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The Role of *E. coli* Infection on IBD Antibodies in Chicken Detected by ELISA Titers

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ABSTRACT

Background: Several factors causing immune suppression in chickens would lead to immune deficiency, *Escherichia coli* (*E. coli*) infection is one of these important determinants, for example, infectious bursal disease (IBD), infectious bronchitis indirect, Newcastle disease, H5 and H9 an acute illness that is extremely communicable and infectious and affects young chicks. The current study aims to determine the level of antibodies in chicks infected with *E. coli*.

Methods: The chicks were divided into two groups: the first group vaccinated chicks against IBD disease at the age of one day and ten days, compared to the second group vaccinated chicks at the age of 10 days only. Five thousand one-day-old Fayoumi chicks were raised in the experiment and divided into two groups for comparison against IBD virus antibodies.

Result: The study revealed that infectious bursal disease (IBD) infected chickens and inoculated with *E. coli* pre or concurrent to infection had lower antibody titers in both groups whether vaccinated at one day old or not, the level of antibodies to IBD disease is low in chicks, whether vaccinated or not vaccinated at one day of age, but when chicks were vaccinated on the ten day old of age with a weak vaccine IBD disease for both groups, it was found that the chicks that were vaccinated on the first day had a higher level of antibodies to IBD disease than the unvaccinated chicks at the age of one day. Fayoumi chicks, mean ELISA titers at one day was (1556±132) increased sharply to (3440±960) at 15 days of age and reach (5596±830) at 20 days of age for group 1 compared to group 2 was the results, mean ELISA titers at one day was (1470±187) increased slightly sharply to (2710±779) at 15 days of age and reach (3560±670) at 20 days of age. All samples tested positive for *E. coli* at one day of age, but turned negative at 15 and 20 days of age. Additionally, the indirect test for Infectious Bronchitis showed a result of 11459±250 at one day of age. In addition, showed that the effect of post inoculation of *E. coli* on the level of ND antibodies was studied in group 1 with *E. coli* infection revealed that the HI titer of all subgroup had arranged of geometric means varied was (7.8), in group 2 was ranged (7) where the effect was not significant.

Key words: Antibody, Chicks, *E. coli,* ELISA titers, Infectious bursal disease.

INTRODUCTION

Several immunizations against common viral diseases are necessary to protect commercial chicken flocks from these illnesses. Young chicks are susceptible to infectious bursal disease (IBD), which impairs immunity and causes large financial losses for the poultry industry worldwide, the illness causes lymphoid depletion in the bursa of Fabricius and impacts lymphatic tissues such as the spleen and bursa of Fabricius (Amajo *et al.,* 2022). The virus belongs to the Birnaviridae family (Waheed *et al.,* 2022). Wagari) 2021 (noted that the bursa of Fabricius in chickens is impacted by a particular disease (IBD). The name comes from Gumboro, Delaware, in the United States, where the first case was documented. Immunosuppression, mortality and a reduced feed conversion ratio are the hallmarks of broiler illness (Mili *et al.,* 2022). The age of the infected birds, the type of chicken, the virus strain, maternally produced antibodies and immunization history are some of the variables that affect the disease's clinical manifestations. Disease symptoms can range from being asymptomatic to more severe clinical signs, including poor feed conversion, diarrhea and death. These indicators are often associated with various conditions in animals and their severity can vary widely depending on the disease. (De Wit *et al.,* 2001). *E. coli*-induced immunosuppression is of primary relevance because to the widespread incidence of infection in commercial hens. Numerous bacterial, protozoa and viral illnesses in hens have been shown to be impacted by IBD infection. Early-life *E. Coli* infection severely impairs chickens' immune responses; however, *E. coli* infection is a secondary localized or systemic disease that arises when host defenses are compromised (Nakamura *et al.,* 1986). The immune response is based on the main organs, such as the thymus gland and bursa

