Introduction

- Infectious bronchitis virus (IBV), is the etiological agent of IB which impairs the respiratory and urogenital tracts of chickens.
- After vaccination, an effective and long-lasting protection against IBV infection requires the activation of both effector & memory humoral (HMI) and cell-mediated immunity (CMI).
- The correlation of development of HMI and CMI with protection in chickens experimentally challenged with IBV have been reported in many studies.
- But the kinetics and relationship between local and systemic HMI and CMI induced by different IBV vaccination programmes remains to be better understood.
- In this study, the protection provided by two live IBV vaccines was assessed against Q1 IBV in broiler chicks in parallel with the progression of cell-mediated and humoral immune responses.
# Materials and Methods

## Samples collection and Laboratory methods:

- **At 0, 4, 7, 14, 21 and 28 days of age (doa):**
  - **Sera:** To detect specific anti-IBV antibodies using a commercial IBV ELISA kit.
  - **Lachrymal fluid and tracheal washes:** For local specific immunoglobulin A (IgA) using commercial IgA chicken ELISA kit.
  - **Peripheral Blood:** To determine the percentage of T-lymphocyte subpopulations using flow cytometry.
  - **Trachea:** Immunohistochemical detection of CD4+, CD8+ and IgA+ cells in tracheal sections.
  - **Challenge:** At 28 days of age, 10 birds from each group were challenged via ocular-nasal route with the Q1 \((10^{4.0} CD50/bird)\).
  - **After 5 days post challenge (dpc)**
  - **Trachea:** For ciliostasis examination.
  - **Kidney and trachea:** Processed for viral load by RT-qPCR and histopathological examination.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Vaccine type and age of vaccination</th>
<th>Volume inoculated and route of vaccination</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>40</td>
<td>H120</td>
<td>100μl, Oculonasally</td>
</tr>
<tr>
<td>II</td>
<td>40</td>
<td>H120+CR88</td>
<td>100μl, Oculonasally</td>
</tr>
<tr>
<td>III</td>
<td>40</td>
<td>Sterile water</td>
<td>100μl, Oculonasally</td>
</tr>
</tbody>
</table>

- **Vaccine type and age of vaccination:**
  - **1 day of age**
  - **14 days of age**
  - **Volume inoculated and route of vaccination:**
  - **H120**
  - **CR88**
  - **Sterile water**
The IgA levels in group II were significantly higher (P>0.05) than group I from 14 doa until 28 doa (the day of challenge).
Results

Immunohistochemical detection of CD4+ cells (a) and CD8+ cells (b) in tracheas of chickens

- **a) CD4+ T cells**
  - At 28 doa, cell count was higher in group II, without statistical significance (P<0.05).

- **b) CD8+ T cells**
  - The number of CD8+ cells were significantly higher in group II than group I at 21 and 28 doa.
Conclusions

- The cytotoxic T cell response in the tracheal tissues and level of IgA in lachrymal fluid were significantly higher in the group vaccinated with H120 and CR88 at day-old followed by CR88 at 14 doa (Group II) in comparison to group vaccinated with H120 alone at day-old followed by CR88 at 14 doa (Group I).

- Combined vaccination of H120 and CR88 of day-old chicks followed by CR88 at 14 doa (Group II) showed higher protection against Q1 owing to higher cytotoxic T cell response in the tracheal tissues and a high level of IgA in lachrymal fluid.

- This study shows the benefits of assessing local and cellular immune response besides serum antibodies in IBV vaccination-challenge studies.

Percentage ciliary protection: Group I, 90%; Group II, 97%; Group III, 12%.

At 5dpc, significant histopathological changes occurred in group III (with mean scores of 10.2) compared to group I & II.

In the kidney, histopathological changes were low compared to tracheal samples.