College of
Veterinary Medicine
UNIVERSITY OF GEORGIA

THINK TWICE

on *Mycoplasma gallisepticum* control

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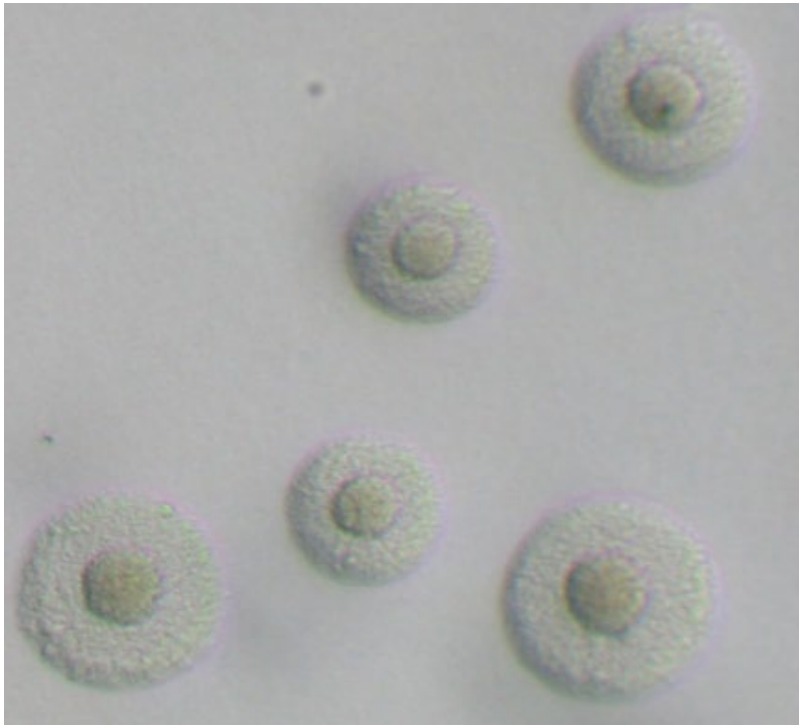
Outline

1. *Mycoplasma gallisepticum* overview
2. Immunity induced by *Mycoplasma*
3. New combined vaccination strategy

Mycoplasma gallisepticum and *Mycoplasma synoviae*

- Bacteria
  - Mollicutes
    - No cell wall
    - Smallest free-living organisms
    - Smallest genome of any free-living organism
- Pathogenic and economically significant avian mycoplasma

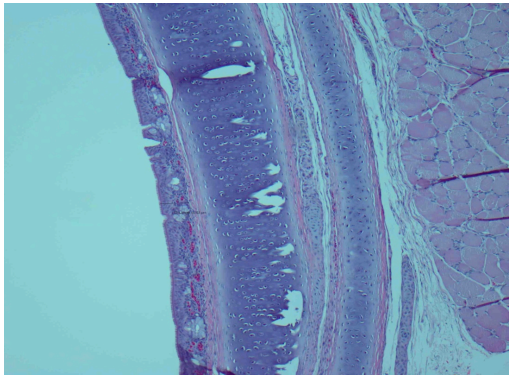


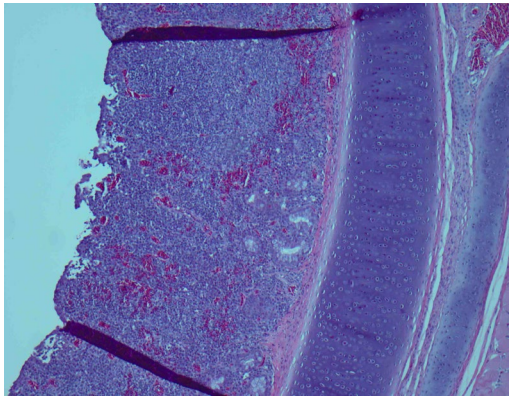
Economically Important Avian Mycoplasmas

- *Mycoplasma gallisepticum* (MG)
  - Low prevalence
  - High significance
- *Mycoplasma synoviae* (MS)
  - High prevalence
  - Low significance

Possible Factors in Pathogenesis of Mycoplasmas

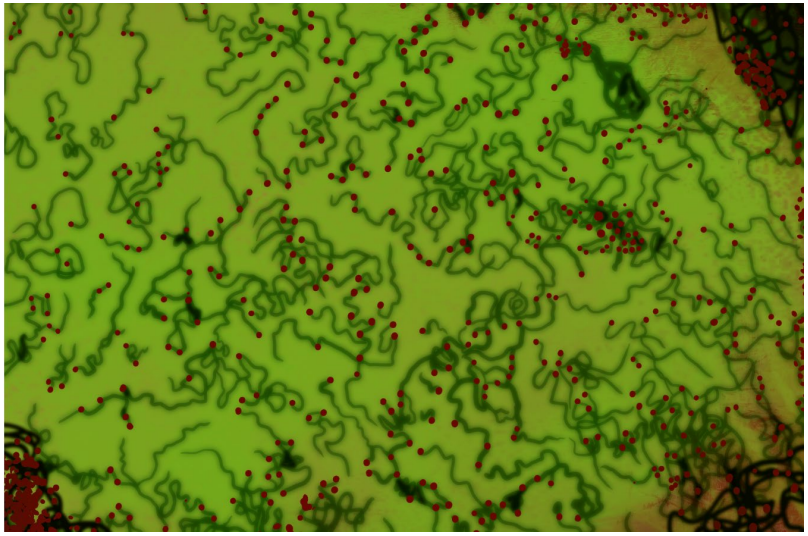
- **Attachment**
- Ciliostasis
- Depletion of cell nutrients
- Local toxins
- Penetration of cells?
- Stimulation of immunopathological reaction
- Effects on lymphoid cells & macrophages
- Antigen variation – immune evasion





Reaching target tissues

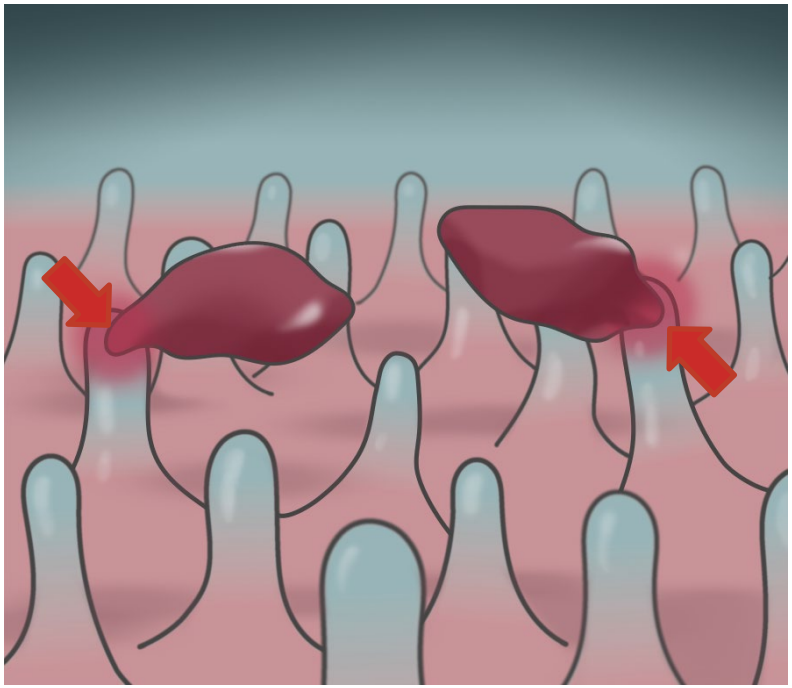
- MG organisms need to go from the conjunctiva and/or the nasal cavity to the trachea of the infected birds. Motility assists MG to reach tracheal epithelial cells.
- The movement of MG has been termed **gliding motility** and is likened to a flock of sheep grazing a field.



Gliding paths of MG (MG in red and paths in green)

Adherence to target tissues

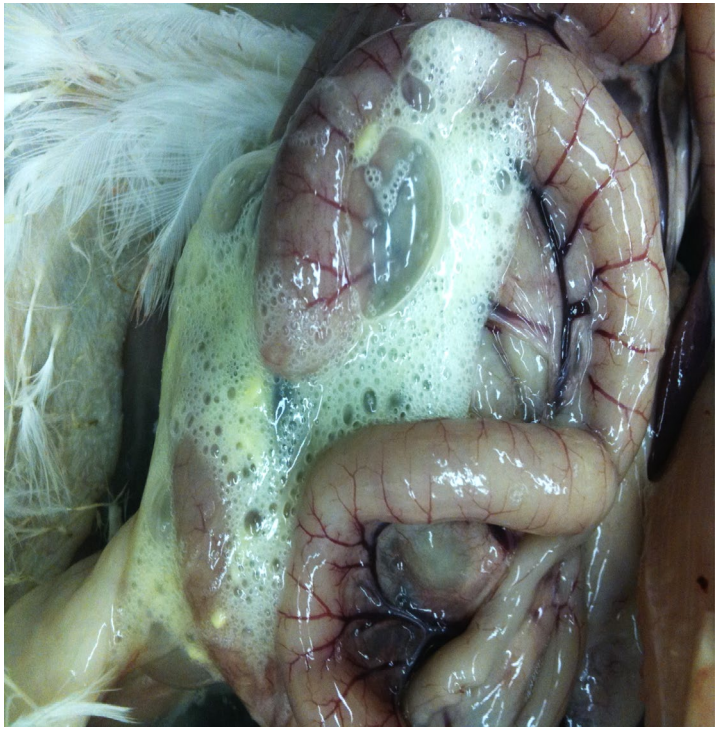
- MG presents specialized terminal tip structures (red arrows).
- They are called terminal tips and mediate adherence of MG to the tracheal epithelial cells.
- MG organisms adhere to tracheal epithelial cells and later, they multiply in the trachea causing loss of ciliary activity.



