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Impacts of Adding Antibiotics to Inactivated Oil in Water Adjuvant Avian Influenza Vaccine on Immunity, Disease Control and Performance of Broiler Chickens

Diaa El-Boraey[1], Mousa A. Ayoub[1], Wael K. Elfeil[2]*, Said Ibrahim Fathalla[3], Ibrahim Said Abu-alya[3], Mohamed Abaza[4], Ahmed R. Gado[5], Mobarez A.A.[6], Nehal K. Alm Eldin[6]

[1]Department of Animal Hygiene and Zoonosis, Faculty of Veterinary Medicine, Damanhur University, Damanhur, 22511, Egypt; [2]Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Suez Canal University, Egypt; [3]Department of Physiology, University of Sadat City, Sadat City, P.O. 32897, Menoufia, Egypt; [4]Department of Poultry and Rabbit Diseases, Faculty of Veterinary Medicine, Benha University, Tukh, Qalyubia, 13736, Egypt; [5]Department of Birds and Rabbit Medicine, Damanhur University, Damanhur, Egypt; [6]Department of Veterinary Public Health, Faculty of Veterinary Medicine, New Valley University, Kharga Oasis, 1062001, Egypt.

Abstract | Generally, the health and well-being of poultry are significantly affected by the repeated handling to administer antibiotics or inactivated vaccines. As a result, it observes a lot of Egyptian chicken farmers started mixing antibiotics with inactivated vaccines then administrating as one shot. Therefore, this study conducted to examine the impact of some antibiotics: amikacin and gentamicin on their effectiveness of an inactivated oil in water adjuvant avian influenza vaccine (H9) in broiler chickens. Four groups of chickens were established as follow: a negative control, a positive control (vaccine only), a group receiving the vaccine with amikacin, and a group receiving the vaccine with gentamicin. Hemagglutination inhibition (HI) titers were measured to assess antibody responses in addition to performance parameters. The obtained results demonstrated a reduction in antibody levels against the H9 strain of Avian Influenza Virus (AIV) reached to 36% in the groups received the vaccines combined with antibiotics. These findings suggested that the addition of antibiotics to inactivated vaccines may compromise the immune response of broiler chickens, potentially increasing the risk of disease outbreaks. The addition of antibiotics oil-adjuvant in activated avian influenza vaccine (H9) didn't improve the bird's performance as it caused decreasing in final body weight, decreasing in conversion rate, and increasing in mortality rate. The study emphasizes the need for alternative strategies to enhance vaccine efficacy, while minimizing antibiotic use, without compromising poultry health or public health. The pharmacokinetics of antibiotics co-administered with adjuvant-containing inactivated vaccines need more further studies.

Keywords: Vaccination, H9, Application, Oil in water adjuvant, Amikacin, Gentamicin

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***Correspondence** | Wael K. Elfeil, Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Suez Canal University, Egypt; **Email:** elfeil@vet.suez.edu.eg
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INTRODUCTION

Poultry farming is an exceptionally efficient and prolific agricultural industry that plays a crucial role in supplying billions of people globally with protein-rich meals (Gržinić *et al*., 2022). Nevertheless, the poultry sector faces additional challenges, including the prevalence of contagious diseases such as avian influenza (Parvin *et al*., 2020). The respiratory pathogens like avian influenza (AI), Newcastle Disease virus (NDV), Infectious bronchitis virus (IBV), adenovirus and Reovirus beside Multidrug resistance (MDR) bacteria as *Esherichia coli*, *Salmonella* and *Pasteurella* spp., associated withs severe losses in poultry industry and subject to regular vaccination regimes in some endemic regions (Eid *et al*., 2019; Sultan *et al*., 2019, 2022; Elfeil *et al*., 2020; Fawzy *et al*., 2020; Sultan *et al*., 2020; Talat *et al*., 2020; Elfeil *et al*., 2022; Mahmoud *et al*., 2022). Avian influenza is a highly contagious viral disease that may rapidly spread among groups of poultry, resulting in significant financial losses, worries about animal well-being, and potential dangers to public health (Diab *et al*., 2019; Wille and Barr, 2022). Influenza viruses have been associated with major outbreaks that have caused significant mortality rates and disruptions to the worldwide chicken trade (Shi and Gao, 2021).

The continuing risk of this disease highlights the critical importance of a limited biosecurity strategy that includes vaccination; the vaccines serve as a first line of defense by stimulating the immune system to produce antibodies against specific pathogens. This immunity helps prevent disease outbreaks and reduces the severity of infections in the event of a breach in biosecurity measures (Dey *et al*., 2023; Nontapan, 2024). Furthermore, vaccines play a role in preventing the shedding of diseases by decreasing the release of pathogens from vaccinated birds. This measure aids in reducing the danger of disease transmission among a group of birds and to other farm locations, thereby protecting the poultry industry (Ahmad *et al*., 2024).

Antibiotics have been extensively used in poultry farming to prevent and treat diseases, thus improving the health of poultry and productivity. Excessive and incorrect usage of antibiotics has contributed to the development of antibiotic-resistant bacteria that are resistant to many antibiotics, presenting a substantial risk to public health (Haque *et al*., 2020; Rady *et al*., 2020). The process of handling and capturing poultry for the purpose of administering antibiotics or inactivated vaccines has a notable impact on the health and overall welfare of the birds. It is widely recognized that repeated catching results in significant physiological stress, this subsequently initiates the secretion of corticosteroids. The stress response can suppress the immune system, rendering the birds more susceptible to other diseases, thereby reducing the effectiveness of the administered vaccine or treatment (Abo-Al-Ela *et al*., 2021).

