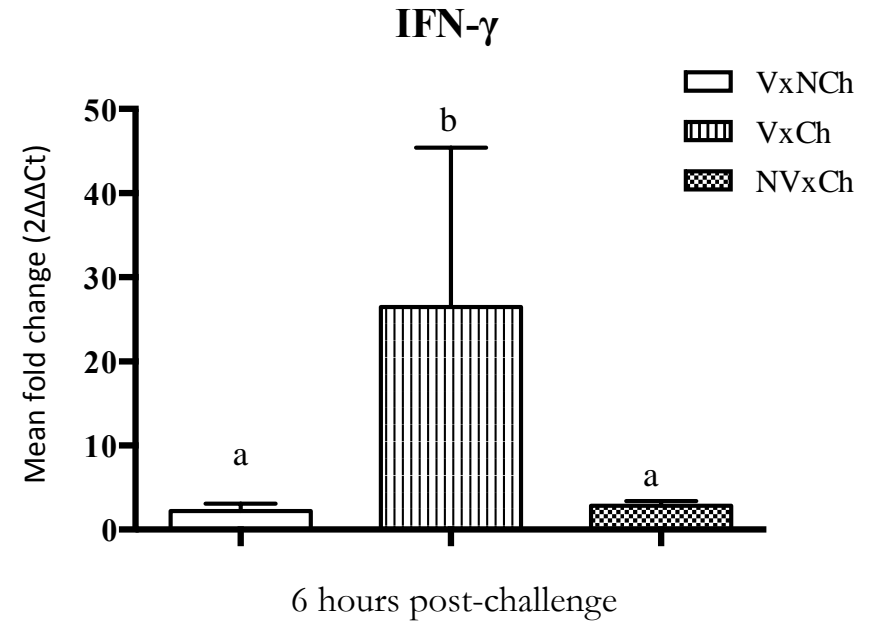
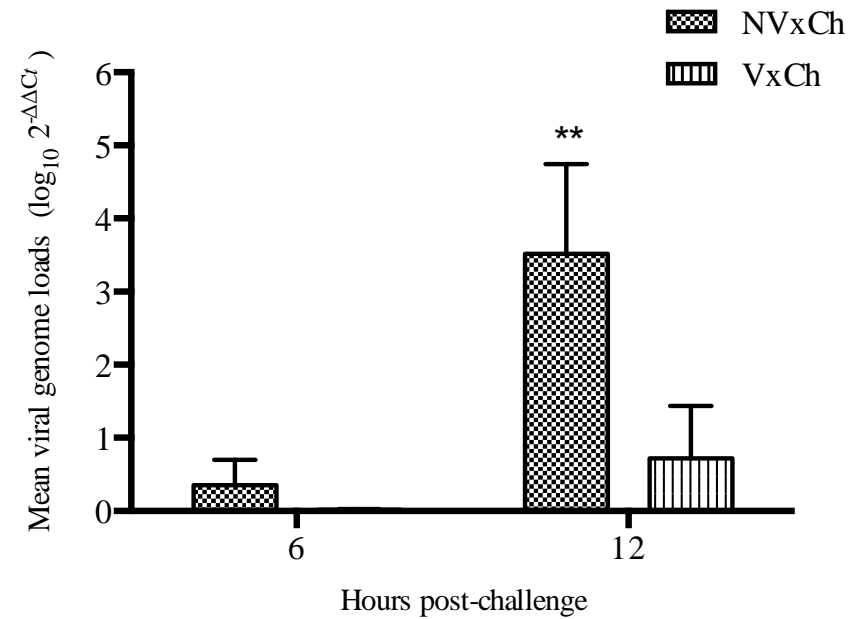
Days Post-infection

# Evaluation of the transcriptional status of host cytokines and viral genes in the trachea of vaccinated and non-vaccinated chickens after challenge with infectious laryngotracheitis virus (ILTV)

Table 3. Cytokine transcripts mean fold changes in tracheas of CEO vaccinated (Vx) and non-vaccinated (NVx) chickens six and 12 hours after mock inoculation (non-challenge, NCh) or inoculation with virulent ILTV strain (challenge, Ch)