The terminal tips of two MG organisms in close proximity to the surface of the tracheal epithelium

Colonization of lower respiratory tract

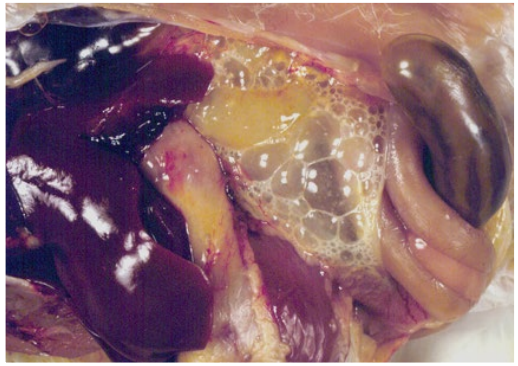
- Multiplication of MG in the tracheal epithelial cells allows MG organisms to penetrate deeper in the respiratory system and colonize the lower respiratory tract.
- Colonization of lower respiratory tract causes **airsacculitis**, one of the most common gross finding of MG infection.

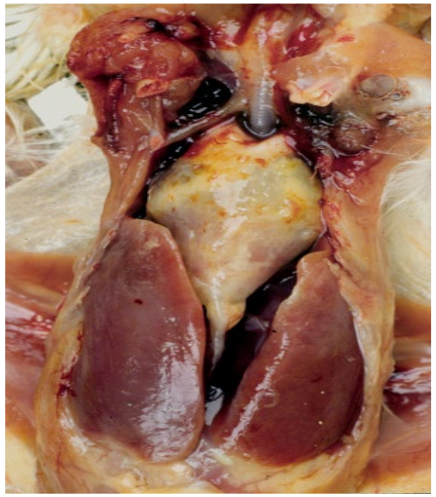


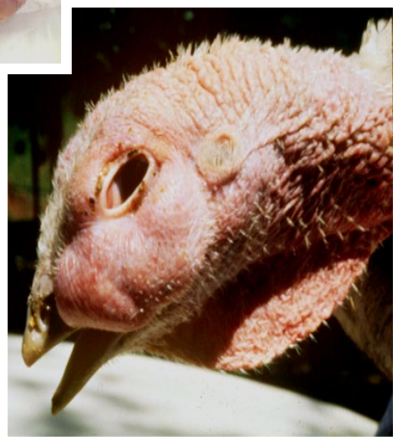
Airsacculitis in bird infected with MG

MG Disease Syndromes

- Infectious sinusitis in turkeys
- Chronic respiratory disease (CRD) in chickens
- Airsacculitis
- Egg production losses
- Complicated respiratory disease – respiratory viruses, *E. coli*

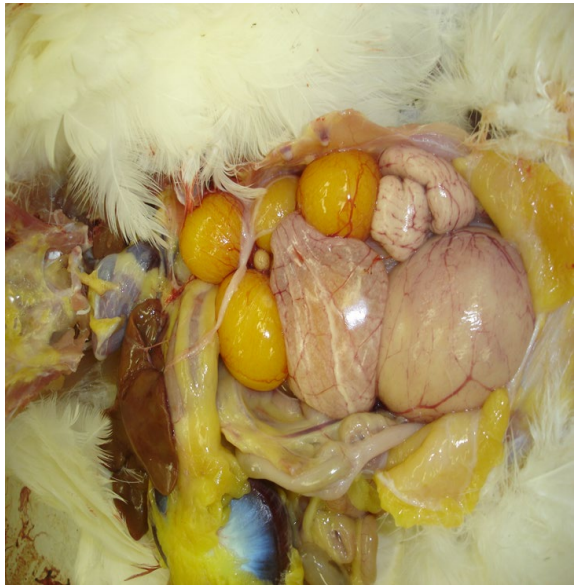




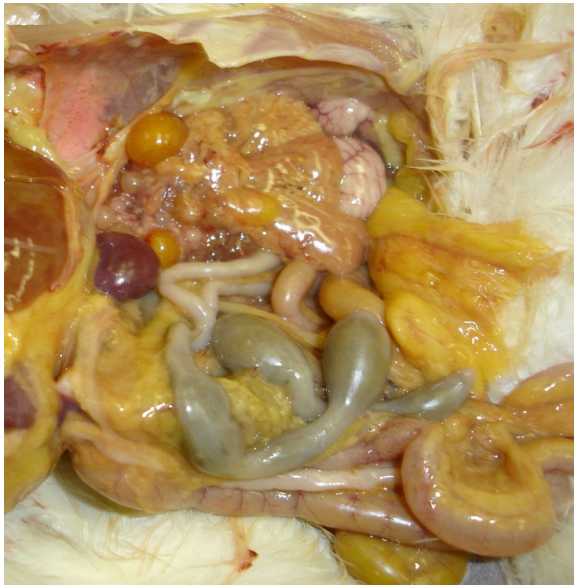


MG in layers

- MG produces respiratory and also reproductive lesions: ovarian regression

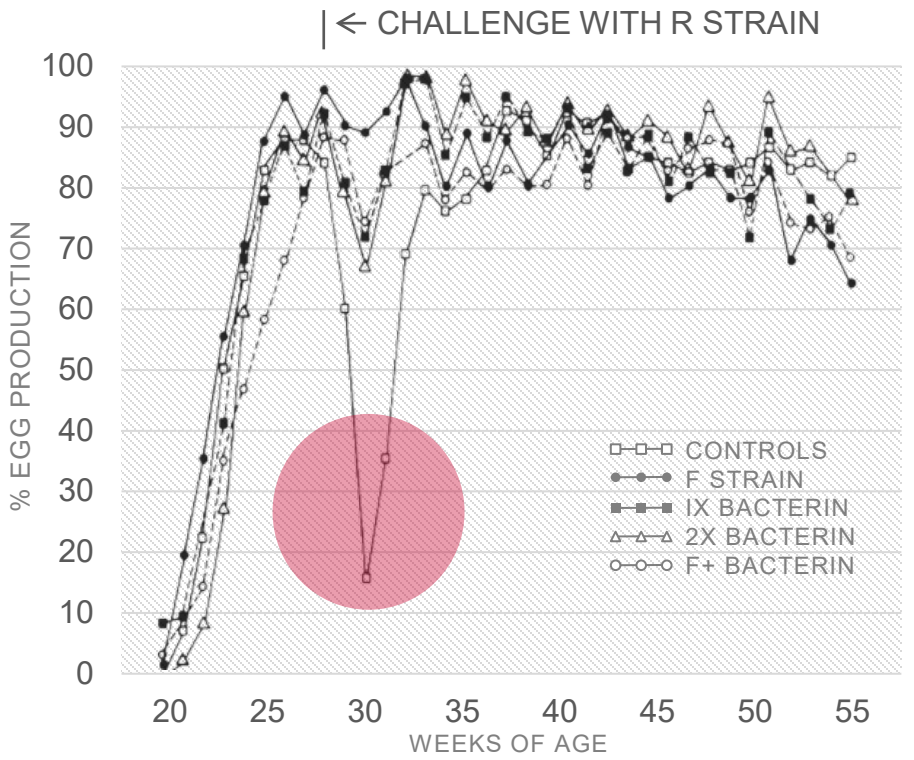


- In laying flocks, MG infection can result in significant egg production losses



MG in broiler breeders

- Drops in egg production & reduction in hatchability
- Vertical transmission resulting in airsacculitis
- Condemnations at the processing plant

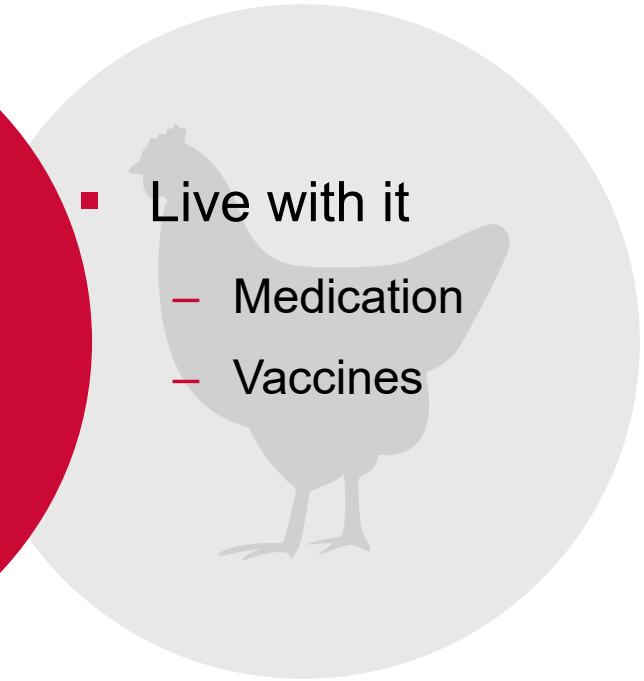


Percentage gg production of vaccinated groups and unvaccinated controls.

Glisson, J.R. & Kleven., S.H. (1984). *Avian Dis,* 28, 406-415.