Moreover, the stress caused by frequent handling has been observed to cause alterations in the birds' behavior, such as decreased food consumption and heightened aggression. These changes have a detrimental impact on the growth rates and productivity of the entire flock. In addition, making multiple efforts to capture birds can result in physical harm, including bruises, fractures, feather loss, and other injuries that are harmful to animal well-being and can be expensive (Akinyemi and Adewole, 2021). To avoid such problems, many poultry producers in Egypt began to mix antibiotics with inactivated vaccines and administrate them as one shot. antibiotics were added to the vaccine to combat inflammation at the injection site or to reduce the risk of infection. It is critical to consider the potential mechanisms underlying these interactions. Antibiotics may interact with the inactivated vaccine in a variety of ways, including chemically interacting with the vaccine's components, making it less effective, altering vaccine structure, and reducing immune response (Shen *et al*., 2024).

Some antibiotics can have immunosuppressive effects, potentially interfering with the body's ability to produce antibodies (Sedeik *et al*., 2018; Qiu *et al*., 2022). Furthermore, antibiotics have the capacity to substantially modify the composition of gut microbiota, which is essential for immune function. This interruption could potentially have an indirect impact on the effectiveness of the vaccine (Ribeiro *et al*., 2020). The purpose of this study was to determine the effect of combining amikacin and gentamicin with inactivated oil in water adjuvant avian influenza vaccines on vaccine effectiveness and broiler chickens' performance.

MATERIALS AND METHODS

Experimental design

Experimental chickens

A total of 140 unsexed, one-day-old Ross-308® chicks were weighed and reared in a deep litter system using sawdust as bedding. They were reared until they reached 5 weeks of age, with an average floor space of 10 birds per square meter. The chickens were given unlimited access to water and feed, and they were continuously monitored for their health and performance. Any cases of mortality were documented. The breeding and handling of birds were conducted in accordance with the guidelines set forth by the ethics committee of Damanhur University, Egypt.

The chicks were divided into four groups randomly, with each group containing 35 birds. Group 1 (G1) serves as the negative control, meaning they were not vaccinated. Group

2 (G2) serves as the positive control, as they were injected with the MEFLUVAC® H9 vaccine subcutaneously (S/C) without antibiotics. Group 3 (G3) received the MEFLUVAC H9 vaccine mixed with amikacin AMIGASOL® (S/C), and group 4 (G4) received the MEFLUVAC® H9 vaccine mixed with gentamycin Gentabiotic® (S/C).

All birds in every group, except G1, were immunized against H5 avian influenza at 7 days old using the MEFLUVAC® H5 PLUS 8 vaccine (Batch No: 2309030101) which was manufactured in September 2023 and will expire in September 2025. The vaccine is registered under Registration Number 927/2023 from MEVAC, an Egyptian company. The vaccine was administered intramuscularly (i/m) at a dose of 0.5 cc. This was done as an internal control to assess the immune system response of the poultry to inactivated vaccines. All groups were subjected to identical rearing conditions and handling methods. The European Broiler Index (EBI) was calculated for each group to assess production efficiency according to (Poultry Performance Plus, 2022).

Monitoring the health of chickens using real-time RT-PCR

The absence of poultry-infecting viruses, including vertically transmitted diseases like Adenovirus, Reovirus, and Chicken Anemia Virus (CAV), was confirmed on the first day of age using the Quantitect RT-PCR Kit (Qiagen, Catalogue No. 204443, Germany). Following regular PCR screenings, conducted every ten days, the chicks were tested to confirm the absence of prevalent poultry infectious illnesses like Infectious bronchitis (IB), Infectious Bursal Disease (IBD), Avian Influenza (H5 and H9), and Newcastle Disease (ND). This kit played a crucial role in guaranteeing that the birds were devoid of viral infections that could potentially affect the immune response to the vaccine. Utilizing this RT-PCR kit enabled accurate and dependable identification of these viruses, thereby guaranteeing the integrity of the immune response findings observed in our study. The primers that were utilized are displayed in Table 1.

Preparation of vaccine-antibiotic mixture

The avian influenza vaccine MEFLUVAC H9 (Batch No: 2402120101) which was manufactured in February 2024 and will expire in February 2026. The vaccine is registered under Registration Number 803/2021 from MEVAC, an Egyptian company was mixed with each antibiotic to create the vaccine and antibiotic mixtures. The amount of each antibiotic was calculated based on the chicks' weight, which was approximately 170 grams at one week of age. Amikacin from AMIGASOL® (Batch No: Drug: 05240723 from

Table 1: The used primers in Real-Time RT-PCR for detection of poultry-infecting viruses.

Item	Pathogen	Type	Primer sequence	Reference
1	Adenovirus	Realtime SYBR Green	52K-fw (ATG GCK CAG ATG GCY AAG G)	Günes *et al.* (2012)
			52K-r (AGC GCC TGG GTC AAAmlG A)	
2	Chicken Anemia Virus	Realtime SYBR Green	CAV Fw (AGAGAGATCCGGATTGGTATCG)	Rimondi *et al.* (2014)
			CAV Rv (TGGGAGCGCGAGCATT)	
3	Reovirus	Realtime SYBR Green	2408C-F (GCGTCTACGGAGTTATTACATCGCT)	Guo *et al.* (2012)
			2408C-R (AGGCGAAAAAGATAGACCATGAC)	
4	IBV	Realtime SYBR Green	UTR-41 (ATGTCTATCGCCAGGGAAATGTC)	Cavanagh *et al.* (2001)
			UTR-11 (GCTCTAACTCTATACTAGCCTA)	
5	NDV (velogenic)	Realtime Probe	F-EGY-Fw (CGS ARG ATM CAA GGG TCT)	Moharam *et al.* (2019)
			F-EGY-Rv (CTA CAC TGC CAA TAA CRG C)	
			F-EGY-probe (6-FAM-AGG AGA CRA AAA CGY TTT ATA GGT GC-BHQ-1)	
6	Influenza Matrix	Realtime Probe	M1-F (AGA TGA GTC TTC TAAmlG AGG TCG)	Fereidouni *et al.* (2012)
			M1.1-R (TGC AAA AAC ATC TTC AAG TYT CTG)	
			M1.2-R (TGC AAA GAC ACT TTC CAG TCT CTG)	
			M1-FAM (FAM-TCA GGCmlC CTC AAA GCC GA-BHQ1)	
7	H9	Realtime Probe	H9-For (ATG GGG TTT GCT GCC)	Monne *et al.* (2008)
			H9-Rev (TTA TAT ACA AAT GTT GCA C (T) CT G)	
			H9 probe (6-carboxyfluorescein TTC TGG GCC ATG TCC AAT GG- 6-carboxytetramethylrhodamine)	
8	IBD	Realtime Probe	Forword primer (5′-GAGGTGGCC-GACCTCAACT-3′)	Moody *et al.* (2000)
			reverse primer (5′-AGCCCGGATTATGTCTTTGAAG-3′)	
			(FAM) TCCCCTGAAGATTGCAGGAGCATTTG	