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of Fabricius. One major issue affecting Egypt's chicken business is vaccination failure. *E. coli* infection causes marked gross and microscopic bursal lesions leading to bursal atrophy which resulted transient humeral immunosuppression Hassan and Hassan (1999). The immunosuppressive effect of some virus such as Newcastle disease (ND), Infectious bursal disease (IBD) and bacterial agents as *E. coli* causing the great economic loses in commercial flocks. Bursal Disease Virus is a major pathogen that destroys B lymphocytes in the bursa of Fabricius, leading to immunosuppression and death in birds between 2 to 8 weeks of age. *Escherichia coli* (*E. coli*) infection is the most common secondary bacterial invaders followed the application of live vaccines as ND and IBD vaccine which are the most common vaccines used on large scale in commercial flocks (Mazariegos *et al.,* 1990).

Engall and Perlmann independently created the enzyme-linked immunosorbent assay (ELISA) in 1971 in order to circumvent the issues that come with radioimmunoassay. ELISAs have been used extensively ever since to assess the levels of monoclonal and polyclonal antibodies in culture media or biological fluids. To identify antigen-specific antibodies after vaccination or to detect antibodies for the identification of infectious diseases, there are already a number of commercial kits and automated systems available (Gheesling *et al.,* 1994). Using antisera or other fluids from immunized experimental animals or human clinical trials, accurate measurement of antibody titers is one of the most essential read-outs to assess the immunogenicity of experimental vaccine candidates. Numerous studies have already been conducted to enhance the ELISA itself (Ravindranath *et al.,* 1994), so that the methodology of indirect ELISAs is well established. The goal of the current study was to investigate the impact of experimental *E. coli* infection on the immune response and IBD vaccination with inter mediate plus IBDV vaccine.

MATERIALS AND METHODS

Chickens

A total of 5000 Fayoumi chicks *E. coli* infection were used during this study. They were divided into two groups: one group was vaccinated at 1 day old and again at 10 days old with inactivated vaccine by under the skin of the neck and the second group was not vaccinated at one day old but was vaccinated at 10 days old. These chicks were raised in the open housing system. Twenty chicks from each group were randomly selected to have blood samples taken at 1, 15 and 20 days of age. Samples of collected blood were centrifuged for five minutes at 3000 rpm. Prior to serological testing using the ELISA titers test, sera were stored at -20°C. Each age group's mean titers were calculated and statistically analyzed. This experiment was conducted from November 2024 to January 2025, in Poultry Breeding Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University.

E. coli strain, chicks infected with *E. coli* were obtained from the farm and this was confirmed by performing a culture for this infection.

IBDV vaccine, is an inactivated vaccine administered subcutaneously.

ELISA test protocol the ELISA test was carried out according to the manufacturer's instructions. Titer-Relates S/P at a 1:500 dilution to an endpoint titer: Log10 titer=1.09 (log10 S/P). ELISA test, Samples 20 sample from both groups was diluted 1:500 in buffer saline and assayed by IBD antibody and infectious bronchitis indirect.

HI test, ND antibody titer, H5 and H9 were determined by the HI test using the standard procedures in micro titer plates using 4 HA units Stephan *et al.* (1998).

Statistical analysis

Data were subjected to two way analysis and their interaction using the General Linear Models (GLM) procedure of SAS User's Guide, Ver.8.2, 2001. Duncan's multiple range tests were used to separate means when separation was relevant.

$$Y_{ikj} = \mu + G_i + A_j + G*A + e_{ik}$$

Where,

μ = Overall mean.

G_i = Group effect.

A_j = Age effect.

G*A = Interaction effect.

e_{ik} = Experimental error.