MG Control Options

- Eliminate Flock
- Quarantine/Isolate
- Treatment
- Biosecurity
- Vaccine

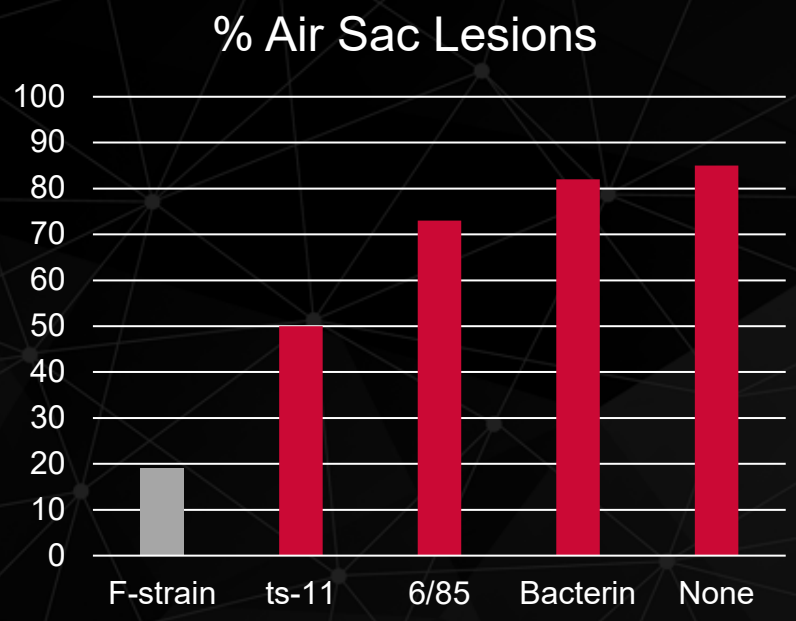


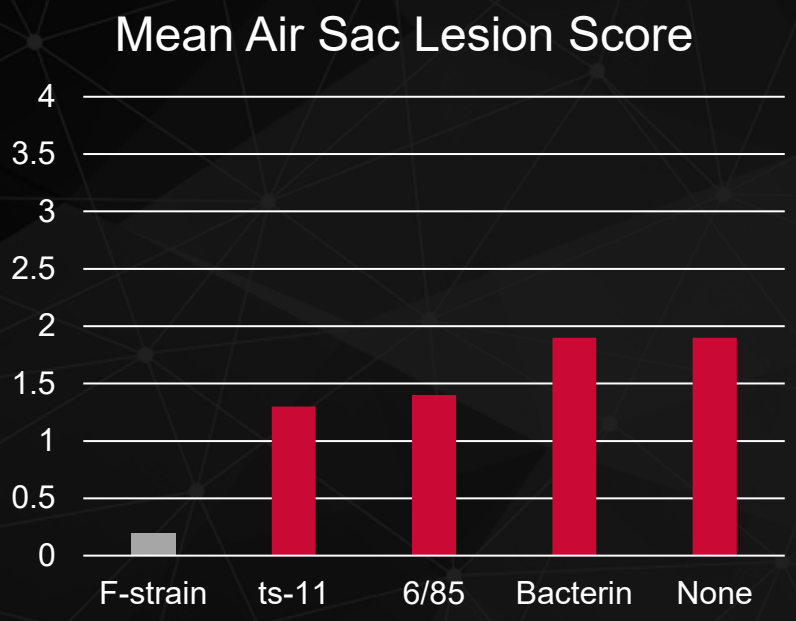
Immunizing Agents Available for MG

- Inactivated oil-emulsion bacterins
- Recombinant MG Vaccine
- Live vaccines
  - F Strain
  - ts-11
  - 6/85
  - K-strain

Efficacy of Live Vaccines and Bacterin

F-strain showed the highest protection against air sac lesions after challenge with the virulent R-strain at 90 days post-vaccination





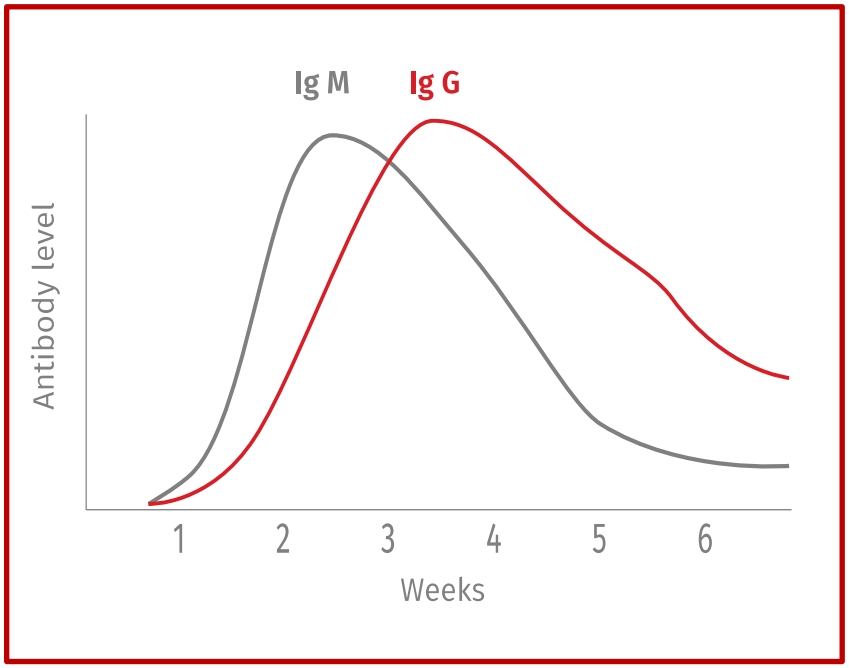
Abd-el-Motelib, T. Y., and S. H. Kleven. Avian Dis 37:981-987. 1993

Immunity against MG

- Systemic humoral immunity
- Mucosal associated lymphoid tissue (MALT)
- Local humoral immunity
- Local cellular immunity

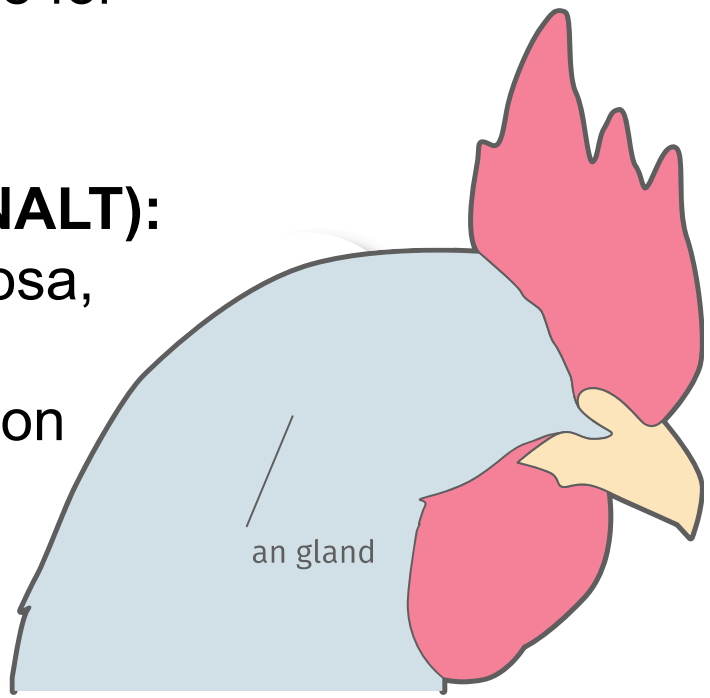
Systemic humoral immunity

- **Systemic antibodies:** MG infection stimulates a systemic humoral immune response mediated by circulating Ig M and Ig G.
- There is not a direct correlation between the levels of systemic Igs and protection.



Mucosal associated lymphoid tissue (MALT)

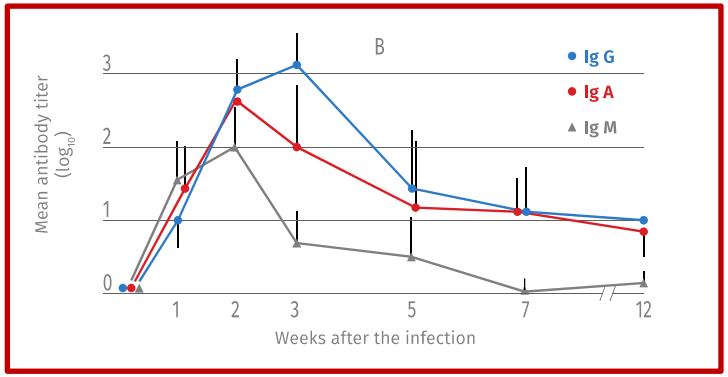
- **Harderian gland (HG):**
  HG contains B-lymphocytes responsible for antibody production (Ig A & Ig G) in the lacrimal fluid.
- **Nasal-associated lymphoid tissue (NALT):**
  B-lymphocytes found in the nasal mucosa, lateral nasal glands and their secretory ducts. MG infection stimulates production of Igs (A, G & M) in the nasal cavity.