ATCO PHARMA company – Egyptian company), a target dose of 15 mg per kg of body weight was used, resulting in a dose of 2.55 mg per chick. The required amount of AMIGASOL® containing 25% amikacin was measured and mixed with a separate sterile vial of MEFLUVAC H9 vaccine from the same bottle. The mixture was then mixed by vortex for 5 minutes. Similarly, Gentamicin sourced from Gentabiotic® (Batch No: Drug: 0354/24 from Arabcomed company – Egyptian company) had a target dose of 5 mg per kg of body weight, so the required dose was 0. 85 mg per chick. The appropriate volume of Gentabiotic® (10% gentamicin) was calculated, withdrawn with a sterile syringe, and added to a sterile bottle containing the MEFLUVAC H9 vaccine. The mixture was then thoroughly mixed using vortex for 5 minutes. To serve as a control, the rest of the bottle of MEFLUVAC H9 vaccine was kept with no antibiotics. All prepared vaccine mixtures were kept at the recommended temperature until administration, as per the manufacturer's instructions for the MEFLUVAC H9 vaccine. Simultaneously, the conditions were kept very sterile to prevent sample contamination.

Immunological measurements

At specific time points (7, 15, 21, 28, and 35 days), blood samples were obtained from 10 randomly chosen birds from each treatment. The samples were collected in tubes without anticoagulants. The purpose was to measure antibody titers against Avian Influenza viruses H5 and H9 using a reference antigen. The purpose of this sampling schedule was to systematically observe and track the development and progression of the immune response as time progressed. The blood samples were dated and labelled based on the quantity of chickens and the respective groups they belonged to. The samples were subjected to centrifugation at a speed of 3000 revolutions per minute for a duration of 10 minutes to obtain serum. The obtained serum was then divided into smaller portions and stored in Eppendorf tubes at a temperature of -20 ºC until further analysis.

Hemagglutination inhibition (HI) test

The serum samples were analyzed using HI assays to determine the antibody titers against Avian Influenza viruses, specifically the H9 and H5 strains. The HI test was conducted according to the established protocols outlined in the Manual of the World Organization for Animal Health (OIE) in 2025 (WOAH, Terrestrial Manual, 2025).

Determining the growth performance of chicks

Performance traits such as feed intake, body weight, body weight gain, feed conversion ratio, and mortality rate were calculated for each age interval and cumulatively from the 7th to the 35th day of age.

Statistical analysis

The data accumulated during the examination was communicated as the mean ± standard error (SE) of the absolute number of animals utilized in each group. Information was exposed to one-way analysis of variance (ANOVA) and Duncan multiple range tests as post-hoc test for the one-way ANOVA to decide the distinction between the exploratory and control groups (Sutherland *et al*., 2003). whereas the Bonferroni test was applied for the repeated ANOVA. All statistical analyses were performed using the SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

An effectively implemented vaccination program is a vital part of a comprehensive biosecurity plan for poultry farms and offers a critical level of protection against contagious diseases (Abdallah *et al*., 2023). However, the success of vaccination programs is heavily reliant on strict adherence to protocols. Any deviation from recommended procedures can undermine vaccine efficacy and possibly lead to disease outbreaks, resulting in significant economic losses (Sharif and Ahmad, 2018). The purpose of this study was to determine the efficacy of adding amikacin and gentamycin to oil in water adjuvant in activated avian influenza vaccine (H9N2 strain) in broilers. PCR was used to confirm that the chicks were free of poultry-infecting viruses and were separated into four groups. Our results as illustrated in Figure 1, found that both antibiotics, gentamicin and amikacin, can interact negatively with the H9 oil in water adjuvant vaccine as HI titers of avian influenza H9 in all

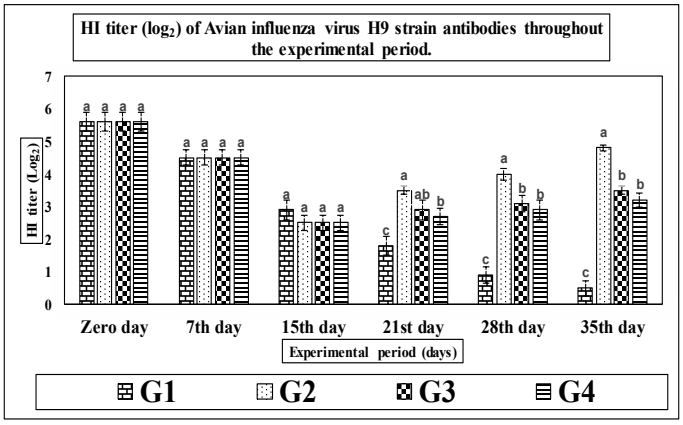

Figure 1: Hemagglutination inhibition (HI) titer (log^2) of avian influenza virus H9 strain antibodies throughout the experimental period (n = 10). All data are expressed as Mean ± SE. Different letters at each observation time indicate significant difference at (P<0.05). G1: control -ve group, G2: control +ve group, G3: inactivated vaccine + amegasol mixture group, and G4: inactivated vaccine + gentamycin mixture group.

