Co-infection of Fowl Cholera with bacterial and viral infection in poultry flocks

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Abstract

This study explores the co-infection dynamics of Pasteurella multocida (P.multocida) in poultry, focusing on Avian Leukosis Virus (ALV), Mycoplasma gallisepticum (MG), and Chicken Anemia Virus (CAV). Co-infections pose significant challenges to poultry health, leading to increased morbidity and mortality rates. The combined impact of co-infection exacerbates respiratory issues, weakens the immune system, and leads to intricate clinical manifestations. Samples from poultry flocks in Telangana and Haryana states of India, suspected for fowl cholera, were collected during 2019–2021. The study confirms co-infections using conventional PCR techniques targeting specific gene regions. Clinical symptoms exhibited by infected birds are described for each pathogen. The epidemiology of co-infections is discussed, and the importance of understanding these dynamics for effective control and prevention strategies is emphasized. Results reveal consistent co-infections over the study period, highlighting the need for further investigations into associations with parasites, bacteria, fungi, or viruses. The study underscores the importance of comprehensive biosecurity measures, vaccination programs, and early detection for managing poultry co-infections and ensuring sustainable production.

Introduction

Poultry co-infections refer to the simultaneous presence of multiple infectious agents within a population of birds. Poultry farms are susceptible to a variety of pathogens, and co-infections can occur when birds are exposed to more than one disease-causing agent concurrently (Sid et al., 2015). This complex scenario can lead to increased morbidity and mortality rates among the poultry, posing significant challenges for farmers and the poultry industry as a whole (Kong et al., 2022).

The co-infection of Mycoplasma gallisepticum and H3N8 low pathogenic avian influenza virus (LPAIV) in chickens poses a complex challenge for poultry health. MG causes chronic respiratory disease, leading to significant economic losses, while H3N8 LPAIV affects the respiratory and digestive systems. The synergistic effects of co-infection exacerbate respiratory issues, increasing susceptibility to secondary infections. This interplay prolongs and intensifies the disease, resulting in decreased feed efficiency, reduced egg production, and an overall decline in the performance of affected flocks (Stipkovits et al., 2012).

The co-infection of chickens with avian influenza virus H9N2 and the Moroccan Italy 02 infectious bronchitis virus presents a multifaceted challenge to poultry health. Both pathogens, individually significant, can intensify respiratory and systemic issues when concurrent. This co-infection may lead to increased susceptibility to secondary infections, complicating the clinical course. Potential outcomes include respiratory distress, decreased egg production, and compromised flock performance. Understanding the intricate mechanisms of synergistic effects is crucial for devising effective control and prevention strategies (Belkasmi et al., 2020).

Respiratory syndromes (RS) in poultry result from complex interactions between pathogens and environmental factors. In broiler chickens, key contributors to RS include low pathogenic avian influenza A viruses, metapneumoviruses, infectious bronchitis virus, infectious laryngotracheitis virus, Mycoplasma spp., Escherichia coli, and Omithobacterium rhinotracheale in turkeys. Respiratory outbreaks may also involve Aspergillus sp., P multocida, Avibacterium paragallinarum, or Chlamydia psittaci. The multifaceted nature of these interactions highlights the complexity of respiratory syndromes in poultry (Croville et al., 2018).

Avian leukosis, known as avian leukosis complex (ALC), encompasses various neoplastic diseases in chickens, with lymphoid leukemia being the most prevalent. Co-infection of P multocida and Avian Leukosis Virus poses a significant challenge to the poultry industry, impacting bird health and overall production. P multocida causes respiratory and systemic infections, while ALV, a retrovirus, induces cancer in chickens. Their co-infection results in severe consequences, including respiratory distress from Pmultocida and immunosuppression from ALV, increasing susceptibility to infections. This synergy leads to complex clinical presentations, with birds exhibiting respiratory signs, reduced immunity, and potential tumor development. The combined effect results in higher mortality rates, reduced egg production, and economic losses for poultry farmers (Bieley, 1943; Zheng et al., 2022).