Local humoral immunity

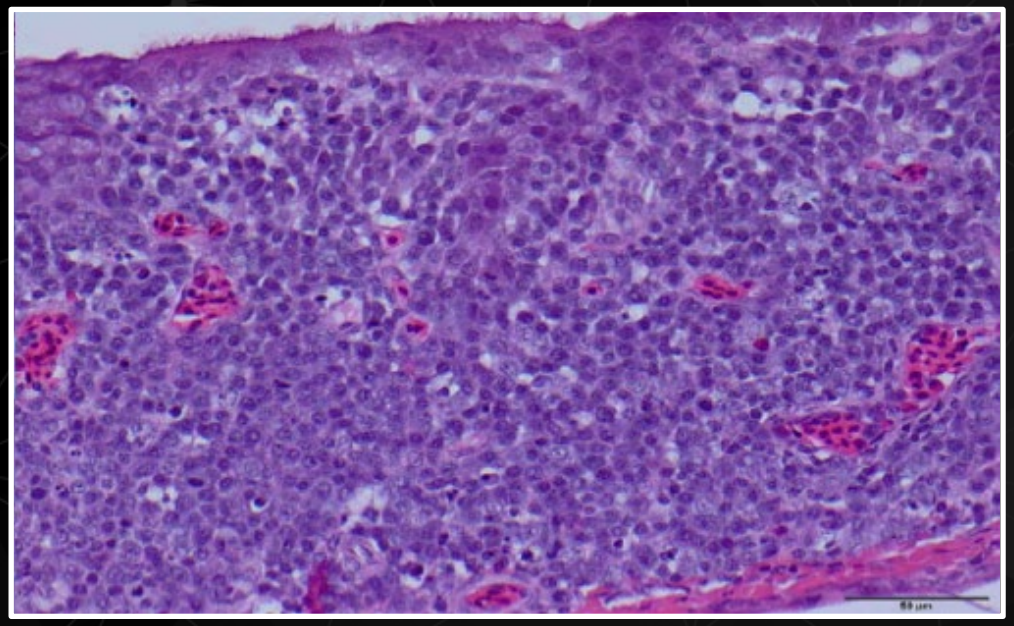
Humoral response in the trachea:

- B-lymphocytes in the trachea produce Igs A, G & M into the tracheal tract lumen.
- Igs present in the respiratory secretions of the trachea represent an important mechanism for protection against MG because they inhibit the adherence and multiplication of MG in the trachea.
- There is correlation between Ig levels in respiratory secretions and protection against MG.



Igs present in the respiratory secretions in the trachea of birds infected with MG

Local humoral immunity

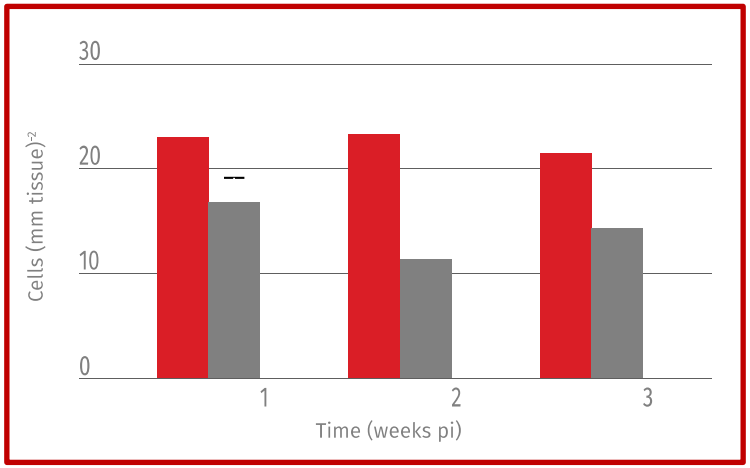


B-lymphocytes in the tracheal mucosa stimulated by MG infection

Local cellular immunity

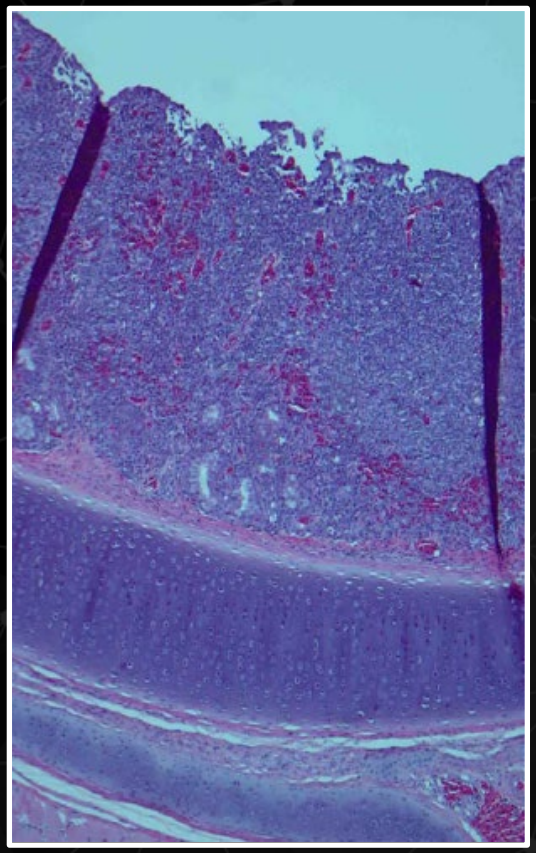
Cell-mediated immunity (CMI) in the trachea:

- Tracheal mucosa reacts to MG infection by producing two types of T-lymphocytes:
  - T-helper cells (lymphocytes CD4+)
  - T-cytotoxic cells (lymphocytes CD8+)
  - The role of T-lymphocytes has not been demonstrated in the protection against MG. Humoral rather than cellular immunity proved to inhibit adherence and multiplication of MG.



Mean concentrations of CD4+ (red bars) and CD8+ (grey bars) in the trachea of birds infected with MG

Local cellular immunity



T-lymphocytes infiltration in chicken tracheal mucosa after MG infection

New combined vaccination strategy

- Current field practice – live plus killed programs proves to be an effective strategy to control MG in high challenge areas.
- Synergies among live + killed vaccines
  - Role of live vaccines
  - Role of killed vaccines

Recent combined vaccination studies

New combined vaccination strategy

Scientific studies were conducted at the University of Georgia with the aim to establish that combining 1 live F strain and 2 inactivated vaccines delivered significant improvements in *Mycoplasma* control.

1. Experimental Design:
   - MG combined program (1L+2K) versus killed vaccination program
2. Experimental Design:
   - MG combined program (1L+2K) versus live vaccination program

Experimental Design 1

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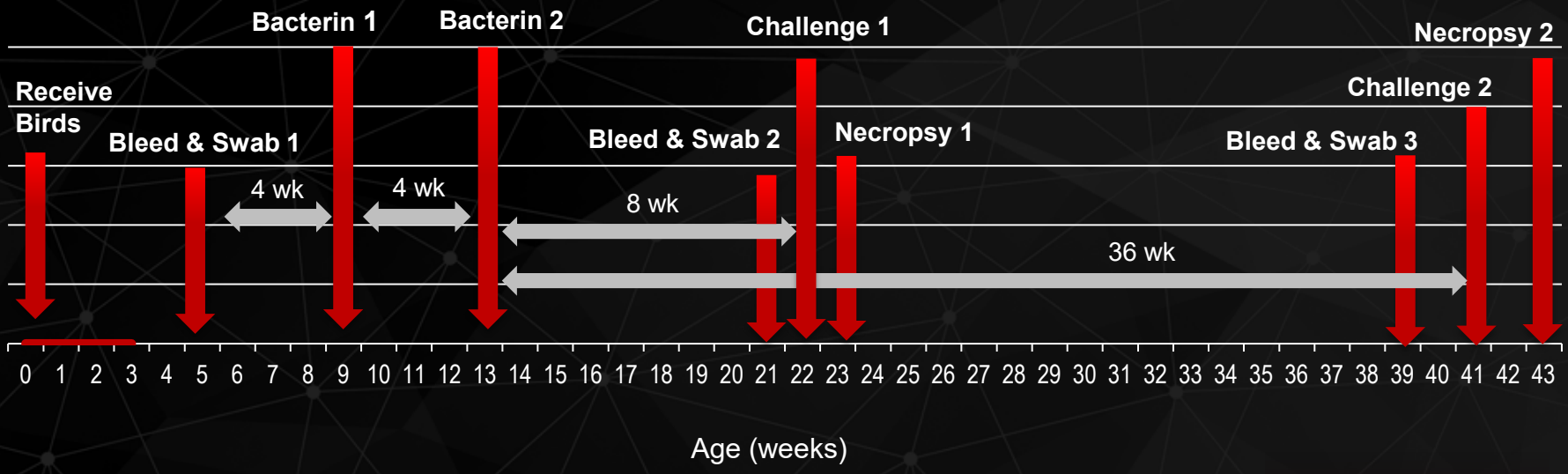
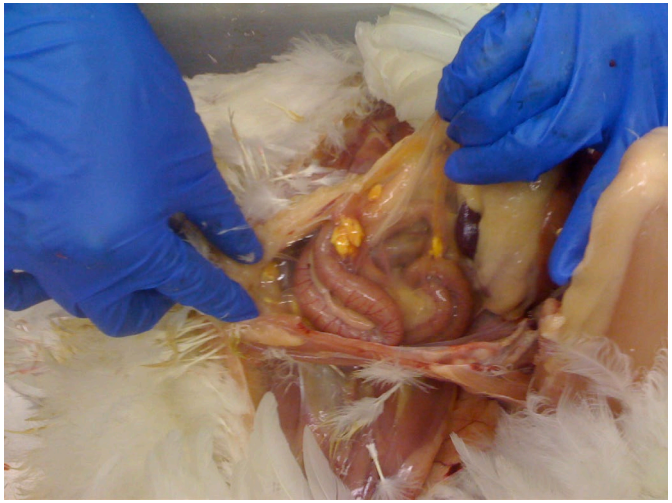
New combined vaccination strategy

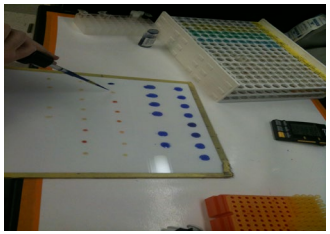


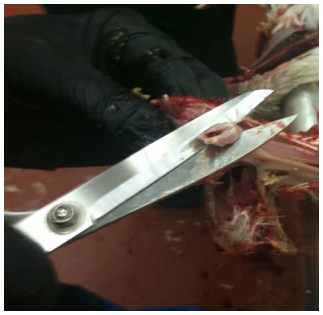
Parameters evaluated

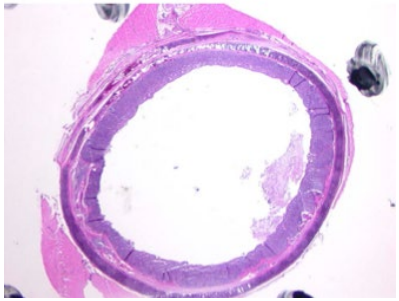
3 New combined vaccination strategy

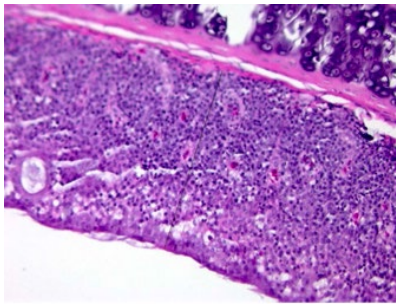
- Serology
- Air sac lesion scores
- Tracheal lesions
- Ovarian Regression
- Quantitative PCR
  - Strain differentiating