groups demonstrated similar initial HI titer values on day zero (log_2 5.60) and no significant differences among all groups on the 7th and 15th day. By day 15, there are. The positive control group (G2) exhibited a significant increase in HI titer on days 21, 28, and 35 (log_2 3.50, 4.00, and 4.80, respectively). Unlike, the lowest HI titer is shown in G1 (control negative group), particularly from day 21, 28, and 35 was log_2 1.80, 0.90, and 0.50, respectively. However, the HI titer values for G3 (the group treated with the vaccine and amikacin) and G4 (the group treated with the vaccine and gentamicin) were lower than those of G2 (the positive control). Specifically, on days 21, 28, and 35, the HI titer values for G3 were log_2 2.90, 3.10, and 3.50, respectively. Similarly, the HI titer values for G4 on days 21, 28, and 35 were log_2 2.70, 2.90, and 3.20, respectively. According to Scott *et al.* (2018), the antibiotics may have prevented the immune response from functioning properly, possibly by affecting the activity of immune cells or the production of cytokines. According to El-Dakroury and Elseify (2014), gentamicin has immunosuppressive effects, which means that it may reduce the body's ability to mount an effective immune response to the vaccine. In terms of immune modulation, amikacin has received less attention than other compounds; however, it is essential to take into consideration the potential impact that it could have on immune function. According to Kocourkova *et al.* (2017), gentamicin and amikacin have the potential to interact with the inactivated components of the H9 vaccine, which could potentially alter the vaccine's structure or antigenicity. Although it is less likely that amikacin play a role in the development of antibiotic resistance, the impact that it has on the microbiome of the gut may have an indirect influence on the overall profile of the gut microbiome (Ramirez and Tolmasky, 2017).

There aren't enough studies that have investigated the effects of adding antibiotics on the efficiency of vaccine. However, a recent study was carried out to evaluate the effects of adding ceftiofur to an AI vaccine on vaccine structure and efficacy on chicks and reported that adding ceftiofur caused disruption of vaccine structure by ceftiofur hydrochloride may be a major factor contributing to poor vaccine absorption at the injection site and reduced H5N8 and H7N9 antibody titer (Shen *et al.*, 2024). A prior study conducted on mice demonstrated that the injection of doxycycline or clarithromycin results in diminished antibody responses subsequent to immunization with tetanus, Hepatitis B, and PPV23 vaccines. Unlike, the administration of ampicillin leads to increased antibody responses following vaccination with a live attenuated *Salmonella* Typhi vaccine (Woo *et al.*, 1999). Another study in calves showed that concurrent administration of oxytetracycline leads to decreased antibody responses to subcutaneous vaccination with *Brucella abortus* vaccine (Smith *et al.*, 1983). The data presented in Figure 2 demonstrates that all groups exhibited a comparable pattern of antibody responses to the H5 strain, except for G1 (negative control). G1 showed a significant increase in HI titer over time, starting at log_2 5.40 on day zero and reaching log_2 4.70 on day 7. However, the HI titer value decreased on day 15, ranging from log_2 2.10 in G2 and G4 to log2 3.8 in G1. Subsequently, the HI titer value in G1 gradually declined until it reached a logarithmic value of 0.70 on day 35. In contrast to other groups, G2, G3, and G4 demonstrated a notable rise in HI titer from day 21 to day 35, reaching log_2 values of 5.80, 5.50, and 5.40, respectively. The antibody response patterns for the H5 strain, which was used as an internal control to evaluate immune responsiveness, followed a similar trajectory. All groups injected with the H5 vaccine, except G1 (the unvaccinated negative control), demonstrated increasing HI titers over time. This pattern confirms that the MEFLUVAC® H5 vaccine alone effectively elicited an immune response, further highlighting the potential detrimental effects of antibiotics when mixed with vaccines. so many studies suggest the negative effect of antibiotics on the efficacy of vaccination process, however, there should be sufficient duration of time for the antimicrobials and the antimicrobial should be administered before minimum of two days before and after administering the vaccination (Sharif and Ahmad, 2018). Unlike other studies suggest that some antibiotics like erythromycin have a special immunomodulating capacity by stimulating the secretion of interleukins by leukocytes that help to maintain strong immunity for the birds (Blondeau, 2022).

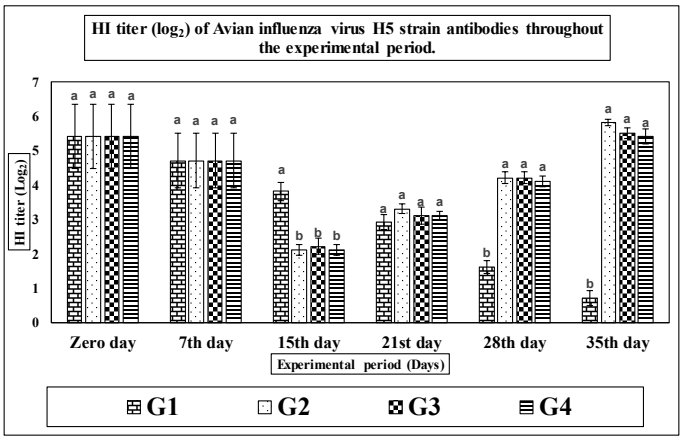

Figure 2: Hemagglutination inhibition (HI) titer (log_2) of avian influenza virus H5 strain antibodies throughout the experimental period (n = 10). All data are expressed as Mean ± SE. Different letters at each observation time indicate significant difference at (P<0.05). G1: control -ve group, G2: control +ve group, G3: inactivated vaccine + amegasol mixture group, and G4: inactivated vaccine + gentamycin mixture group.

