Infectious Coryza Modelling in Chickens Using Indonesian *Avibacterium paragallinarum* Isolates

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Abstract. *Avibacterium paragallinarum* is the causative agent of infectious coryza in chickens, characterized by reduced egg production and elevated morbidity. Despite the extensive reporting of this illness, data on the pathogenicity and infection dynamics of local Indonesian isolates are limited. This research aimed to evaluate the infection model for local Indonesian isolates of *A. paragallinarum* in chickens. Four groups of chickens were utilized: a control group, an *A. paragallinarum* inoculation group, a *Staphylococcus aureus* coinfection group, and a natural transmission group. The bacteria were inoculated into the infraorbital sinus, and clinical symptoms were observed, daily average scores were recorded, and confirmation was performed by PCR using HPG-2 primers. The test findings demonstrated mild clinical impacts, including swelling of the infraorbital sinus, in the inoculated group, while the natural transmission group showed no clinical symptoms. The mean daily score in the *A. paragallinarum* inoculation group rose from 0.3 on day 7 to 0.4 on day 14. Simultaneously, the coinfection cohort showed a decline in score from 0.3 on day 7 to 0.1 on day 14. The HPG-2 PCR test identified the infection prior to the onset of clinical manifestations. The peak of infection occurred on day 7, with 6 of 10 chickens in the inoculation group, 10 of 10 in the coinfection group, and 8 of 10 in the natural transmission group positive. The results validate that local Indonesian *A. paragallinarum* isolates exhibit varying levels of pathogenicity, and the infection model developed may represent disease dynamics for further study and the development of management strategies.

1 Introduction

Infection Coryza (IC) is a significant respiratory disease in poultry caused by *Avibacterium paragallinarum* (*A. paragallinarum*). This disease is not directly related to public health, as the infection is limited to poultry. The mortality rate of this disease is low, but the morbidity rate is high. In laying hens, egg production reductions of up to 40% have been reported [1]. In recent years, IC has occurred worldwide, including Indonesia, but the data on the prevalence and molecular characterization of this disease are still limited. So far, *A. paragallinarum* has been successfully identified from swab samples of layer chickens with coryza symptoms in the Sleman area of Yogyakarta [2].

A. paragallinarum is divided into three serogroups: A, B, and C. Meanwhile, the Kume classification further divides the bacterium into nine serovars: A-1, A-2, A-3, A-4, B-1, C-1, C-2, C-3, and C-4 [3]. Both classifications are determined by the results of the hemagglutination inhibition (HI) test. However, the HI test for serotypes is quite complicated and requires the production of hemagglutination antigens and reference antisera. Serovar differentiation is critical because cross-protection among different serovars has not been consistently demonstrated [4]. Vaccination programs need to be improved to prevent the spread of the disease. Many commercial vaccines for coryza have been distributed in Indonesia, both imported and local. However, test results show that the use of commercial vaccines in the field still does not provide optimal immunity against *A. paragallinarum*. Although antimicrobial therapy can be used to manage infectious coryza, certain antibiotics primarily reduce the severity and duration of clinical signs rather than achieving complete pathogen elimination.

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The incidence of infectious coryza in developing countries remains challenging due to multiple contributing factors. The disease burden may be exacerbated by coinfections with other pathogens, including *Escherichia coli, Pasteurella multocida, Bordetella avium,* and *Mycoplasma gallisepticum* [5]. Clinically affected chickens typically present with infraorbital sinus exudate, conjunctivitis, and unilateral or bilateral facial swelling during the acute stage, whereas more advanced cases may exhibit serous to caseous sinusitis. In severe and prolonged infections, the condition may progress to more complex manifestations, such as chronic respiratory disease, swollen head syndrome, airsacculitis, tarsal arthritis, and septicemia [6]. This disease can spread rapidly from one chicken to another in a flock or from one flock to another. Direct transmission occurs through contact between infected or carrier and susceptible chickens. Reference and field strains have been shown to vary in pathogenicity. Therefore, this study aimed to evaluate the infection model of Indonesian field isolates of *A. paragallinarum* in chickens.