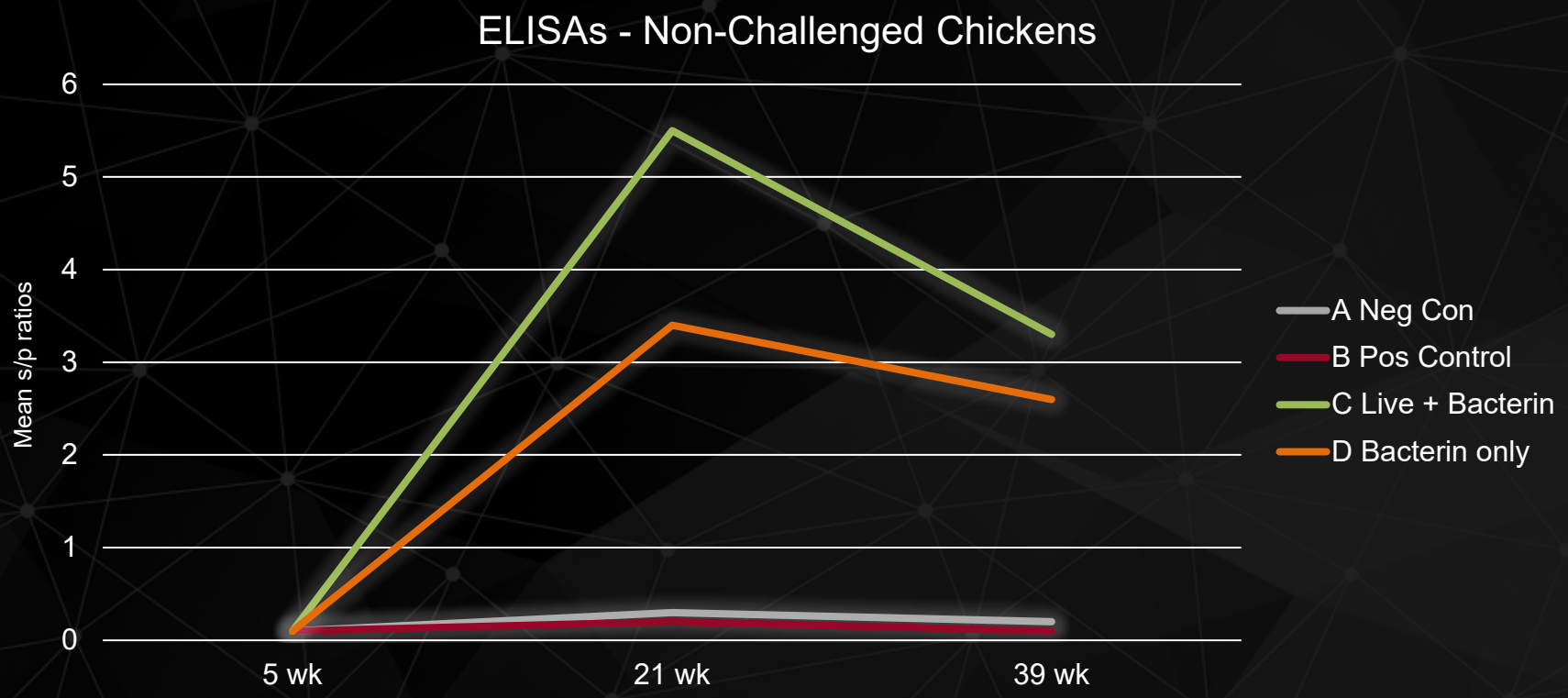




Serological Response

Combined program showed the highest ELISA mean titer

3 New combined vaccination strategy



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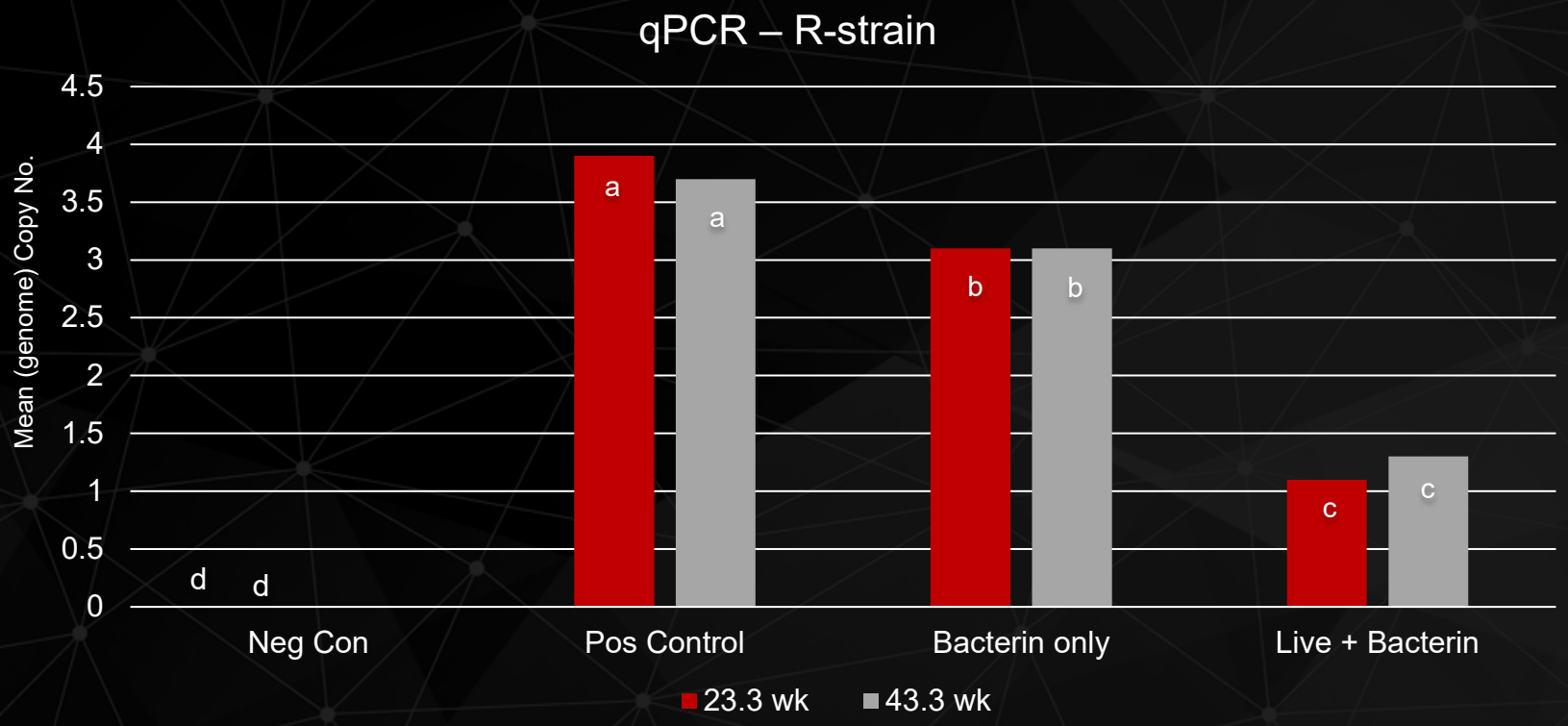
Quantitative PCR

Combined program showed the lowest replication of challenge strain

3 New combined vaccination strategy



Values with a different lower case letter are significantly different ($P \leq 0.05$)