The impact of antibiotic-vaccine mixtures on performance was also significant. At the beginning of the experiment, there were no significant differences in the weights of the broiler chicks, while the final body weights of the birds

had significant differences between the groups, where G1 recorded the highest weights (2480.00 gm), followed by G2, then G3, while the lowest weight appeared in G4 (2160.50 gm). These results were compatible with the total feed intake (FI) results, as G1 recorded the highest value, followed by G2 and G3, While G4 also was the least feed-consuming group; 3454 gm, 3371 gm, 3192 gm, and 3279 gm, respectively, as shown in Figure 3. The Feed Conversion Ratio (FCR) reflected similar trends, with the best FCR in G1 and the poorest in G4 as shown in Figure 4. FCR was 1.42 in broilers in G1, while G2 and G3 were almost close (1.44 and 1.47, respectively), but G4 is the least efficient group and had the worst conversion rate (1.55). Mortality rates also differed among groups, with the highest mortality observed in G4, the gentamicin group. The increased mortality may be linked to immunosuppressive effects that rendered the broilers more vulnerable to secondary infections or other health issues. In contrast, G1, which did not receive the vaccine, recorded the lowest mortality, while G2 and G3 had intermediate levels. The observed trends in FCR and mortality collectively emphasize the need to consider the potential risks associated with co-administering antibiotics and vaccines, as the interaction could negatively impact health, particularly with gentamicin. These results can be explained by the vaccine mixed with gentamycin in group G4 might have negatively impacted the birds' health or metabolism, leading to reduced feed intake and higher mortality (Arafat *et al*., 2017). Antibiotics have the potential to disrupt the microbiota in the gut, which is an essential component of digestive system health and optimal digestion process. Indirectly, the performance parameter of the birds be impacted because of this disruption (Ramirez *et al*., 2020).

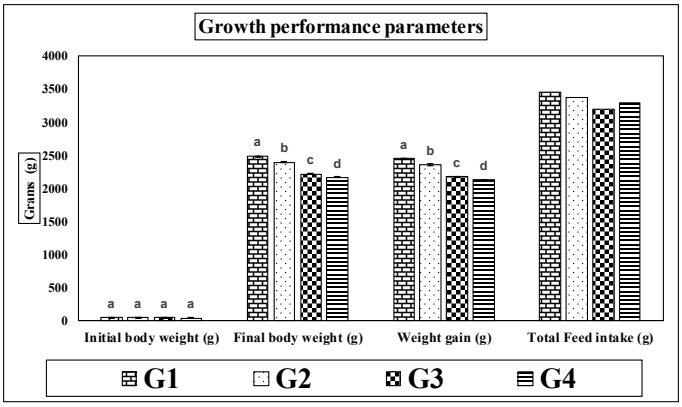

Figure 3: Performance parameters, initial body weight, final body weight, weight gain, and feed intake, of broiler chicks throughout experimental period (n = 35). All data are expressed as Mean ± SE except total feed intake (g). Different letters at each observation time indicate significant difference at (P<0.05). Total feed intake was not exposed to statistical analysis as there were no replicated for each group. G1: control -ve group, G2: control +ve group, G3: inactivated vaccine + amegasol mixture group, and G4: inactivated vaccine + gentamycin mixture group.

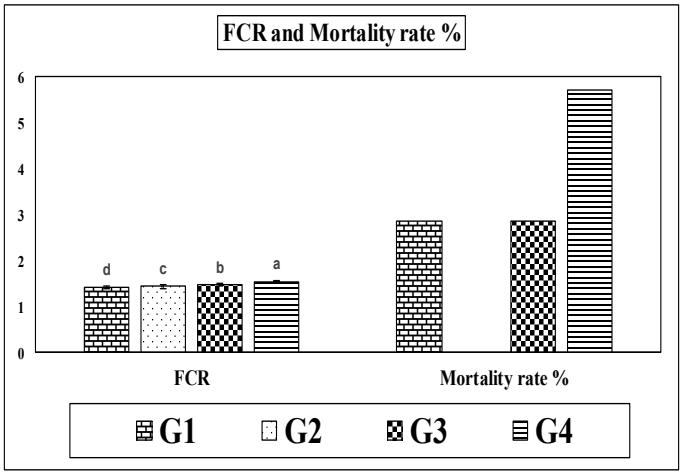

Figure 4: FCR and mortality rate% of broiler chicks throughout experimental period (n = 35). All data are expressed as Mean ± SE except Mortality rate %. Different letters at each observation time indicate significant difference at (P<0.05). FCR was not exposed to statistical analysis as there were no replicates for each group. G1: control -ve group, G2: control +ve group, G3: inactivated vaccine + amegasol mixture group, and G4: inactivated vaccine + gentamycin mixture group.

The EBI which evaluates production efficiency, supported these findings. Group 1 (407), which did not receive any vaccine, exhibited the highest production efficiency, suggesting that the absence of vaccination or antibiotic interventions contributed to optimal growth, feed conversion, and survival. Group 2 (398), the positive control vaccinated with MEFLUVAC® H9 alone, demonstrated high efficiency, indicating that vaccination without antibiotics did not significantly impair performance compared to G1. Group 3 (372), which received the vaccine combined with Amikacin, showed lower efficiency, potentially due to the impact of antibiotic administration on gut microbiota or overall health (Ribeiro *et al*., 2020). Group 4 (314), which received the vaccine mixed with Gentamycin, exhibited the lowest EBI, suggesting that the use of Gentamycin alongside vaccination might have negatively affected performance. These findings suggest that the combination of vaccines with antibiotics may influence broiler production efficiency. Due to insufficient data on the pharmacokinetics of antibiotics combined with inactivated vaccinations, further studies should be conducted on the pharmacokinetics of antibiotics when administered together with inactivated vaccines containing adjuvants. The depot effect of antigens is further enhanced by the slow-release properties of adjuvants and may also affect the release profile of the antibiotic. This interaction may affect plasma antibfiotic levels and lead to antibiotic residue in the food chain. Hence, it is important to evaluate the pharmacokinetics and toxicity of such antibiotic synergistic in vaccines.