Chicken Infectious Anemia (CIA), resulting from the chicken anemia virus, is an immunosuppressive disease in poultry characterized by aplastic anemia, immunosuppression, growth retardation, and atrophy of lymphoid tissue(Liu et al., 2022; Pope,
The co-infection of poultry with *P. multocida* and Chicken Anemia Virus poses a significant threat to the poultry industry, affecting bird health and overall production. *P. multocida* primarily targets the bone marrow, leading to anemia and immunosuppression. CAV induces anemia, weakness, and increased susceptibility to infections. The combination of these infections results in a more complex and severe clinical presentation. Respiratory symptoms caused by *P. multocida* worsen the anemic condition induced by CAV, leading to higher mortality rates and decreased flock performance. Additionally, CAV's immunosuppression enhances the severity and persistence of *P. multocida* infections, making the co-infection challenging to manage (Liebhart et al., 2023).

*Mycoplasma gallisepticum*, a bacteria devoid of a cell wall, is the causative agent of Chronic Respiratory Disease (CRD). CRD is marked by symptoms such as coughing, respiratory rales, nasal discharge, infraorbital sinusitis, and air sacculitis. This disease has been documented in layers and breeders, leading to embryo mortality and notable declines in egg production (Saif and Jarosz, 1978). *P. multocida* causes respiratory and systemic infections, while *Mycoplasma gallisepticum* leads to respiratory, ocular, and reproductive issues. The co-infection results in severe and complex consequences, including respiratory distress, nasal discharge, ocular lesions, and systemic infections. This combination presents a complicated clinical picture, with birds exhibiting a range of respiratory and systemic signs (Paudel et al., 2017). This co-infection often results in increased morbidity, decreased egg production, and economic losses for poultry farmers. The respiratory symptoms, coupled with potential reproductive issues caused by *Mycoplasma gallisepticum*, can significantly impact the overall flock health and productivity (Boulianne et al., 2020; Fatoba and Adeleke, 2019).

The Indian poultry sector struggles with several challenges that must be addressed to assure sufficient egg and meat production (Hafez and Attia, 2020). Co-infections in poultry may involve various combinations of viral, bacterial, parasitic, or fungal pathogens (Yehia et al., 2023). The interactions between these agents can result in more severe clinical symptoms, immune system suppression, and difficulties in disease diagnosis and management (Gowthaman et al., 2019). Parasite-induced infections not only incur significant financial burdens through mortality rates but also entail costs related to preventive measures and control efforts. The stress induced by co-infections can compromise the overall health and welfare of the birds, ultimately resulting in economic losses for poultry farmers (Attia et al., 2022; Meng et al., 2018).

The aim of the present study was to understand the co-infections of *P. multocida* with Avian Leukosis virus, *Mycoplasma Gallisepticum* and Chicken Anemia Virus based on the field samples suspected for fowl cholera, avian leukosis, mycoplasmosis and Chicken anaemia infections.

**Materials and methods**

**Samples for the study:**

The archived homogenized chicken organ samples and glycerol preserved isolates that were positive for *P. multocida* infection in addition to a suspected co-infection with another virus or bacteria during the study period of 2019–2021 from poultry flocks were included in this study. The study samples were obtained from Telangana and Haryana states of India, where veterinarians have suspected for different bacterial and viral infections in poultry flocks based on the respective symptom of the disease. All these organ samples were homogenized and confirmed for the infection by performing conventional PCR at Globion India Private Limited. The present study was reviewed and approved by the Institutional Ethical Committee.