	6 hours post-inoculation			12 hours post-inoculation		
	VxNCh	VxCh	NVxCh	VxNCh	VxCh	NVxCh
IFN $\beta$	1.346 <sup>*</sup> (0.261)	2.760 (0.490)	1.509 (0.062)	0.948 (0.179)	0.513 (0.084)	0.897 (0.088)
IFN $\gamma$	2.209 (0.883)	<b>26.47<sup>a</sup></b> <b>(18.94)</b>	2.852 (0.553)	0.962 (0.219)	4.411 (2.789)	1.026 (0.167)
IL-1 $\beta$	1.027 (0.158)	0.708 (0.329)	1.030 (0.192)	1.024 (0.160)	0.995 (0.151)	1.183 (0.122)
IL-6	1.767 <sup>a</sup> (0.140)	<b>0.992<sup>b</sup></b> <b>(0.174)</b>	<b>0.882<sup>ab</sup></b> <b>(0.188)</b>	1.800 (0.609)	0.614 (0.082)	0.794 (0.024)
IL-8	0.765 (0.023)	1.292 (0.808)	0.682 (0.022)	0.703 (0.113)	0.752 (0.123)	0.899 (0.207)
IL-18	0.468 (0.086)	0.523 (0.074)	1.026 (0.187)	0.593 (0.058)	1.185 (0.269)	1.792 (0.319)

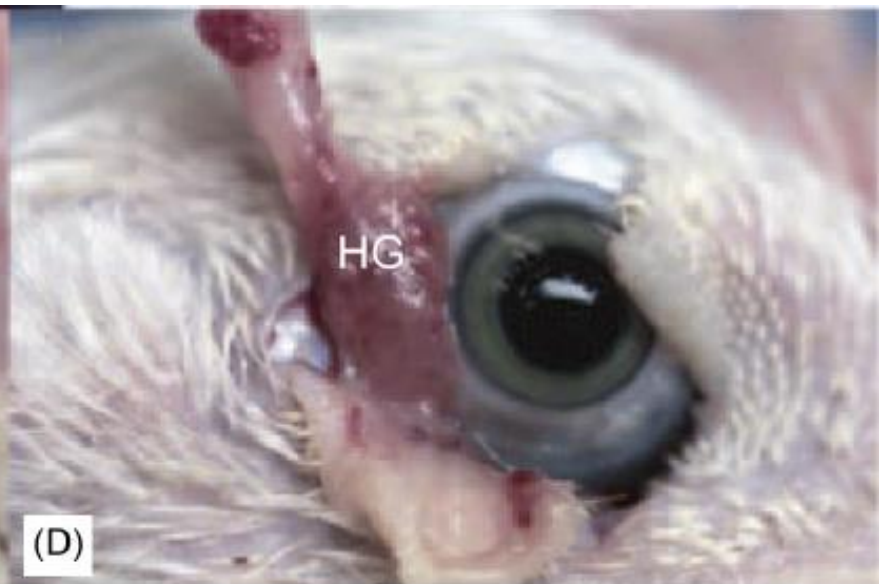
- Why CEO? Gold standard protection ILTV



