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Research Article

Monitoring of Non-Vaccinated Chickens for Avian Metapneumovirus and Co-Infection with Avian Pathogenic Escherichia coli.

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Abstract

Brazil is the world's second-largest producer of broiler chicken, and monitoring avian diseases is crucial for both global nutrition and the economy. High rates of animal carcass losses from aerosacculitis are caused by avian metapneumovirus (aMPV) infection, and these effects can be exacerbated by co-infection with pathogenic bacteria, especially Escherichia coli (APEC). The current study assessed the clinical consequences of co-infection with APEC and the seroprevalence of the primary aMPV subtypes in unvaccinated broiler chickens from Brazilian poultry farms. A total of 1000 samples, including blood, respiratory swabs, femurs, liver, and spleen, were taken from 100 batches of poultry production. The history of respiratory and systemic clinical symptoms was taken into consideration when choosing the production batch. According to the findings, two lots tested positive for aMPV-B, and 20% of the lots had serological evidence of aMPV. Co-infection between aMPV and APEC was found in 45% of batches. The findings highlight the necessity of targeted vaccination campaigns, viral surveillance, and vaccination programs, all of which may lessen clinical issues and, in turn, the need for antibiotics to treat bacterial co-infections.

Keywords : *clinical signs; slaughter convictions; virus–bacteria co-infection; colibacillosis.*

INTRODUCTION

Global activity is constantly challenged by the spread of respiratory agents in poultry farming; losses are linked to a decline in zootechnical performance and have a direct impact on the afflicted animals' quality of life [1].Given that viral respiratory diseases like avian influenza (IA), Newcastle disease (NCD), infectious bronchitis virus (IBV), and avian metapneumovirus (aMPV) lack pathognomonic symptoms, it is challenging to make a presumptive diagnosis of these conditions [2].

When infected, the aMPV virus, which is a member of the Metapneumovirus genus and Pneumoviridae family, primarily affects the respiratory and reproductive systems of birds [3]. The envelope glycoproteins (G, F, and SH) of aMPV can be used to classify it; the G glycoprotein is the primary one and is in charge of binding to the host cell receptor [4].Only four aMPV subtypes are identified based on their antigenicity: A, B, C, and D [5]. Variations in certain amino acids found in the genetic material can change the subtypes. Additionally, two intermediate subtypes have been identified [6].For instance, subtypes A and B are more alike than subtype C [5]. The first case of aMPV was documented in Brazil in the middle of the 1990s [7], despite the fact that the illness is still relatively new there and that not many epidemiological studies have been conducted. The majority of continents have already identified the aMPV, and it was initially described in South Africa in Turkey as Turkish Rhinotracheitis (TRT) [8]. Since its initial emergence, aMPV has been identified in a number of different regions in a matter of years. In addition to migratory birds, individuals moving across continents can also play a major role in this spread [9].

Bacteria like Escherichia coli can be isolated from or frequently linked to upper respiratory tract infections brought on by aMPV [1]. Compared to viral monoinfections, coinfection-related viral damage and persistence might change [12]. Both primary and secondary infections can be brought on by avian pathogenic Escherichia coli (APEC) [13,14].

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Predisposing factors, such as compromised skin or mucous membrane integrity, poor hygiene practices, the influence of immunosuppressive factors, inadequate ventilation, and the presence of viral diseases, can disrupt the host's organic balance and condition the development of secondary infections by APECs [13–15].

As demonstrated by global health and nutrition, Brazil is the world's second-largest producer and exporter of chicken meat in terms of both economy and poultry production. The states of Paraná, Santa Catarina, Rio Grande do Sul, São Paulo, Goiás, and Minas Gerais are particularly notable when it comes to the slaughter of broiler chickens; taken as a whole, they account for 88.33% of all the birds killed in Brazil and exported [19]. The state of Cerá distributes its output for domestic consumption in Brazil [19]. This high percentage of birds housed in a geographic region can pose health risks to the animals' health,mainly through the transmission of respiratory infectious agents [20]. An epidemiologicalstudy conducted between 2004 and 2008 in Brazil,involving 228 samples from broilers,broiler breeders, and turkeys,revealed a prevalence of 6.57% for aMPV-A and 10.08% for aMPV-B [7].