**Suspected disease and samples collected:**

Three samples were included in this study, a sample each from three consecutive years 2019–2021. All three samples are positive for fowl cholera infection caused by *P. multocida* and each suspected for one of the other three different infections. Based on the underlying symptoms, veterinarians had collected the organ samples and sent the samples to the laboratory for testing. The suspected co-infection samples along with *P. multocida* infection including the yearly and region wise data represented in the table.
Table 1
Yearly and region wise distribution of suspected co-infection samples

<table>
<thead>
<tr>
<th>Year</th>
<th>Region</th>
<th>Sample number</th>
<th>Suspected for</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019</td>
<td>Telangana</td>
<td>Sample-1</td>
<td>P.multocida, ALV</td>
</tr>
<tr>
<td>2020</td>
<td>Telangana</td>
<td>Sample-2</td>
<td>P.multocida, MG</td>
</tr>
<tr>
<td>2021</td>
<td>Haryana</td>
<td>Sample-3</td>
<td>P.multocida, CAV</td>
</tr>
</tbody>
</table>

i. *Avian Leukosis virus* (ALV) infection: Liver & Femur bone  
ii. *Mycoplasma Gallisepticum* (MG) infection: Liver, Kidney, Nasal swab, Air Sac swab, & Long bone  
iii. *Chicken Anemia Virus* (CAV) infection: Liver, Spleen, Thymus, & Femur bone

Nucleic acid extraction:

The genetic material of ALV virus is RNA, so RNA was extracted from liver sample for ALV detection using SPIneasy® RNA Kit for Tissue (Without Lysing Matrix), (MP Bio- Medicals, California); DNA was extracted from liver, spleen, and thymus for CAV detection and from nasal swab & air-sac swab for MG detection using QIAamp® DNA mini kit (QIAGEN, Hilden, Germany), DNA was extracted from the glycerol preserved isolates using Himedia genomic DNA purification kit (HiPurA® Bacterial Genomic DNA Purification Kit). All the above extraction methods were performed as per manufacturer’s instructions.

Confirmation of co-infection:

The study focused on confirmation of all four infections in this study along with the *P.multocida* infection by performing polymerase chain reaction (PCR) targeting specific gene regions to detect the suspected infections.

Polymerase chain reaction:

cDNA synthesis was performed using RevertAid™ First Strand cDNA Synthesis kit (Thermo Scientific™, Waltham, Massachusetts, United States) for RNA extracted from ALV suspected homogenized liver sample using Veriti™96-Well Fast Thermal Cycler, (Applied Biosystems™, Foster City, California, USA). Conventional PCR was further performed for direct DNA and cDNA samples. The PCR was carried out using the primers targeting the 23s rRNA for *P. Multocida* earlier published by Miflin et.al, the gp85 env gene for ALV earlier published by Daniel et.al, the c2 gene for MG earlier published by Abdelwhab et.al & the orf2 gene for CAV earlier published by Dr Holger Klaproth (unpublished data), usingVeriti™96-Well Fast Thermal Cycler, (Applied Biosystems™, Foster City, California, USA) (Miflin and Blackall, 2001; Häuptli et al., 1997; Abdelwhab et al., 2011; Holger, 2013). Amplified products underwent analysis through electrophoresis on a 1.8% agarose gel, employing Ethidium Bromide as an intercalating dye for band visualization. The observed bands were examined using a UV transilluminator. Details regarding the PCR primers, the anticipated product size and cycling conditions of PCR were presented in table no.1.
### Results

Clinical symptoms presented by chickens:

The infected birds that were selected for the study presented with the characteristic clinical symptoms for each of the infection such as runny nose, cough or unusual breathing sounds, and swollen or puffy eyelids and face for *Mycoplasma Gallisepticum* infection, inappetence, weakness, diarrhea, dehydration, and emaciation, become depressed before death, enlarged bursa, enlarged liver, laying fewer eggs for Avian Leukosis virus infection, and a pale comb, wattle, eyelids, legs and carcass, anorexia, weakness, stunting, unthriftiness, weight loss, cyanosis, petechiation and ecchymoses, lethargy, and sudden death for Chicken Anemia virus. Whereas, all the chickens suspected for co-infection presented with ruffled feathers, a faster breathing rate, and later on, diarrhoea, anorexic, dull, and melancholic post-mortem examination revealed petechial haemorrhages, overall hyperaemia, and an enlarged liver with necrotic foci that are characteristic symptoms for *P. multocida* infection.