CONCLUSIONS AND RECOMMENDATIONS

The study's findings indicated that incorporating amikacin and gentamicin into an oil-in-water adjuvant in the activated avian influenza vaccine (H9) not only diminished the vaccine's efficacy by inhibiting the immune response but also presented substantial public health risks by reducing vaccine effectiveness against the disease, potentially contributing to the concerning increase in antibiotic efficacy. Furthermore, the incorporation of antibiotics into the oil-in-water adjuvant of the avian influenza vaccine (H9) resulted in a reduction in final body weight, a decline in conversion rate, and an increase in fatality rate. Further research is required to comprehensively define the immune response resulting from the combination of various antibiotics and vaccines, as well as to investigate the chemical interactions between them. Further research is necessary to examine the pharmacokinetics of antibiotics when delivered concurrently with inactivated vaccinations containing adjuvants.

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NOVELTY STATEMENT

This study challenges the prevailing belief among poultry farmers regarding those positive effects of co-administering antibiotics with vaccines. By analysing antibody titters and performance parameters, our study provides unequivocal evidence that this combination, specifically amikacin and gentamicin with an inactivated oil-in-water adjuvant avian influenza vaccine (H9), leads to adverse effects. The findings demonstrated the potential immunosuppressive impact of antibiotic-vaccine co-administration, reducing vaccine efficacy and negatively impacted bird health. Consequently, our results directly challenge current practices and recommend further research to develop more effective vaccination strategies without compromising poultry health or productivity.

AUTHOR'S CONTRIBUTION

D. El-Boraey, M. A. Ayoub and W. K. Elfeil conceptualized the study and designed the experiments, ensuring alignment with poultry health and immunology standards. W. K. Elfeil coordinated the project, overseeing animal handling, ethical compliance, and vaccine-antibiotic preparation protocols. S. I. Fathalla contributed to the design and execution of immunological assessments and provided expertise in data interpretation. I. S. Abu-Alya performed statistical analysis and data visualization, supporting a robust interpretation of vaccine efficacy and poultry performance metrics. M. Abaza and A. R. Gado assisted in immunological assays and collaborated in assessing the impacts of antibiotics on immune responses. N. K. Alm Eldin and Mobarez A.A. drafted the manuscript, incorporating feedback from all authors, and facilitated the critical review and final editing, enhancing the manuscript's clarity and scientific rigor. All authors reviewed, revised, and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdallah N, Tekelioğlu BK, Kurşun K, Baylan M, Ümit ELÇ (2023). Vaccination and poultry (chicken) production. J. Agric. Food, Environ. Anim. Sci., 4(1): 119-136.

Abo-Al-Ela HG, El-Kassas S, El-Naggar K, Abdo SE, Jahejo AR, Al-Wakeel RA (2021). Stress and immunity in poultry: Light management and nanotechnology as effective immune enhancers to fight stress. Cell Stress Chaperones, 26: 457–472. https://doi.org/10.1007/s12192-021-01204-6

Ahmad A, Anjum AA, Chaudhry M (2024). Viral shedding evaluation is critical for determining efficacy of avian influenza (H9) vaccines in broiler chickens. Pak. J. Zool., 56(4): 1569-1575 https://doi.org/10.17582/journal.pjz/20220322040347

Akinyemi F, Adewole D (2021). Environmental stress in chickens and the potential effectiveness of dietary vitamin supplementation. Front. Anim. Sci., 2: 775311. https://doi.org/10.3389/fanim.2021.775311

Arafat N, El-Shafei RM, Awadin W, Farag V, Saleh R (2017). Consequences of hepatorenal toxicity induced by gentamicin in chickens. Assiut. Vet. Med. J., 63: 154. https://doi.org/10.21608/avmj.2017.170781

Ayoub MA, Elfeil WK, El-Boraey D, Hammam H, Nossair MA (2019). Evaluation of some vaccination programs in protection of experimentally challenged broiler chicken against Newcastle disease virus. Am. J. Anim. Vet. Sci., 14(3): 197-206. https://doi.org/10.3844/ajavsp.2019.197.206

Blondeau JM (2022). Immunomodulatory effects of macrolides considering evidence from human and veterinary medicine. Microorganisms, 10(12): 2438. https://doi.org/10.3390/microorganisms10122438

Cavanagh D, Mawditt K, Sharma M, Drury SE, Ainsworth HL, Britton P, Gough RE (2001). Detection of a coronavirus from turkey poults in Europe genetically related to infectious bronchitis virus of chickens. Avian Pathol., 30(4): 355-368. https://doi.org/10.1080/03079450120066368

Dey P, Ahuja A, Panwar J, Choudhary P, Rani S, Kaur M, Sharma A, Kaur J, Yadav AK, Sood V, Suresh BAR (2023). Immune control of avian influenza virus infection and its vaccine development. Vaccines, 3: 593. https://doi.org/10.3390/vaccines11030593

Diab MS, Abd El-Hafez MS, Ashry MA, Elfeil WK (2019). Occurrence of avian influenza h5n1 among chicken, duck

farms and human in Egypt. Am. J. Anim. Vet. Sci., 14(1): 26-32. https://doi.org/10.3844/ajavsp.2019.26.32