MATERIALS AND METHODS

Sample Collections

One hundred batches of broiler chickens (Gallus gallus domesticus) from all over Brazil were examined. The period of collection was August through December 2021. The definition of the states where the samples were collected was with respect to the proportionality of broiler chicken production; ten chickens were sampled per batch, totaling one thousand chickens, coming from the South Region (states of Paraná (n = 30 batches),Santa Catarina (n = 15 batches), Rio Grande do Sul (n = 15 batches), Southeast Region(states of São Paulo (n = 10 batches), and Minas Gerais (n = 10 batches)) and NortheastRegion (state of Ceará (n = 20 batches)), which represent 80% of chicken meat productionin Brazil [19]. The regions and collection areas are depicted in.

Based on the clinical evaluation conducted by the accountable veterinarians at each farm, the batches were chosen based on the history of respiratory issues and animals exhibiting respiratory disorders, such as sneezing, rales, snoring, nasal secretions, swollen heads, and suspected colibacillosis. Moreover, the birds did not receive an aMPV vaccination. Consultation with the Ethics Committee on the Use of Animals (no 4434190521/Federal University of Santa Catarina) All biological samples evaluated here were donated by farms that conduct routine inspections, negating the need for an ethics committee because they are leftover biological samples gathered by routine health surveillance services.Blood samples were taken between 15 and 21 days following the initial collection in order to perform a serological evaluation for aMPV. Twenty-animal pools were used for collection, and batches of sera were kept separately for storage.

Clinical Indications in Groups

Anamnesis was performed in order to survey the clinical signs of the sampled batches, and each batch's individual sanitary control sheet—which included data on average weight, feed consumption, clinical signs, and medications used—was examined.

aMPV Serological Detection

Using the BioChek commercial kit (Reeuwijk, The Netherlands), the ELISA (Enzyme Linked Immuno Sorbent Assay) method was employed in accordance with the manufacturer's instructions to detect antibodies against aMPV. As a positive control, the commercial Freeze-Dried Reference Serum CR300 (BioChek) kit from Reeuwijk, the Netherlands, was employed.The results were analyzed according to an optical density (OD) using the BioChek ii software (version 2015) with sample/positive ratios (SP) > 0.5 (titer ≥ 0.501), indicating the averagetiter of the 20 birds evaluated per batch against possible natural exposure to aMPV, sincethey are not birds vaccinated against the pathogenic agent.

RT-PCR-Based Molecular Identification of aMPV

The RNeasy® Mini kit (QIAGEN, Hilden, Germany) was used to extract the samples' total RNA in accordance with the manufacturer's instructions. In accordance with the manufacturer's instructions, reverse transcription was carried out using the M-MLV Reverse Transcriptase kit (Promega, Madison, WI, USA). The G protein gene was utilized in the polymerase chain reaction (PCR) to identify subtypes A and B (Table 1), with the following chemicals and concentrations: To make 25 µL, combine 2 mM magnesium chloride, 0.25 mM deoxyribonucleotide phosphates, 0.3 µM of each primer, 1~° Green GoTaq® Reaction Buffer (Promega, Madison, WI, USA), 1 U of Taq DNA polymerase GoTaq® DNA Polymerase, 3 µL of sample, and sterile ultrapure water. The reactions were carried out in a thermocycler, using the following cycling parameters: 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 63 °C for 30 s, 68 °C for 3 min; and a final cycle of 72 °C for 10 min [21].

RESULTS

Lesions and Clinical Signs in Batches

Twenty-eight batches had more than two clinical signs (attribute "++"), 29 batches had just one clinical sign (attribute "+"), and 43 batches had no clinical signs at all (attribute "0"). Table 3 lists the batches in order of evaluation based on clinical signs gleaned from batch health control sheets, which include details on the drugs taken and clinical signs noted.71% of the

batches exhibited clinical respiratory symptoms, such as rales, sneezing, nasal discharge, infraorbital sinus enlargement, and swollen head, during sample collection. During the housing phase, 13.3% of the batches from the southern states (Santa Catarina and Paraná) used antibiotics; these medications included florfenicol, sulfachlorpyridazine+trimethopim, and ciprofloxacin. Just 5% of the batches in the Southeast (São Paulo and Minas Gerais) exhibited clinical symptoms, and on the day of collection, ciprofloxacin was administered to this batch. When the batch control sheets were examined, the clinical signs found varied amongst the batches.Particularly noteworthy are the southern Brazilian states of Rio Grande do Sul, Paraná, and Santa Catarina, where 83.3% of the batches displayed different respiratory clinical symptoms. Furthermore, at some stage of the production cycle, 36.6% of these batches underwent antibiotic therapy. However, only 5% of the batches in the Southeast states of São Paulo and Minas Gerais displayed clinical symptoms of respiratory illnesses, and one of these batches also contained antibiotics.