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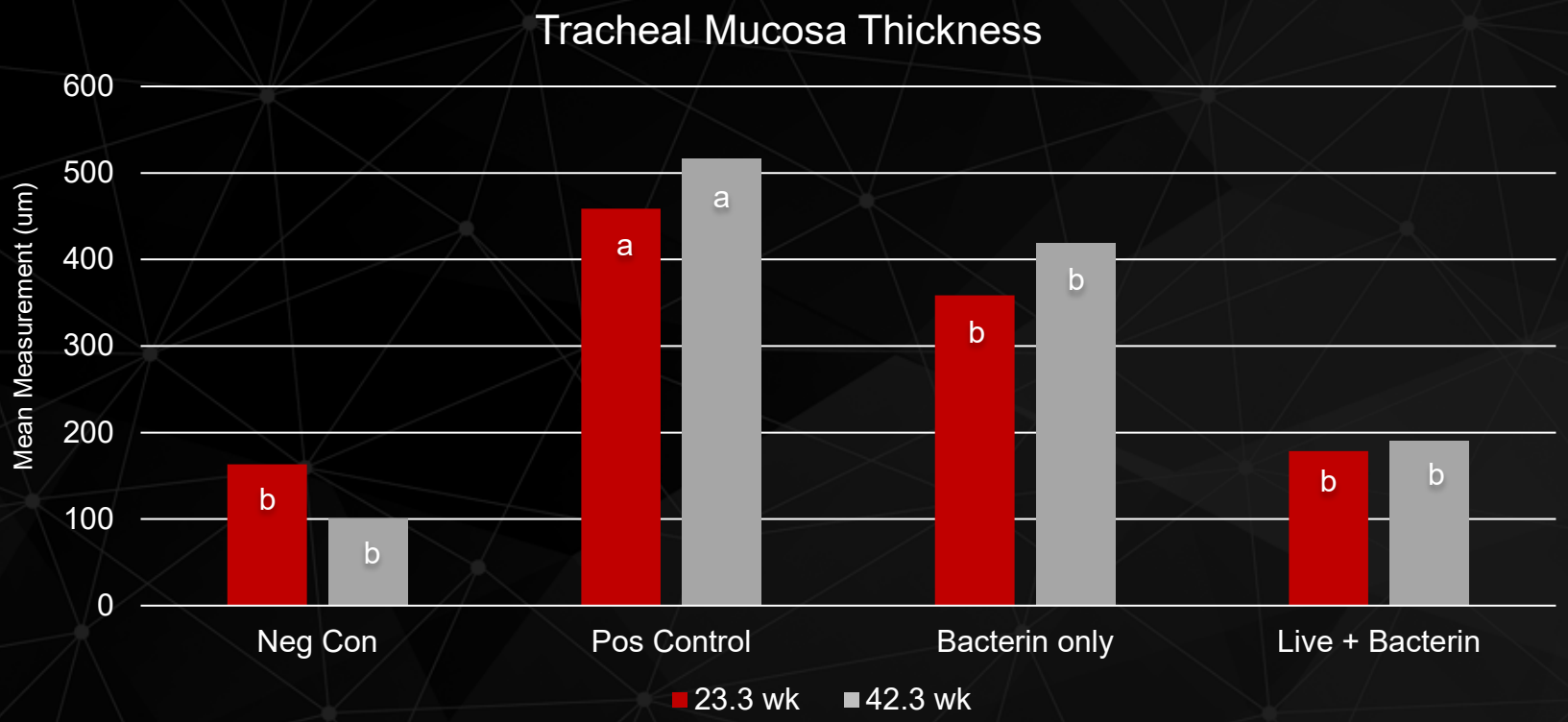
Tracheal Lesions

Combined program showed the lowest tracheal thickness

3 New combined vaccination strategy



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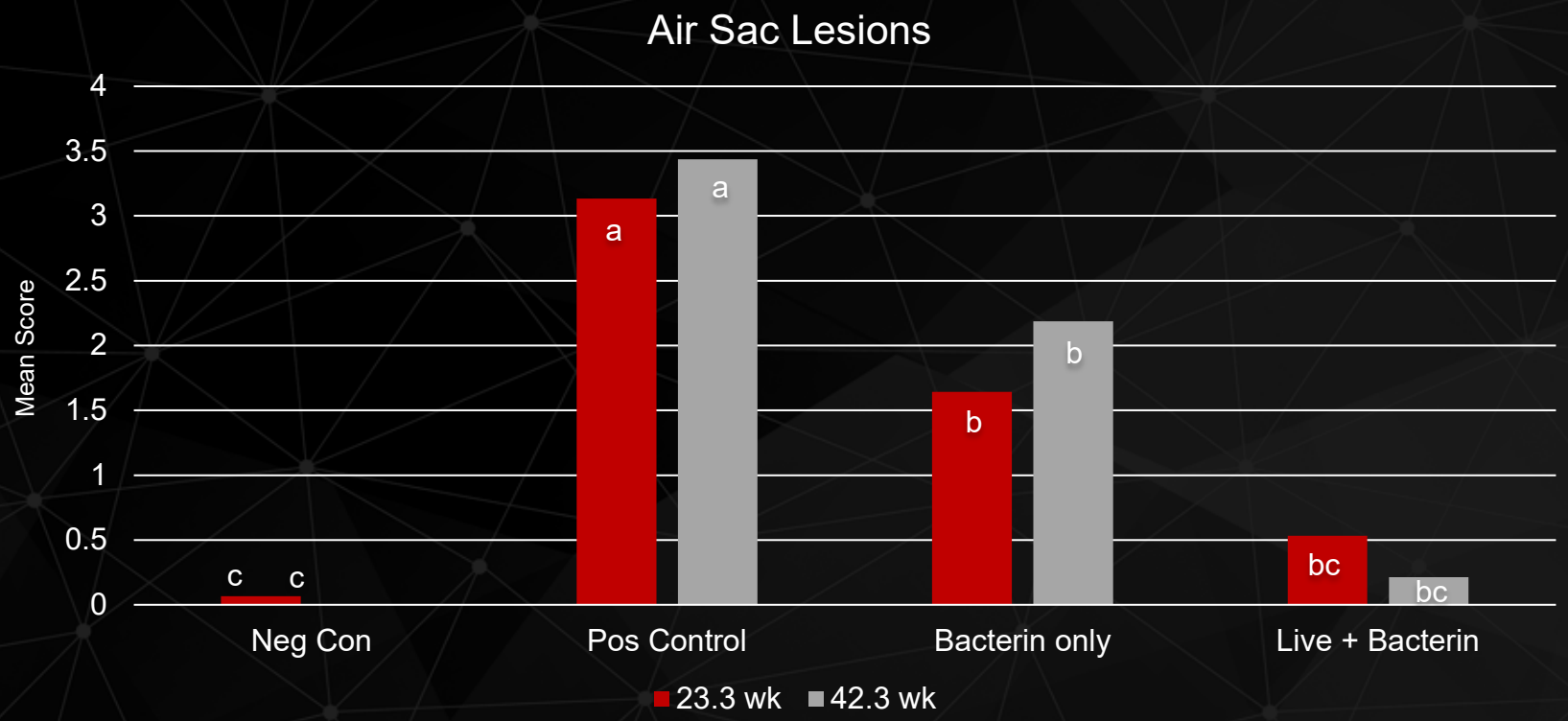
Air Sac Lesions

Combined program showed the lowest air-sac lesions

3 New combined vaccination strategy



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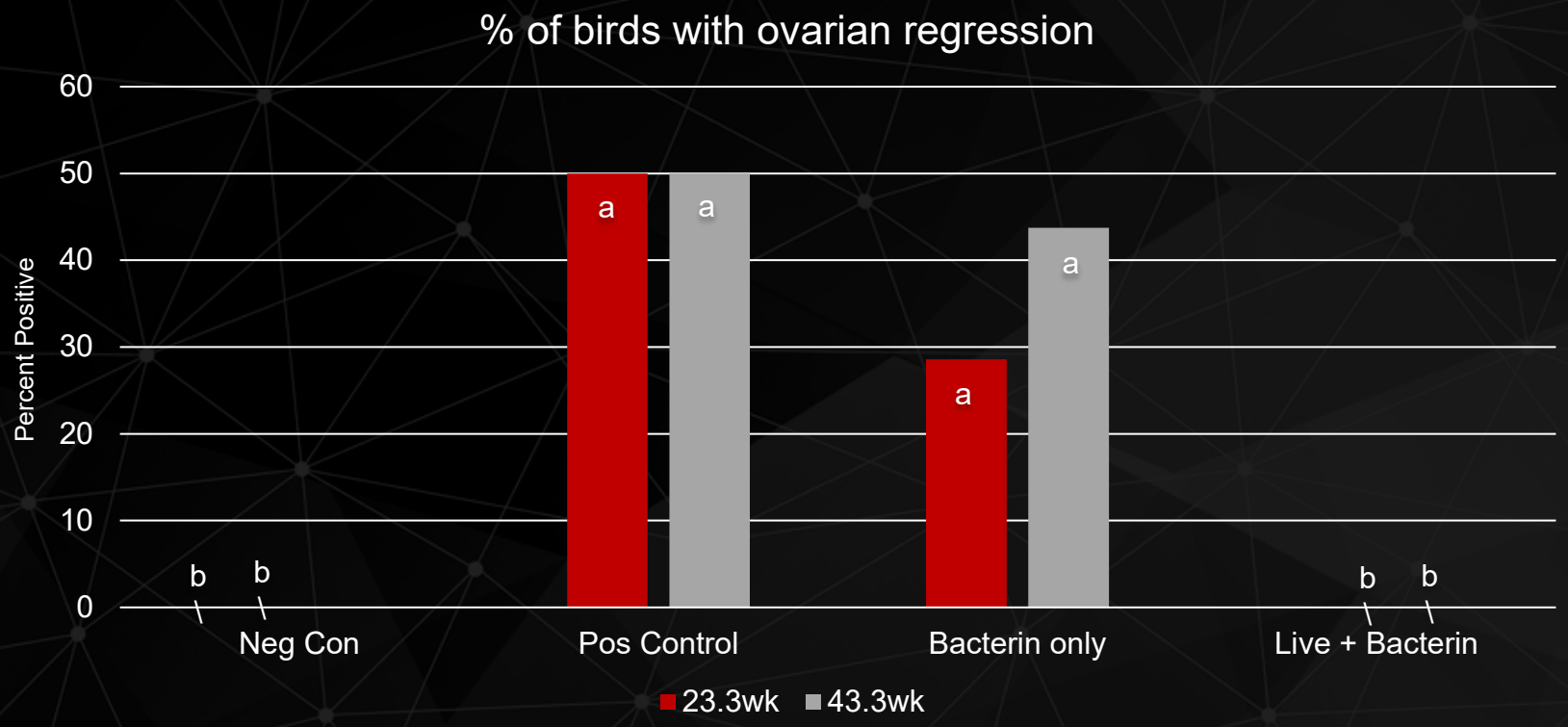
Ovarian Regression