2 Materials and Methods

2.1 Animal ethics

All experimental procedures involving chickens were reviewed and approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, with approval number 293/KEH/SKE/II/2025.

2.2 Bacteria

The bacteria used in this study were obtained from an archived isolate maintained at PT Medika Satwa Laboratoris. The bacteria were inoculated onto blood agar and showed satellite-shaped colonies. Confirmation with PCR-HPG 2 identified the isolate as *Avibacterium paragallinarum*. Colonies were suspended in sterile phosphate-buffered saline (PBS) and adjusted to the required concentration. All experimental infections were performed once at the beginning of the study.

2.3 Infection modeling *Avibacterium paragallinarum*

In the infection modeling, 40 Hy-Line Brown layer chickens aged 4 weeks were divided into four groups, as shown in Table 1. Chickens in group P1 were challenged with 0.1 ml of inoculum (1×10^8 colony-forming units (CFU) per ml) through the infraorbital sinus of both the left and right eyes. Group P2 was challenged with inoculum (0.1 ml; 1×10^8 colony-forming units (CFU) per ml) mixed with accompanying bacteria, namely *Staphylococcus aureus*. Group P3 was not inoculated but was placed in the same cage as groups P1 and P2. In contrast, the control group received no treatment. The severity of nasal discharge and facial swelling in infected chickens was evaluated using a clinical scoring systems as follows: score 0, no observable clinical signs; score 1, mild nasal discharge or slight swelling of the infraorbital sinus; score 2, nasal discharge accompanied by infraorbital sinus swelling; and score 3, marked infraorbital sinus swelling with or without conjunctivitis, associated with depression and isolation behavior [7]. Clinical symptoms were evaluated on days 2, 5, 7, and 14 post-infection using the standardized scoring system described above. At the same time points, infraorbital sinus swabs were obtained from each chicken for PCR testing.

Table 1. Treatment groups for modeling *A. paragallinarum* infection

<table>
<tr><th>Group</th><th>Treatment</th><th>Number of chickens</th><th>Pathogen preparation</th><th>Administration Route</th><th>Observation</th></tr>
<tr><td>P0</td><td>Control</td><td>10</td><td>Without treatment</td><td rowspan="4">Infraorbital sinus in the left and right eyes</td><td rowspan="4">Days 2, 5, 7, and 14 post-infection</td></tr>
<tr><td>P1</td><td>A. paragallinarum infection</td><td>10</td><td>0.1 ml inoculum</td></tr>
<tr><td>P2</td><td>A. paragallinarum + Staphylococcus aureus infection</td><td>10</td><td>0.1 ml inoculum</td></tr>
<tr><td>P3</td><td>Transmission</td><td>10</td><td>Without treatment</td></tr>
</table>

https://doi.org/10.1051/bioconf/202622901006

2.4 DNA extraction

Bacterial DNA was extracted using the boiling method. Suspected *Avibacterium paragallinarum* colonies were suspended in 500 µL of nuclease-free water in a sterile microcentrifuge tube. The suspension was heated to 98°C for 10 minutes in a dry-bath incubator, followed by freezing for 5 minutes. The samples were then centrifuged at 10,000 rpm for 1 minute. Subsequently, 50 µL of the supernatant containing genomic DNA was collected and stored at -20°C until further analysis.

2.5 Species-specific PCR

PCR amplification was performed according to the protocols described by Chen et al. (1996) and Putra et al. (2023), with minor modifications [2,8]. The total reaction volume was 50 µL, consisting of 25 µL MyTaq HS Red Mix, 2 µL of each forward and reverse HPG-2 primer, 16 µL nuclease-free water, and 5 µL of DNA template. Amplification was performed in a thermal cycler under the following conditions: an initial denaturation at 95°C for 1 minute, followed by 30 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at 72°C for 10 seconds. PCR products were analyzed by agarose gel electrophoresis in 1× TAE buffer and stained with ethidium bromide for visualization. The primer sequences used were as follows: forward (F) 5′-TGAGGGTAGTCTTGCACGCGAAT-3′ and reverse (R) 5′-CAAGGTATCGATCGTCTCTCTACT-3′.