aMPV Molecular Detection and Seropositivity

Consequently, antibodies against aMPV were detected in 20% of the samples. With 70% of the positive samples originating from Paraná and the remaining 30% from Santa Catarina, the southern part of Brazil was home to the majority of the samples. With an average titratable weight of 6881.4 IU, the results show a seroprevalence for aMPV of 46.6% of the batches evaluated in the State of Paraná, with 14 out of the 30 batches sampled showing positivity. Six out of the fifteen batches sampled in Santa Catarina showed positive results, with a titratable average of 780 IU and an aMPV seroprevalence of 40% of the batches assessed (Figure 2).From the femurs, a set of sixty-three E. Coli isolate characteristics were obtained. 58 (92%) of the 63 E. coli isolates were found to exhibit three to five of the genes identified as minimum virulence indicators for APEC strains using qualitative PCR.

DISCUSSION

In the southern part of Brazil, specifically in the states of Paraná and Santa Catarina, which rank first and second in the nation's poultry production rankings, respectively, the current study showed seroconversion to aMPV in batches of broiler chickens that were not vaccinated against aMPV [19]. Twenty out of every 100 batches in this study showed seroconversion to aMPV, and RTPCR was used to identify 2/100 of these as aMPV-B. The detection of the viral genome and isolation of aMPV represents a considerable challenge, since the virus has a relatively short period of persistence in the host and is often detected in the early stages of infection, without demonstrating characteristic clinical signs [24].Viral spread in poultry flocks is made possible by the high concentration of poultry farms in some areas and the frequent non-use of vaccinations to prevent aMPV. Given that subtypes A and B are primarily found in chickens and turkeys, it is also significant that there is a high production of turkeys in southern Brazil, which may help spread and sustain the virus in the area [25]. Globally, the aMPV is widely dispersed [2,26,27]. The first report in Latin America was published in 1995 [28], and they identified subtype A using chicken embryo cells and field samples of aMPV. An increase in aMPV cases was initially noted, primarily in long-lived chickens and turkeys. Ref. [29] described aMPV-B's initial appearance in Brazil in 2011. Even though aMPV is found in poultry flocks and frequently ignored in broiler chickens, it seriously harms the poultry industry. It was discovered that aMPV thickens the tracheal mucosa following viral infection [30]. This happens because of edema, congestion,and mononuclear cell infiltration in the tracheal lamina propria, which typically manifests three to seven days following infection. Additionally, focal disciliation and epithelial cell flattening were noted, which could promote the development of secondary infections and exacerbate clinical symptoms.

Brazil's southern region accounts for 88% of all batches medicated in the country, indicating a significant concentration of medication use during production cycles. The main goal of these treatments was to manage opportunistic bacteria or those that are naturally found in birds. In 45% of the batches where seroconversion to aMPV occurred, isolates identified as APEC were obtained, indicating the presence of co-infection.This was connected to the birds' clinical state, which results in both the loss of killed birds and production losses over the flock's lifetime.Regardless of its primary or secondary function, the identification and characterization of APEC in aMPV-positive batches in Brazilian states highlights the significance of this agent, particularly in batches that were medicated to lessen the effects of co-infection with aMPV linked to APEC [37].

CONCLUSIONS

According to the study, 20% (20/100) of the batches examined in Brazil had seroprevalence of aMPV, subtype B was found, and 45% (9/20) of the batches showed more clinical issues when APEC co-infection was present.This study emphasizes the necessity of developing ongoing monitoring plans to combat aMPV in the poultry industry and lower viral and bacterial co-infection rates. When taken as a whole, these factors will undoubtedly boost output in order to safeguard livestock, enhance animal health, and ultimately lower the need for antibiotics.

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