Epidemiology of the study samples:

Among the three samples selected in this study presented with co-infection along with *P. multocida* during 2019–2021, two samples were from Telangana region collected in 2019 & 2020 suspected for the infection with ALV & MG respectively. In 2021 the sample from Haryana region was suspected for CAV by the veterinarian.

Conventional PCR:

PCR was conducted for the cDNA for ALV & DNA for MG, CAV, and *P. multocida*. The PCR assays were performed with a positive and negative control for each assay. The expected band sizes were observed to be same for each of the assays when compared with the positive controls as 466bp for ALV, 300bp for MG, 350bp for CAV, 1432bp for FC as represented in Fig. 1.

Discussion

While numerous reports exist regarding co-infections in poultry involving various viral and bacterial agents, particularly fowl cholera disease, there is a notable scarcity of studies specifically addressing the co-infection of Avian Leukosis Virus, *Mycoplasma gallisepticum*, and Chicken Anemia Virus in chickens and there is an absence of detailed information regarding the clinicopathological patterns exhibited by these two agents during natural co-infection (Fatoba and Adeleke, 2019; Nishitha et al., 2021; Stipkovits et al., 2012; Zheng et al., 2022).

Despite the elevated prevalence observed in various neighbouring Indian countries, there exists limited information pertaining to the prevalence and circulation of diverse respiratory pathogens within Indian poultry flocks. This research contributes valuable
insights by employing molecular methods to confirm the separate detection of *P. multocida* with ALV, MG, and CAV. Notably, this study marks the inaugural identification of *P. multocida* and ALV in the Indian context. Examination of liver samples disclosed that all examined flocks exhibited positivity for more than one pathogen, with fowl cholera consistently detected across all instances (Nishitha et al., 2021).

Research findings have highlighted the substantial role of *P. multocida* in instigating respiratory and systemic infections in poultry, resulting in economic losses and adversely affecting the overall productivity of poultry farms. Renowned for its capacity to induce various diseases such as respiratory infections, septicemia, and other systemic manifestations, this bacterium poses a considerable threat to the poultry industry (Ookanti, 2022).

Effective management of poultry co-infections involves implementing comprehensive biosecurity measures, vaccination programs, and proper hygiene practices on farms. Additionally, regular monitoring and surveillance for early detection of potential pathogens can aid in preventing the spread of diseases and minimizing the impact of co-infections (Hafez and Attia, 2020). Understanding the dynamics of co-infections in poultry is crucial for developing targeted control strategies and maintaining the overall health and productivity of poultry flocks (Adams et al., 2023).

The co-infection of *P. multocida* with ALV, MG, and CAV (with each separately) represents a significant departure from the traditional understanding of individual pathogen impact in poultry. The investigation paves the way for additional scientific inquiry into the complex interplays among these pathogens, delving into potential complementary effects that could amplify the severity of diseases. Understanding the mechanisms underlying such co-infections is crucial for developing effective control and prevention strategies to safeguard poultry health and ensure sustainable production.

**Conclusion**

This research reveals the presence of *P. multocida* co-infections. Over the three consecutive years (2019, 2020, and 2021), we observed co-infections, encompassing both bacterial and viral co-occurrences alongside *P. multocida* infections in poultry. Consequently, additional investigations into co-infections are essential to comprehend potential associations between *P. multocida* and other pathogens such as parasites, bacteria, fungi, or viruses.

**Declarations**

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Conflict of Interest: The authors declare no conflict of interest.

**Contributions**

O.S.K. conceptualization, methodology, resources acquisition, writing—original draft, B.S.S writing—review & editing, formal analysis, data curation, V.Y. supervision, writing—review & editing, M.B. supervision, formal analysis, writing—review & editing.

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Figures

Figure 1

A. *P. multocida* PCR gel image B. ALV, MG, CAV gel image

*S: Sample