Eid HM, Algammal AM, Elfeil WK, Youssef FM, Harb SM, Abd-Allah EM (2019). Prevalence, molecular typing, and antimicrobial resistance of bacterial pathogens isolated from ducks. Vet. World, 12(5): 677-683. https://doi.org/10.14202/vetworld.2019.677-683

El-Dakroury MF, Elseify MM (2014). A study on the immunomodulating effect of gentamicin on broilers vaccinated with Newcastle disease virus. Kafrelsheikh Vet. Med. J., 12(2): 63-77. https://doi.org/10.21608/kvmj.2014.110147

Elfeil WK, Ezzat ME, Fathi A, Alkilany MA, Abouelmaatti RR (2020). Prevalence and genotypic analysis and antibiotic resistance of salmonella species isolated from imported and freshly slaughtered chicken. Am. J. Anim. Vet. Sci., 15(2): 134-144. https://doi.org/10.3844/ajavsp.2020.134.144

Elfeil WK, Youssef H, Sedeek A, El-Shemy A, Abd-Allah EM, Elkady MF, Abdallah MS (2022). Protective efficacy of inactivated H9N2 vaccine in Turkey poults under both experimental and field conditions. Vaccines, 10(12): 2178. https://doi.org/10.3390/vaccines10122178

Fawzy M, Ali RR, Elfeil WK, Saleh AA, El-Tarabilli MM (2020). Efficacy of inactivated velogenic Newcastle disease virus genotype VII vaccine in broiler chickens. Vet. Res. Forum, 11(2): 113-120.

Fereidouni SR, Globig A, Starick E, Harder TC (2012). Effect of swab matrix, storage time, and temperature on detection of avian influenza virus RNA in swab samples. Avian Dis., 56(4s1): 955-958. https://doi.org/10.1637/10146-033012-ResNote.1

Gržinić G, Piotrowicz-Cieślak A, Klimkowicz-Pawlas A, Górny RL, Ławniczek-Wałczyk A, Piechowicz L, Olkowska E, Potrykus M, Tankiewicz M, Krupka M, Siebielec G (2023). Intensive poultry farming: A review of the impact on the environment and human health. Sci. Total Environ., 858: 160014. https://doi.org/10.1016/j.scitotenv.2022.160014

Günes A, Marek A, Grafl B, Berger E, Hess M (2012). Real-time PCR assay for universal detection and quantitation of all five species of fowl adenoviruses (FAdV-A to FAdV-E). J. Virol. Methods, 183(2): 147-153. https://doi.org/10.1016/j.jviromet.2012.04.005

Guo S, Xu D, Xu HX, Wang T, Li JL, Lu LQ (2012). Suppression of RNA interference pathway *in vitro* by grass carp reovirus. Virol. Sin., 27: 109-119. https://doi.org/10.1007/s12250-012-3230-4

Haque MH, Sarker S, Islam MS, Islam MA, Karim MR, Kayesh ME, Shiddiky MJ, Anwer MS (2020). Sustainable antibiotic-free broiler meat production: Current trends, challenges, and possibilities in a developing country perspective. Biology, 9(11): 411. https://www.poultryperformanceplus.com/information-database/broilers/285-european-broiler-index-or-european-production-efficiency-factor, https://doi.org/10.3390/biology9110411

Kocourkova A, Honegr J, Kuca K, Danova J (2017). Vaccine ingredients: components that influence vaccine efficacy. Mini Rev. Med. Chem., 17(5): 451-466. https://doi.org/10.2174/1389557516666160801103303

Mahmoud SI, Zyan KA, Hammoud MM, Khalifa E, Dardir S, Khalifa R, Elfeil WK (2022). Effect of co-infection of low pathogenic avian influenza H9N2 virus and Avian pathogenic *E. coli* on H9N2 vaccinated commercial broiler chickens. Front. Vet. Sci., 9(5): 918440. https://doi.org/10.3389/fvets.2022.918440

Moharam I, Abd El-Razik A, Sultan H, Ghezlan M, Meseko C, Franzke K, Harder T, Beer M, Grund C (2019). Investigation of suspected Newcastle disease (ND) outbreaks in Egypt uncovers a high virus velogenic ND virus burden in small-scale holdings and the presence of multiple pathogens. Avian Pathol., 48(5): 406-415. https://doi.org/10.1080/03079457.2019.1612852

Monne I, Ormelli S, Salviato A, De Battisti C, Bettini F, Salomoni A, Cattoli G (2008). Development and validation of a one-step real-time PCR assay for simultaneous detection of subtype H5, H7, and H9 avian influenza viruses. J. Clin. Microbiol., 46(5): 1769-1773. https://doi.org/10.1128/JCM.02204-07

Moody A, Sellers S, Bumstead N (2000). Measuring infectious bursal disease virus RNA in blood by multiplex real-time quantitative RT-PCR. J. Virol. Methods, 85(1-2): 55-64. https://doi.org/10.1016/S0166-0934(99)00156-1

Nontapan N (2024). Role of biosecurity measures in controlling infectious diseases in poultry farms in Thailand. J. Anim. Health, 4(2): 51-62. https://doi.org/10.47604/jah.2768

Parvin R, Nooruzzaman M, Kabiraj CK, Begum JA, Chowdhury EH, Islam MR, Harder T (2020). Controlling avian influenza virus in Bangladesh: Challenges and recommendations. Viruses, 12(7): 751. https://doi.org/10.3390/v12070751

Poultry Performance Plus (2022). European Broiler Index or European Production Efficiency Factor. Retrieved September 17, 2024.