2.6 Data analysis

Observation results were recorded and calculated in the form of a total score. The average daily clinical score was determined by dividing the cumulative daily score by the total number of chickens in each group. Data were analyzed descriptively using figures, tables, and graphs depicting the observation results and PCR electrophoresis visualization.

3 Results

Isolates were inoculated into the infraorbital sinuses of the left and right eyes. Groups P1 and P2 began to show clinical symptoms on day 5. Meanwhile, group P3 showed no clinical symptoms until day 14 of observation. The control group (P0) did not exhibit any observable clinical signs. The symptoms were mild with swelling in the infraorbital sinus. The average daily score in group P1 increased from day 7 to day 14. Meanwhile, group P2 experienced a decrease in the score on day 14 (Figure 1).

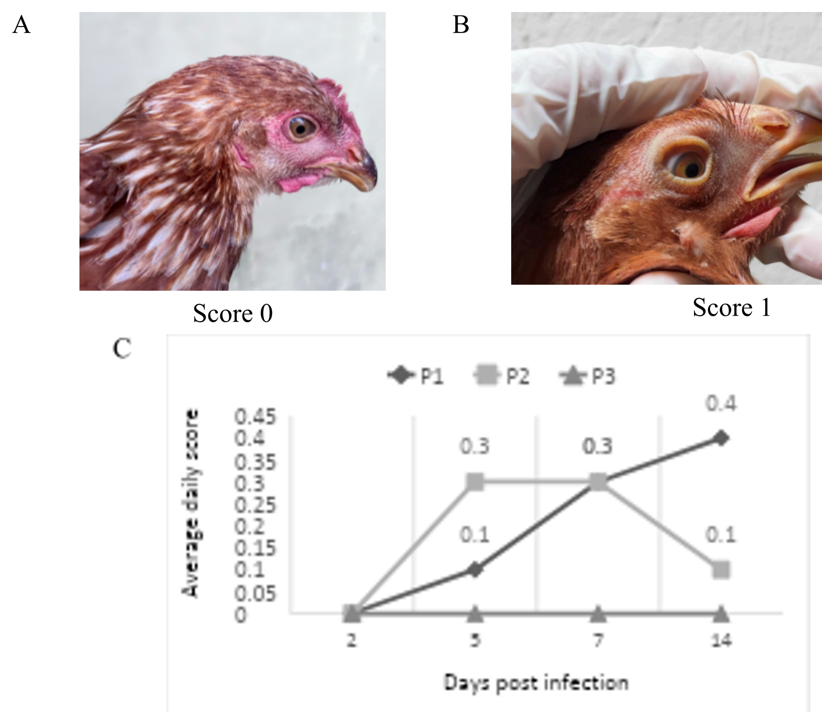

Fig. 1. Results of clinical symptom observations. (A) Clinical symptoms with a score of 0 (no clinical symptom), (B) Clinical symptoms with a score of 1 (mild), and (C) Graph of the average daily score of clinical symptoms.

Table 2. Results of modeling *A. paragallinarum* bacterial infection

Group	Number of chickens	Age (weeks)	Observation result post-infection							
			Day 2		Day 5		Day 7		Day 14	
			CS	PCR	CS	PCR	CS	PCR	CS	PCR
P0	10	4	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
P1	10	4	0/10	4/10	1/10	6/10	3/10	6/10	4/10	1/10
P2	10	4	0/10	4/10	3/10	5/10	3/10	10/10	1/10	1/10
P3	10	4	0/10	3/10	0/10	4/10	0/10	8/10	0/10	0/10