RESULTS AND DISCUSSION

Fig (1) showed that all samples tested positive for *E. coli* at one day of age, but turned negative at 15 and 20 days of age. In Table (1) that ELISA test was adapted to determine infectious bursal disease (IBD) antibodies in chick's sera at 1, 15 and 20 days of age. ELISA titers at one day was (1556±132) increased sharply to (3440±960) at 15 days of age and reach (5596±830) at 20 days of age for group 1 compared to group 2 was the results, mean ELISA titers at one day was (1470±187) increased slightly sharply to (2710±779) at 15 days of age and reach (3560±670) at 20 days of age. (Fig 2) showed that infectious bronchitis indirect (11459±250) at one day of age and non-significant in both group 1 and 2. Also, (Fig 3) showed that the effect of post inoculation of *E. coli* on the level of ND antibodies was studied in group 1 with *E. coli* infection revealed that the HI titer of all subgroup had arranged of geometric

Table 1: ELISA titers of IBDV in fayoumi breed.

Groups	IBD		
	1 day	15 day	20 day
Group 1	1556±132	3440^{A}±960	5596^{A}±830
Group 2	1470±187	2710^{B}±779	3560^{B}±670
P = value	NS	*	*

^{a,b}Means within column with different are significantly different, NS = not significant. *Significant at P≤0.01.

means varied was (7.8), in group 2 was ranged (7). Also, showed that H5 and H9 was (6.5 and 7.4) respectively in group 1 but in group 2 was (7 and 7.1) respectively.

These results agreed with those of previous studies they came to the conclusion that the ELISA test is useful for measuring and detecting IBD antibodies as well as for evaluating the decrease in antibody titer. When BF is damaged by bacterial (*E. coli*) or viral (IBD) agents, the humeral immune response is directly impacted. A study by Shinde *et al.* (2021) investigated the prevalence of IBDV in both vaccinated and non-vaccinated flocks. Despite vaccination efforts, IBDV was detected in 38.88% of the flocks, indicating that vaccination alone may not provide complete protection. This underscores the importance of monitoring antibody levels through ELISA to assess vaccine efficacy and detect potential failures. Kannaki *et al.* (2019) found that Con A-S-ELISA for the detection and quantification of anti-Newcastle disease-antibodies proved to be sensitive, specific and accurate. Moreover, Con A-S-ELISA is rapid, costeffective and easy to perform; hence, this assay can be effective used in the field for sero-monitoring. The research of Hassan and Hassanein (1999) supported this observation. The results showed that it is better to vaccinate the chicks at one day of age against IBD disease because the level of antibodies began to rise and appear on the 20th day of age. The immune antibodies to this disease were high in the first group compared to the second group, whose immune antibodies were lower than the first group, as they were not vaccinated on the first day of age and were vaccinated on the tenth day of age.

A study by Choudhary *et al.* (2012) investigated the incidence of infectious bursal disease (IBD) in and around Ranchi. This study provides valuable information on the seroprevalence and seasonal variability of IBD, which can be relevant when considering lymphoid organ pathology in poultry. According to Sharma *et al.* (2016) observed significant lymphocytic depletion in the bursa of Fabricius and spleen following experimental *E. coli* inoculation. Because, it is known that Fayoumi chickens are very sensitive to salmonella and *E. coli*, so they get infected easily. Therefore, in this experiment, we brought infected chicks and vaccinated them with an inactivated vaccine and measured the level of antibodies. Also, we made a culture for *E. coli*. At first, we found that the level of antibodies to IBD was low in both groups. Then we started treating the *E. coli* and made a culture. We found that it had completely disappeared and the result was negative. We repeated the vaccination on the tenth day and found that the level of antibodies was rising, knowing that the vaccination on the first day with an inactivated vaccine began to appear on the 20th day, when the antibodies to infectious bursal disease (IBD) increased.