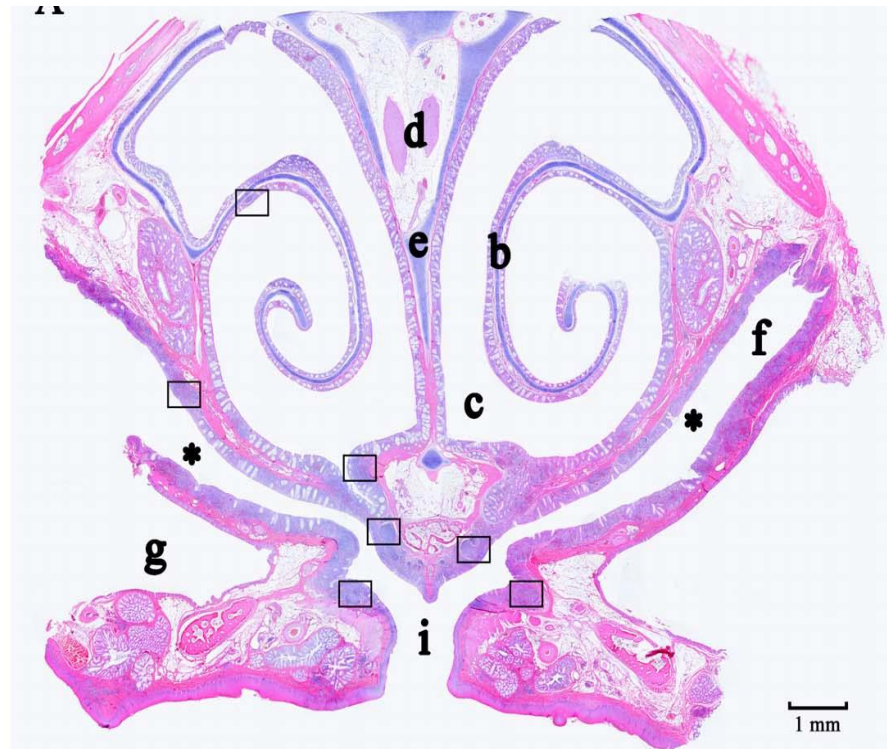
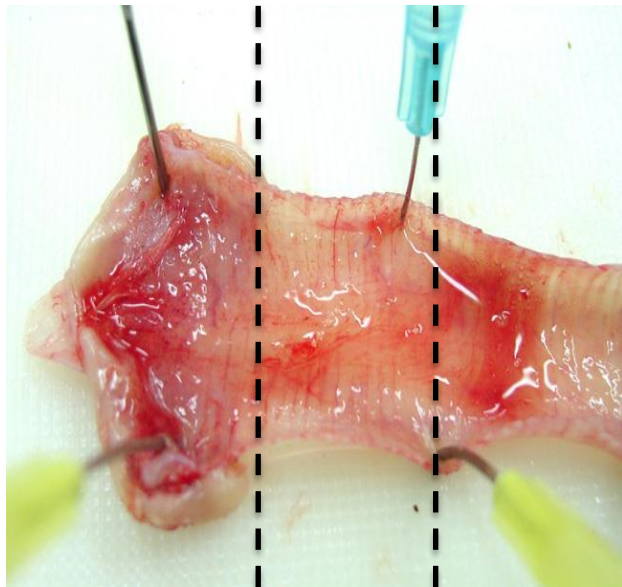
## What Tissues???

- Conjunctiva, sinus cavity and Harderian gland are anatomically connected to the respiratory system are the first to come in contact with the virus during vaccination or natural infection.
- These structures are both are sites where replication of ILTV and are functionally essential sites where immune responses are generated and