Combined program protected against ovarian regression

3 New combined vaccination strategy



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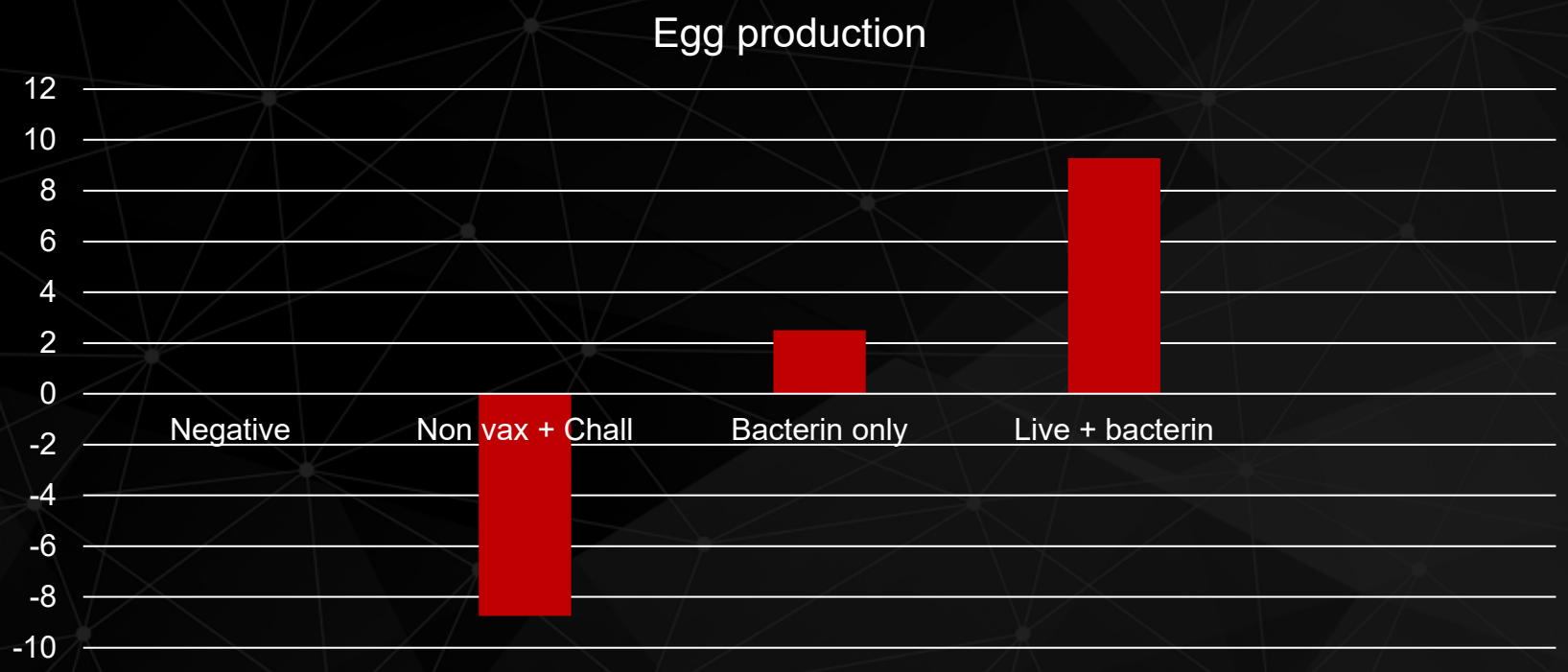
Egg production

Combined program limited egg production drops

3 New combined vaccination strategy



Change in egg production following challenge with R-strain at 41 weeks of age; expressed as percentage change between 10 days before challenge and 10 days after challenge.

Summary results

Combined program (1L+2K) versus killed vaccination program

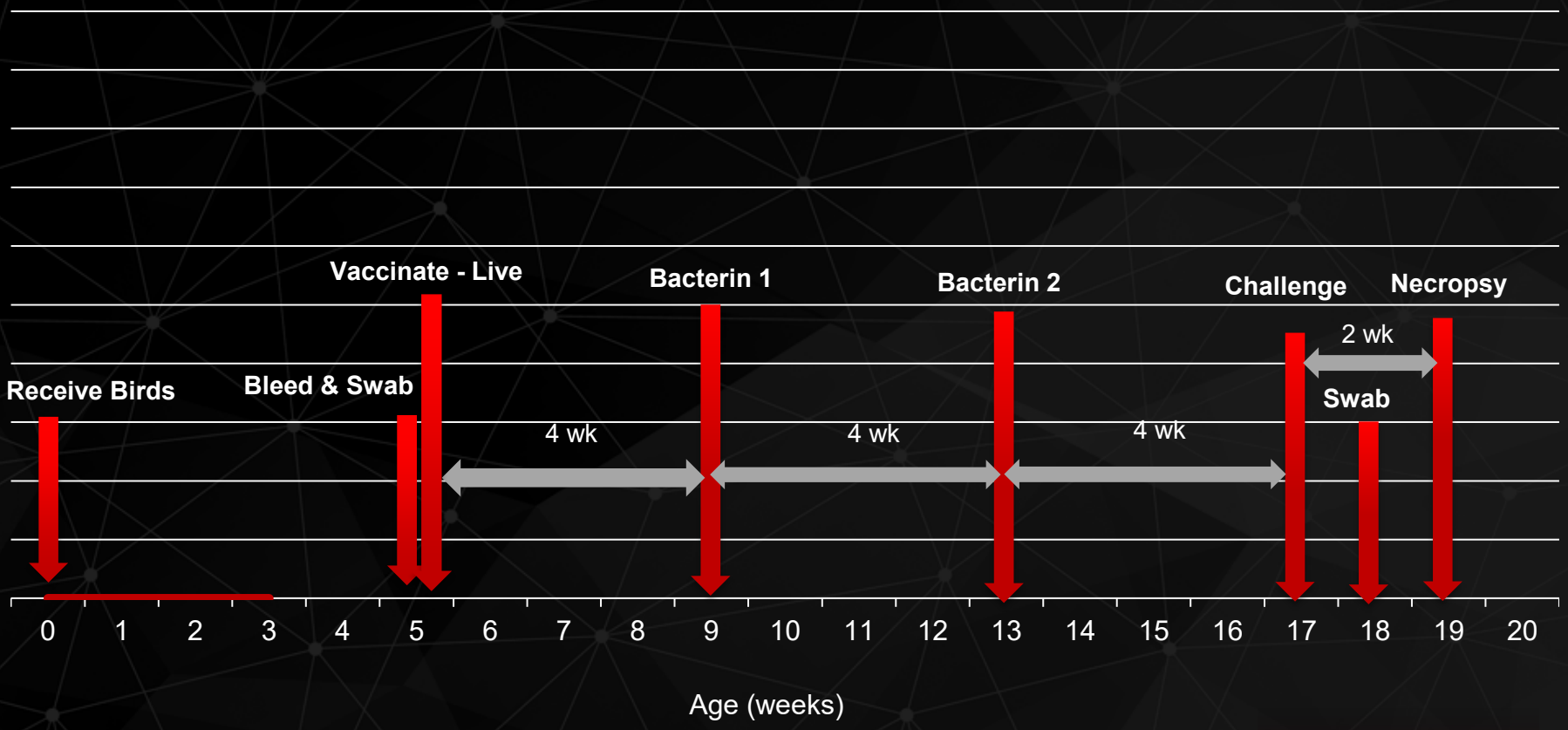
3 New combined vaccination strategy

Combined program (1L+2K) was superior to the killed vaccination program in the following parameters:

- Higher peak of antibody response
- Lower percentage of air sac lesions
- No presence of ovarian regression
- The highest number of eggs (not statistically significant)

Experimental Design 2

3
New combined vaccination strategy



Parameters evaluated

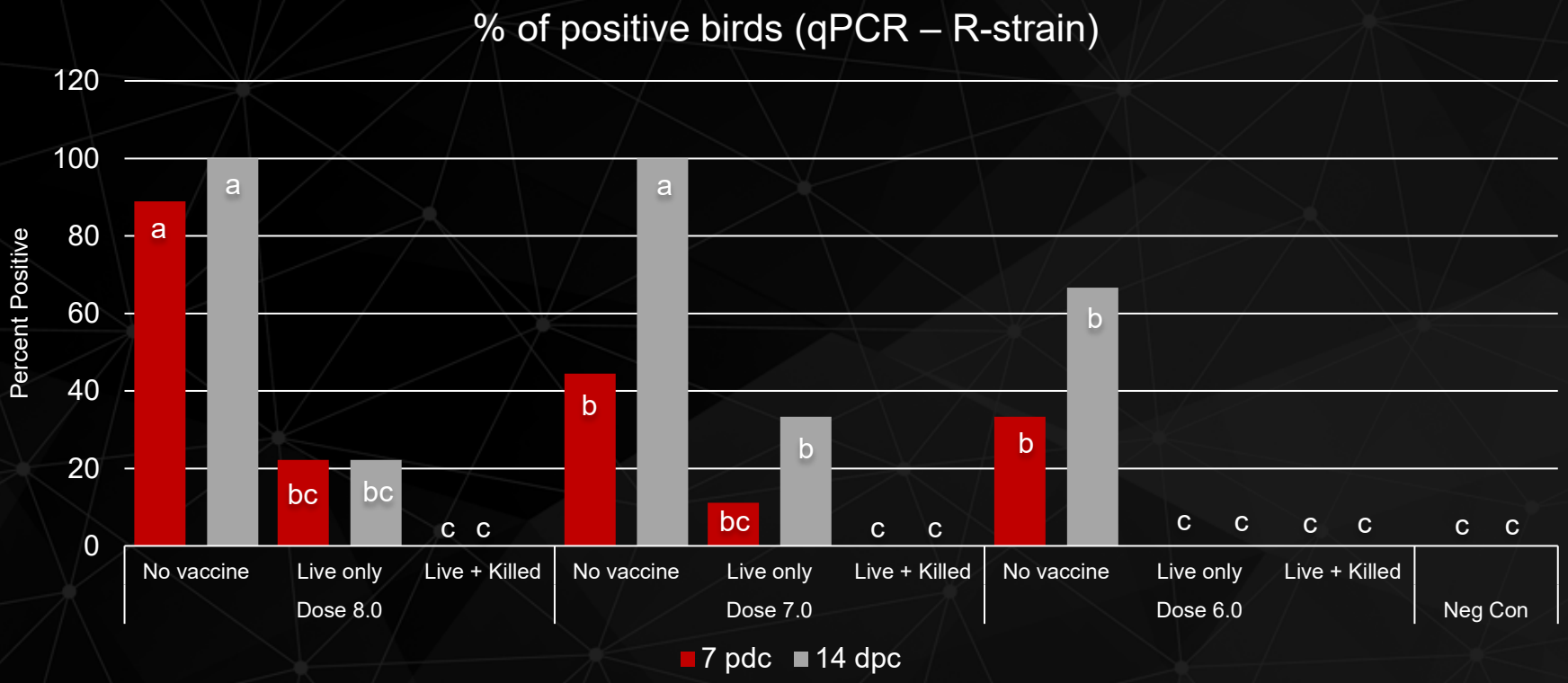
- MG colonization – quantitative PCR (trachea)
  - 7 dpc and 14 dpc
- Air sac lesion scores