Abbreviations: CS = clinical symptoms, PCR = *Polymerase Chain Reaction*

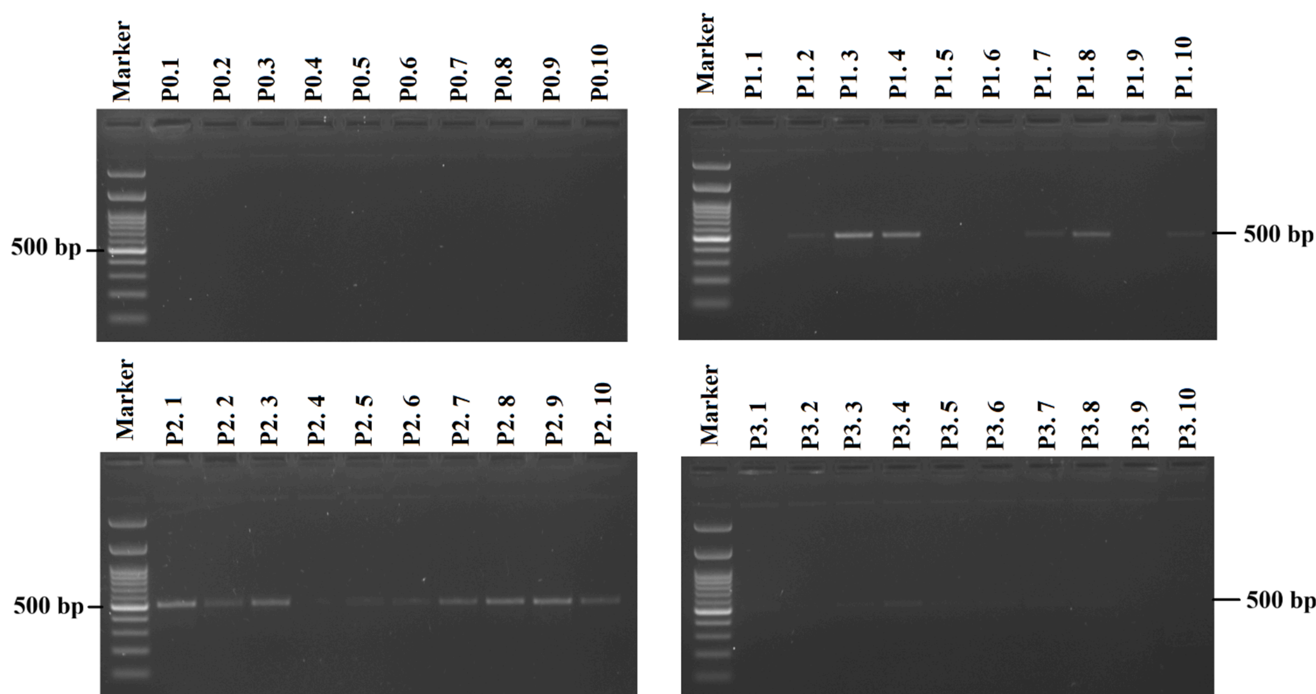

Fig. 2. HPG-2 PCR on day 7 of observation. Positive results are indicated by a 500 bp band.

HPG-2 PCR testing showed that several chickens in all three treatments tested positive for coryza on day 2, even though they had not yet shown any clinical symptoms. An increase in positive PCR results occurred on day 7, with 6 out of 10 chickens in group P1 and all chickens in group P2 (*Avibacterium paragallinarum* + *S. aureus*) testing positive (Figure 2). On day 14, the number of positive PCR chickens decreased significantly. Although chickens in group P3, or the transmission group, showed no clinical symptoms during the observation period, three out of 10 tested positive on day 2, increasing to eight on day 7 (Table 2).