Qiu W, Chen B, Tang L, Zheng C, Xu B, Liu Z, Magnuson JT, Zhang S, Schlenk D, Xu EG, Xing B (2022). Antibiotic chlortetracycline causes transgenerational immunosuppression via NF-κB. Environ. Sci. Technol., 56(7): 4251-4261. https://doi.org/10.1021/acs.est.1c07343

Rady M, Ezz-El-Din NA, Mohamed KF, Nasef S, Samir A, Elfeil WK (2020). Correlation between ESβL Salmonella serovars isolated from broilers and their virulence genes. J. Hellenic Vet. Med. Soc., 71(2): 2163-2170. https://doi.org/10.12681/jhvms.23645

Ramirez J, Guarner F, Bustos FL, Maruy A, Sdepanian VL, Cohen H (2020). Antibiotics as major disruptors of gut microbiota. Front. Cell. Infect. Microbiol., 10: 572912. https://doi.org/10.3389/fcimb.2020.572912

Ramirez MS, Tolmasky ME (2017). Amikacin: Uses, resistance, and prospects for inhibition. Molecules, 22(12): 2267. https://doi.org/10.3390/molecules22122267

Ribeiro CF, Silveira GG, Candido ED, Cardoso MH, Espinola CCM, Franco OL (2020). Effects of antibiotic treatment on gut microbiota and how to overcome its negative impacts on human health. ACS Infect. Dis., 6(10): 2544-2559. https://doi.org/10.1021/acsinfecdis.0c00036

Rimondi A (2014). Estudio de la patogenia del Virus de Influenza Aviar de baja patogenicidad en aves de corral inmunosuprimidas por la afección con el virus de la Anemia Infecciosa Aviar (Doctoral dissertation, Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales).

Scott NA, Andrusaite A, Andersen P, Lawson M, Alcon-Giner C, Leclaire C, and Mann ER (2018). Antibiotics induce sustained dysregulation of intestinal T-cell immunity by perturbing macrophage homeostasis. Sci. Trans. Med., 10(464): eaao4755. https://doi.org/10.1126/scitranslmed.aao4755

Sedeik ME, Awad AM, Rashed H, Elfeil WK (2018). Variations in pathogenicity and molecular characterization of infectious

bursal disease virus (IBDV) in Egypt. Am. J. Anim. Vet. Sci., 13(2): 76-86. https://doi.org/10.3844/ajavsp.2018.76.86

Sharif A, Ahmad T (2018). Preventing vaccine failure in poultry flocks. Immun. Vaccine Adj. Deliv. Syst. Strategy, 10: 80-94. https://doi.org/10.5772/intechopen.79330

Shen X, Zhang A, Zhao R, Yin L, Yin D, Dai Y, Hou H, Wang J, Hu X, Pan X, Zhang D (2024). Effects of adding antibiotics to an inactivated oil-adjuvant avian influenza vaccine on vaccine characteristics and chick health. Poult. Sci., pp. 104135. https://doi.org/10.1016/j.psj.2024.104135

Shi W, Gao GF (2021). Emerging H5N8 avian influenza viruses. Science, 372(6544): 784-786. https://doi.org/10.1126/science.abg6302

Smith RA, Thedford TR, Espe BH, Woodson PD, Burrows GE (1983). Effect of oxytetracycline administration on antibody response to *Brucella abortus* vaccination in calves. J. Am. Vet. Med. Assoc., 183(1): 70-71.

Sultan HA, Arafa AE, Talaat S, Gaballa AA, Kilany WH, Elfeil WK, Amarin N (2019). Efficacy of clade 2.3.2 H5-recombinant baculovirus vaccine in protecting muscovy and pekin ducks from clade 2.3.4.4 H5N8 highly pathogenic avian influenza infection. Avian Dis., 63(sp1): 219-229. https://doi.org/10.1637/11880-042718.1

Sultan HA, Elfeil WK, Nour AA, Tantawy L, Kamel EG, Eed EM, Talaat S (2022). Efficacy of the Newcastle disease virus genotype VII.1.1-matched vaccines in commercial broilers. Vaccines, 10(1): 29. https://doi.org/10.3390/vaccines10010029

Sultan HA, Talaat S, Elfeil WK, Selim K, Kutkat MA, Amer SA, Choi KS (2020). Protective efficacy of the Newcastle disease virus genotype VII–matched vaccine in commercial layers. Poult. Sci., 99(3): 1275-1286. https://doi.org/10.1016/j.psj.2019.10.063

Sutherland EK, Hutchinson TF, Yaussy DA (2003). Introduction, study area description, and experimental design. Characteristics of mixed-oak forest ecosystems in southern Ohio prior to the reintroduction of fire. Edited by EK Sutherland and TF Hutchinson. USDA For. Serv. Gen. Tech. Rep. NE-299, pp. 1-16. https://doi.org/10.2737/NE-GTR-299

Talat S, Abouelmaatti RR, Almeer R, Abdel-Daim MM, Elfeil WK (2020). Comparison of the effectiveness of two different vaccination regimes for avian influenza H9N2 in broiler chicken. Animals (Basel), 10(10): 1-12. https://doi.org/10.3390/ani10101875

Wille M, Barr IG (2022). Resurgence of avian influenza virus. Science, 376: 459-460. https://doi.org/10.1126/science.abo1232

Woo PC, Tsoi HW, Wong LP, Leung HC, Yuen KY (1999). Antibiotics modulate vaccine-induced humoral immune response. Clin. Diagn. Lab. Immunol., 6: 832-837. https://doi.org/10.1128/CDLI.6.6.832-837.1999

World Organization for Animal Health (2021). OIE terrestrial manual, Chapter 3.3.4. Avian Influenza (Including Infection with High Pathogenicity Avian Influenza Viruses), version adopted in May 2021. https://www.WOAH,TerrestrialManual2025.org/fileadmin/Home/eng/Health_standards/tahm/A_summry.htm. 2024.