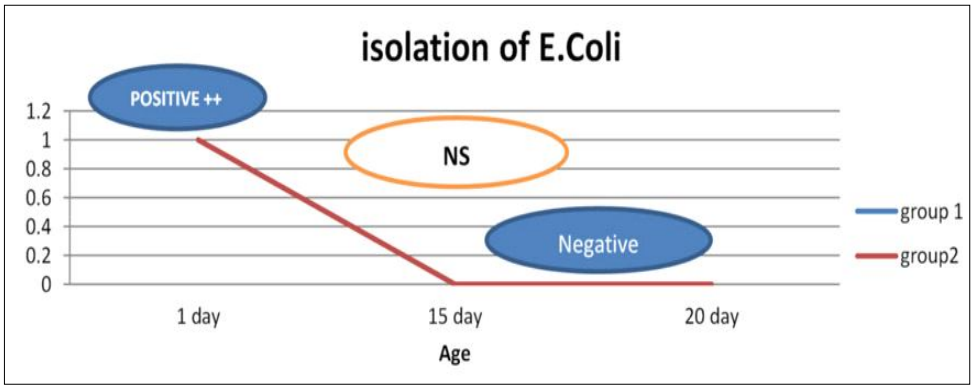


Fig 1: Isolation of *E.coli* in Fayoumi breed.

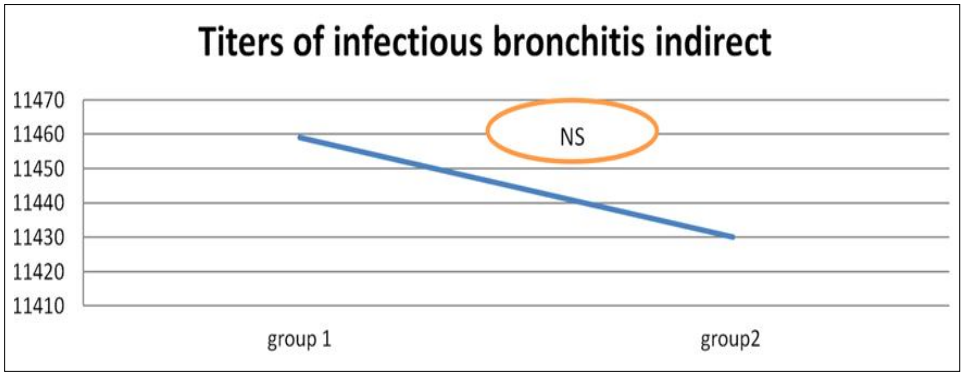


Fig 2: Mean ELISA titers of infectious bronchitis indirect in Fayoumi breed.

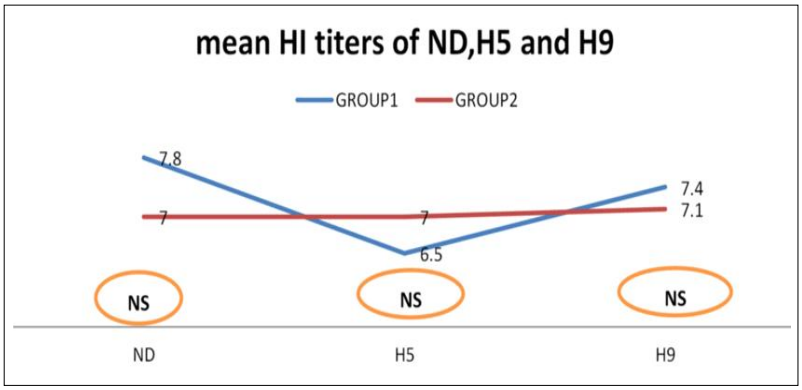


Fig 3: Mean HI titers of Newcastle disease (ND), H5 and H9 in Fayoumi breed.

CONCLUSION

Infectious bursal disease (IBD) is one of the most dangerous diseases affecting chickens, causing significant economic losses. Therefore, through research, we aimed to determine whether vaccinating chickens at one day old would help maintain a high level of antibodies against IBD throughout their lives. Our findings suggest that the best vaccination schedule to achieve optimal antibody levels against IBD involves vaccinating at one day of age, followed by additional doses at 10 and 20 days of age. This vaccination schedule results in a high percentage of antibodies against the disease.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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