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TÍTULO DO TRABALHO: Evaluation Of Three Apec Field Isolates In Broiler Chickens For Experimental Colibacillosis Model Standardization

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RESUMO:

Evaluation of three APEC field isolates in broiler chickens for experimental colibacillosis model standardization

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Avian colibacillosis is a bacterial disease of major economic importance in intensive poultry production, characterized by systemic and respiratory infections caused by avian pathogenic *Escherichia coli* (APEC). The genetic and phenotypic variability among field isolates of *E. coli* directly influences their ability to colonize, disseminate, and induce lesions in the host. Therefore, identifying highly virulent strains is essential for the development of standardized experimental infection models. This study aimed to compare the infectivity and virulence of three APEC field isolates in broiler chickens to determine the most suitable strain for consistent reproduction of colibacillosis under experimental conditions. A total of 48

broiler chickens, 14 days old, were divided into three groups (n = 16): G1, G2, and G3. Chicks were challenged intratracheally with different APEC field isolates at a bacterial dose of 2×10^{12} CFU. Chicks were monitored daily for clinical signs and mortality. Fourteen days post-challenge, all chicks were necropsied, and air sacs, heart, liver, and spleen were collected for analysis. Tissues were fixed in 10% neutral buffered formalin for histopathological evaluation using hematoxylin and eosin (HE) staining. *E. coli* isolation was performed by enrichment in Brain Heart Infusion (BHI) broth at 37°C for 18–24 hours, followed by selective plating on Levine Eosin Methylene Blue (L-EMB) agar and incubation at 37°C for an additional 18–24 hours. Bacteriological analysis revealed differences among the challenged groups regarding the frequency of *E. coli* isolation from tissues. Group G3 exhibited the highest rates of bacterial recovery: 92.3% from air sacs, 100% from hearts, 75.0% from livers, and 85.7% from spleens. This was followed by group G2, with isolation rates of 91.7% (air sacs), 80.0% (hearts), 80.0% (livers), and 40.0% (spleens). Group G1 showed the lowest frequencies: 80.0% (air sacs), 72.7% (hearts), 57.1% (livers), and 28.6% (spleens). Histopathological examination revealed similar lesion patterns across all groups. All three field isolates were capable of establishing infection. However, the strain used in group G1 showed reduced tissue colonization, especially in the spleen, suggesting a lower ability to invade and persist systemically. In contrast, the notably high isolation rate of *E. coli* from the spleens of group G3 probably indicates a greater potential for systemic dissemination. Nevertheless, despite the evidence of septicemia, mortality rates and lesion severity were lower than desirable for a robust experimental infection model. In conclusion, the isolate used in group G3 exhibited the greatest ability to invade systemic tissues and produce colibacillosis-like lesions, making it the most promising candidate for use in standardized infection models. To better replicate the natural course of the disease, further studies will be conducted using this strain, applying higher bacterial doses, alternative inoculation routes, and/or the inclusion of immunosuppressive agents. A well-established experimental model is essential for advancing studies on APEC pathogenesis, vaccine evaluation, and therapeutic strategies in poultry.

Keywords: Avian pathogenic *Escherichia coli*; poultry; virulence; septicemia; experimental infection model.

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