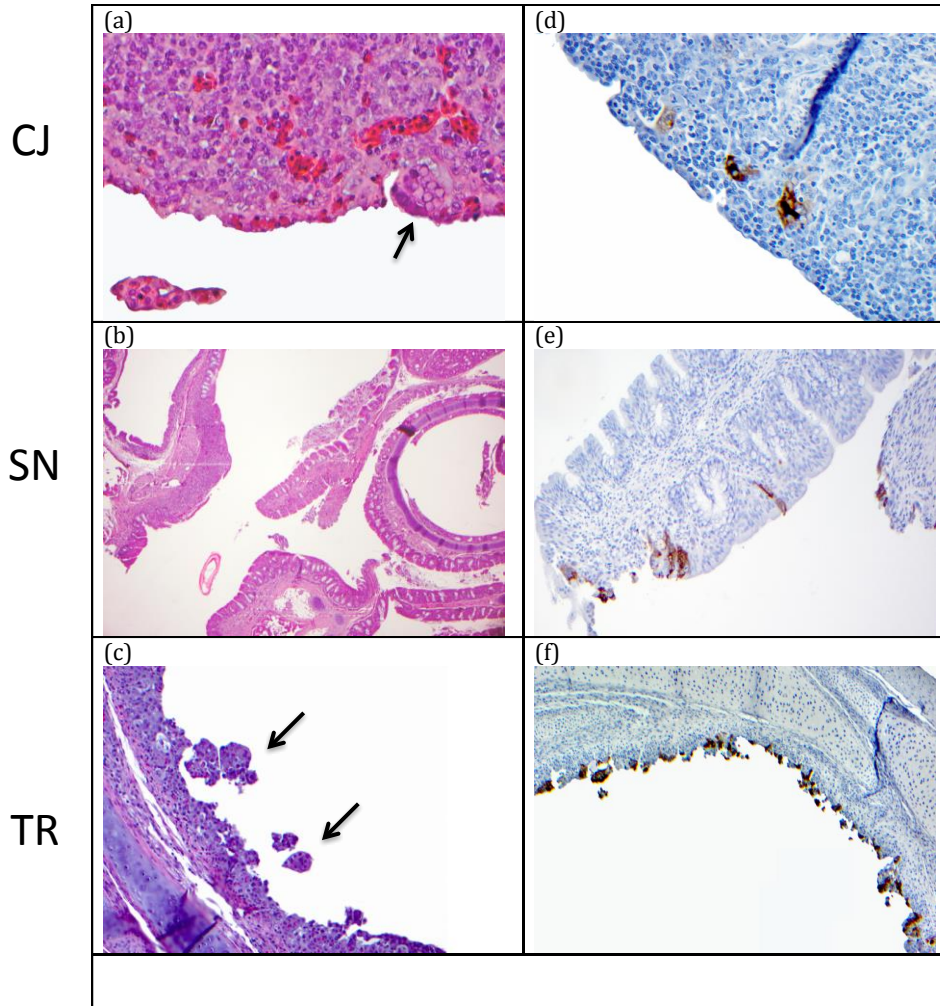




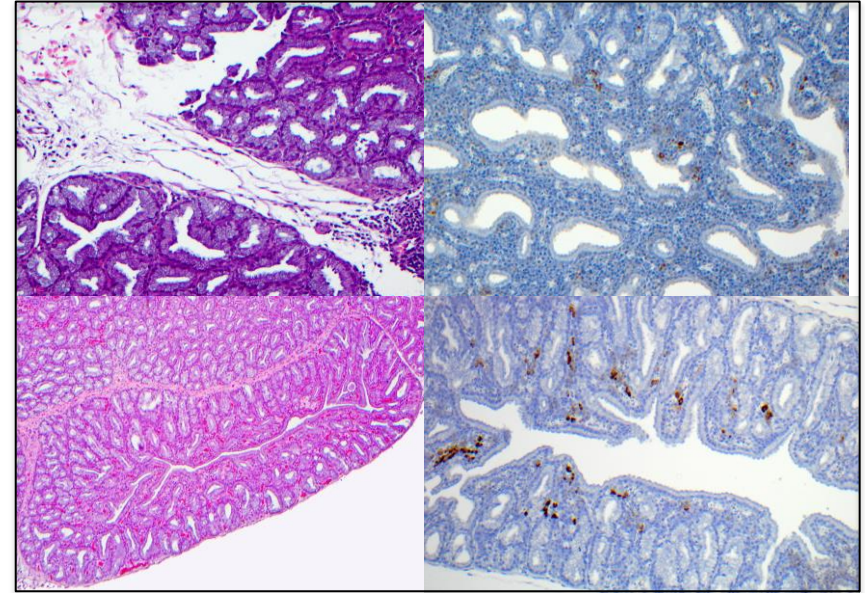
From Avian Immunology 2<sup>nd</sup> Edition



# Chicken embryo origin (CEO) Vaccine



Harderian Gland



No LT lytic replication in HG but  
antigen uptake by cells??

# Positive tissues in chickens inoculated with CEO vaccine

## Histopathology lesion/IHC

	Day 3				Day 5			
Tissues	OC	OR	IN	IT	OC	OR	IN	IT
<i>Conjunctiva<sup>d</sup></i>	+/+	+/+	-/-	-/-	+/+	+/+	+/+	-/-
<i>HG<sup>c, e</sup></i>	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
<i>Sinus<sup>d</sup></i>	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
<i>Trachea<sup>d</sup></i>	-/-	-/-	-/-	+/+	-/-	-/-	-/-	+/+



# Results

- These experiments demonstrated that entry of ILTV through mucosal tissues of the nasal cavity, conjunctiva and para-ocular harderian gland (HG) do influence the replication patterns of ILTV both vaccine or virulent isolate
- The CEO vaccine and 63140 isolate showed evidence of replication in the conjunctiva and sinus but not in trachea when administered via the ocular, oral and intranasal routes (natural routes of viral entry)
- Independently of the route of inoculation both 63140 and CEO inoculated groups chickens showed the presence of **viral antigen** in not-identified cells of the **Harderian gland** in the absence of ILTV pathognomonic lesions characteristics of lytic viral replication.
- Ongoing cytokine mRNA expression in conjunctiva, trachea, HG

## Next Step

- Develop and ex-vivo system with trachea, HG, sinus cavity, conjunctiva associated lymphoid tissue (CALT) **ILTV** infected chickens (SPFs)
- Viral replication
- Measure INF $\gamma$ , IL-8 (ELISAs) and NO (Greiss assay)
- Ex-vivo for IBV, Mycoplasma, maybe?? LPAI

# Immunophenotyping Avian Leukocytes in the Mucosal Tissues During Aging in White Leghorns

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