Quantitative PCR for R-strain

Combined program increases resistance of birds to infection

3

New combined vaccination strategy



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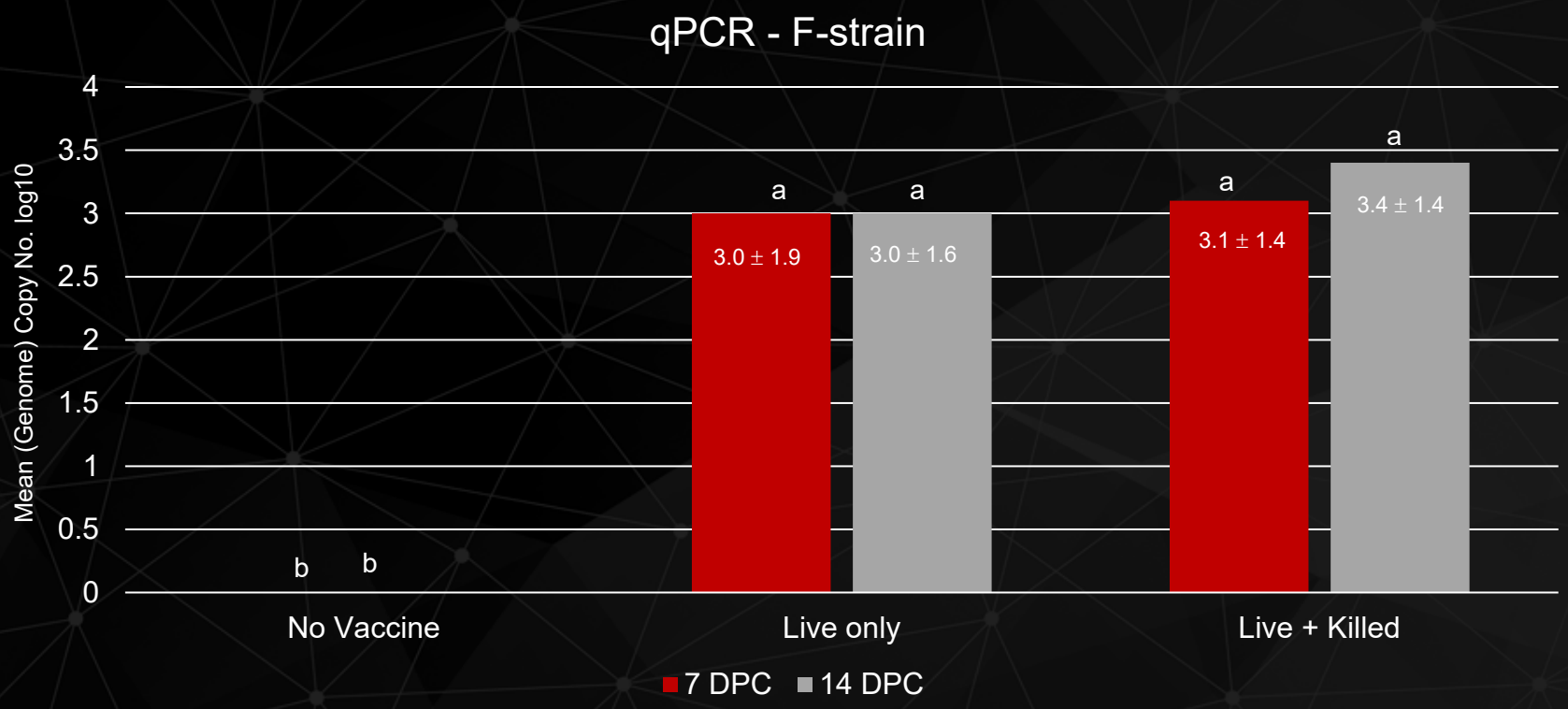
Quantitative PCR for F-strain

Effective live F strain replication

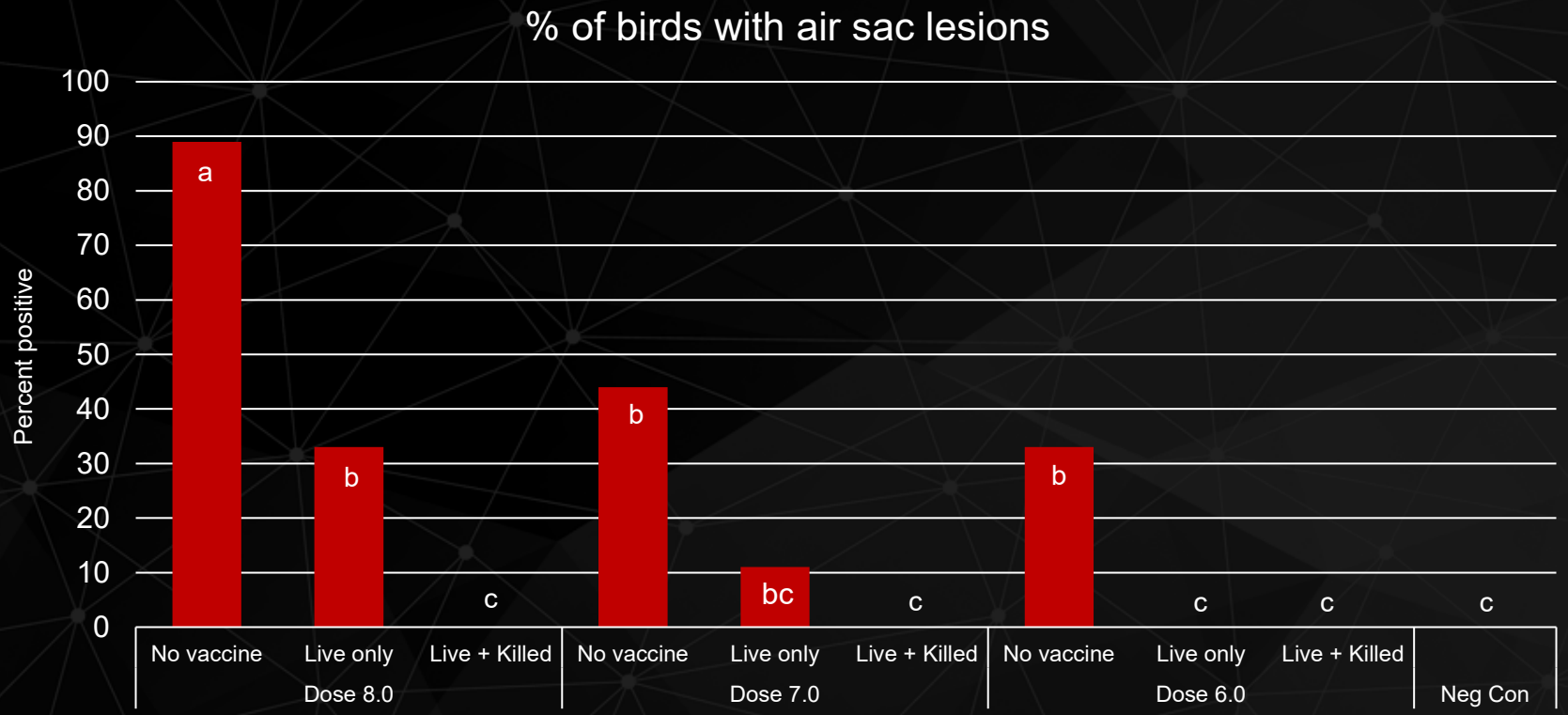
3 New combined vaccination strategy



Air Sac Lesions

Combined program provided stronger protection against air-sac lesions

3 New combined vaccination strategy



Summary results

Combined program (1L+2K) versus live vaccination program

- Live+ Killed Program increases resistance of birds to infection ($P < 0.05$).
- Lower percentage of air sac lesions in group vaccinated with live plus killed groups ($P < 0.05$).
  - The combined program performed better than the single live vaccination program which until now was considered to provide the best protection.

Conclusions

3
New combined vaccination strategy

Recent research, supported by Elanco, is the first to demonstrate the efficacy of a combined MG vaccine approach.

New combined MG vaccine approach provided:

Greater reduction of MG replication.

Stronger protection against lesions in the respiratory tract.

Combined MG vaccination protocols (1L+2K) provide the best protection against the *Mycoplasma* challenge.

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THANK YOU

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