4 Discussion

The results of this study indicate that the local isolates successfully produced mild clinical symptoms. The pathogenicity of *A. paragallinarum* strains varies between both field and reference strains. Morbidity rates depend on the strain; some strains cause 100% clinical symptoms, while others cause only mild symptoms and low morbidity. Furthermore, Soriano et al. (2004) [9] research indicates that virulence exists among serovars, with all serovars being pathogenic to chickens. The highest clinical symptom score was obtained by serovar C-1. Even serogroup B was reported not to produce clinical symptoms. Variations in results may be influenced by the route of infection, dose, and observation period. Another study reported that coryza cases lasted 7 days, with the highest clinical symptom score on day 3 and decreasing from day 5

post-infection [10]. Susceptible chickens that acquire infection through direct contact with infected individuals typically develop clinical manifestations within 24–72 hours. In cases without concurrent co-infection, the disease generally resolves spontaneously within approximately 2–3 weeks.

The inclusion of *Staphylococcus aureus* in group P2 was associated with a 100% PCR detection rate, despite a reduction in the mean clinical score from 0.3 on day 7 to 0.1 on day 14. This is likely influenced by the role of *S. aureus* in producing additional Nicotinamide Adenine Dinucleotide (NAD) for *A. paragallinarum,* increasing bacterial establishment during the acute phase of infection [11]. However, as the immune response progressed and the disease entered a recovery phase, clinical signs diminished despite continued molecular detectability of the pathogen. This findings indicate that PCR positivity does not directly correlate with disease severity and highlight the importance of integrating molecular and clinical parameters when interpreting infection dynamics.

Group P3 is intended to represent natural infection conditions. PCR positivity in this group may indicate a subclinical infection or carrier state, in which chickens harbor and intermittently shed the pathogen without overt clinical disease. IC is transmitted horizontally via direct contact and aerosol exposures. Birds that are carriers or have subclinical infections often remain asymptomatic but can intermittently shed through respiratory secretions. Consequently, subclinically infected and carrier birds play a critical role in the epidemiology and spread of IC [1]. Inoculation via the infraorbital sinus is a more powerful challenge method than natural exposure. Therefore, the score of naturally infected chickens should be counted twice the score of chickens infected via infraorbital sinus inoculation [12].

Several virulence factors also influenced the pathogenicity of *A. paragallinarum*, including hemagglutinin (HA), capsule, and toxin. The hemagglutinin protein is encoded by the *HMTp210* gene and plays an important role in hemagglutination activity and bacterial colonization. *A. paragallinarum* that lacks the *HMTp210* gene has been reported to lose HA activity and fail to induce HI antibodies when inoculated into chickens [13]. On the other hand, loss of the bacterial capsule has been shown to enhance hemagglutination and adhesion while reducing overall virulence in *A. paragallinarum*. The capsule is thought to be protective by preventing phagocytosis and complement-mediated killing, making it an important virulence determinant. However, the presence of a capsule may also hinder bacterial attachment to host cell receptors, thereby potentially interfering with effective colonization [14]. In addition, cytolethal distending toxin (CDT) is a virulence factor produced by various Gram-negative pathogenic bacteria. This toxin is typically composed of three subunits, designated CdtA, CdtB, and CdtC. *A. paragallinarum* carries the *cdtABC* gene cluster, which has been associated with DNA damage, cell cycle arrest, and apoptosis in host cells [15].

Overall, the pathogenicity of *Avibacterium paragallinarum* is influenced by multiple factors. Although experimental pathogenicity tests serve as valuable models for studying disease mechanisms, their outcomes should be interpreted cautiously when extrapolated to field conditions. Natural variables, such as environmental stressors, coinfections, exposure dose, and routes of infection, can markedly alter clinical manifestations. Continued identification and molecular characterization of local isolates are essential to enhance understanding of the pathogenic diversity and disease dynamics of *A. paragallinarum*.

5 Conclusion

The experimental infection model successfully demonstrated the pathogenic potential of the local *Avibacterium paragallinarum* isolate in chickens. The test findings demonstrated mild clinical impacts, including swelling of the infraorbital sinus. HPG-2 PCR results revealed that infection could be detected earlier than the onset of clinical signs, with the highest detection rate occurring on day 7. These findings indicate that the developed infection model effectively represents the clinical and molecular progression of mild signs of Infectious Coryza under experimental conditions and may serve as a valuable platform for future studies of vaccine efficacy and pathogenesis.

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