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Effects Of Salmonella Pullorum Infection on Vaccinal Immunity Against Newcastle and Avian Influenza Diseases in Broiler Chickens

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Abstract: A study aimed to evaluate the effect of Salmonella Pullorum infection on vaccinal immunity against ND and AIV in a broiler. Before starting the study collected several samples from suspected infected flocks with S. Pullorum (OM988162.1), and identified by several biochemical test then molecular test PCR and confirmed isolates by sequencing. then take two hundred and fifty (Ross 308) Five groups of broiler chicks were formed. Each group has 50 chicks., as follow first group: infected with *S. Pullorum* and vaccinated against (NDV, AIV). Second group: infected with *S. Pullorum* and treated by (ciprofloxacin 10% 1ml / liter or 20 % 0,5 L for 7day and colistin 200million IU/0.5L for 7 days) also vaccinated against (NDV, AIV). Third group: infected with *S. Pullorum* and treated by (ciprofloxacin 10% 1ml/ liter or 20 % 0,5 L for 7day and colistin 200million IU/0.5L for 7 days) only. Fourth group: infected with *S. Pollorum* only as consider control positive. Five group: uninfected and unvaccinated as consider control negative. Antibodies titre (IgG and IFN-γ) against ND and AIV were significantly increased in G1 with the least histopathological changes in liver, kidney, bursa of fabricius, and thymus. G1 and G4 growth performance was significantly lower than that of treatment groups G2 and G3. Conclusion: S. Pullorum infection increased vaccinal immunity (IgG and IFN-γ) against Newcastle and influenza. Ciprofloxacin and colistin treatment improved body weight and weight gain.

Key Word: ELISA Test, IgG, IFN-γ, Histopathological changes, Body weight

Introduction

Pullorum disease (also known as BWD) is one of the most significant global poultry illnesses due to its economic impact, global spread, and disease prevention challenges (Endris et al., 2013). High mortality rates, reduced egg and chick production, and high human and animal medicine costs have caused an economic loss (Netsanet et al., 2012). Newcastle disease (ND) is a viral disease that has a significant economic impact on the poultry industry. The amino acid patterns at the F-cleavage glycoprotein site of NDV play a role in the virus's pathogenicity. (Nordin et al., 2001). Hygiene and vaccination are the two most important methods for controlling NDV. The number of viruses in the cells, as well as the strain's virulence, can have an impact on the specific reactions that occur after infection. (Lowenthal *et al.,* 1995). Macrophage activation and cell-mediated immunity are boosted by IFN produced by NK cells and T lymphocytes. Chickens that have been exposed to NDV twice have cytotoxic T lymphocytes (CD8) in their spleens (Sick *et al.*, 2000). Cell-mediated immunity, specifically a T helper cell type 1 (Th1) response, can induce a humoral response from the host. .(Lambrecht *et al.,* 2004). Avian influenza viruses are classified into subtypes using hemagglutinin and neuraminidase (Brown *et al.,* 2010). There are influenza viruses in birds' respiratory secretions and in their excreta and fecal matter. Good biosecurity and hygiene can reduce the risk of virus transmission to poultry. Farmers should avoid contact with any other domestic or wild birds, mechanical vectors, and fomites such as water sources (Swayne, 2020). Poultry flocks benefit from all-in/all-out flock management. The 16 different strains of influenza A virus have no vaccine that provides

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complete immunity. Both vaccination and infection with the AI virus trigger a humoral antibody response. Antibodies to both HA and NA are protective because they neutralize and protect against pathogens (Suarez and Schultz, 2000) Cytotoxic T cells, which are typically CD^{+8} cells, appear to be the primary immune cells involved in cell-mediated defense (Swayne *et al.*, 2020).

Methodology

Experimental animal

The study was conducted to evaluate vaccinal immunity against NDV and AIV with Pullorum infection, take two hundred and fifth (Ross 308) dividing the broiler chickens into five groups 50 chicks per group, with each group given the following care:

first group: infected with *S. Pullorum* and vaccinated against (NDV, AIV). Second group: infected with *S. Pullorum* and treated by (ciprofloxacin 10% 1ml / liter or 20 % 0,5 L for 7day and colistin 200 million IU/0.5L for 7 days) also vaccinated against (NDV, AIV). Third group: infected with *S. Pullorum* and treated by (ciprofloxacin 10% 1ml / liter or 20 % 0,5 L for 7day and colistin 200million IU/0.5L for 7 days) only. Fourth group: infected with *S. Pullorum* only as consider control positive. Five group: uninfected and unvaccinated as consider control negative.

Blood samples

Blood was collected from chickens at 7, 14, 21, 28, and 35 days of age using sterile needles and immediately placed into 15 ml tubes no containing heparin. After separating a serum sample from blood clots, the collected sample was kept frozen in a refrigerator for it was analyzed**.**

ELISA Test

The procedures for detection of NDV antibody in chicken using a rapid serological test (ELISA) was carried out according to the manufacture company ProFlock® NDV ELISA kit and AIV ELISA kit (Synbiotics – USA).

Detection of Chicken IFN-γ concentrations

Using a commercially available ELISA kit, the level of interferon- (IFN-) in chicken sera was evaluated for the quantitative measurement of IFN- concentrations in serum. Picograms per milliliter (Pg/ml) were used to express the results, with a detection range of 2.8 to 180 Pg/ml. Methods specified by the manufacturer were used in this assay. (SunLong Biotech Co.LT).

Body weight

The average weight of the chicks was determined by weighing five at random every seven days and then dividing the total weight by the total number of chicks in each group.

Body weight gain

After calculating the weight of each group individually through 7, 14, 21, 28 and 35 weight gain was calculated after deducting the initial weight, on the basis of the formula below:

Weight gain= Weight every seven days - Weight at the beginning of the first seven days.

Feed Conversion Ratio (FCR)

The following equation was used to calculate for each group during the experiment:

$$\text{Feed conversion ratio} = \frac{\text{Feed consumption rate (gm)}}{\text{Weight gain rate (gram)}}$$

Feed conversion efficiency (FCE)

The following formula was used to calculate the feed conversion efficiency for each category::

$$\text{Feed conversion efficiency} = \frac{\text{Body weight gain rate (gm)}}{\text{Feed consumption rate (gm)}}$$

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Result

Immunity against Newcastle disease

Following the random selection of 10 serum samples following the slaughter of 260 1-day-old chicks, the maternal immunity to ND could be determined. ELISA results showed a healthy immune response, with a mean value of. (8342±354) of antibodies against ND in all groups

Table 1 .Antibody titer (Means ± SE) against Newcastle disease in broiler chickens in different periods by ELISA test

Periods	7 days	14 days	21 days	28 days	35 days
Groups	ND antibody titre Means ± Stander error				
G1	3299.2±231 **A**	3811.2±245.7 A	4232.7±564.2 A	5676±421 A	6783.3±563.1A
G2	2987.4±211.1 **B**	2733±232.1 B	3077.8±321 B	3543.7±428.2 B	4342.6±456.2B
G3	2766.1±223.3 **B**	1004±144.2 E	177.2±225 D	0±0 C	0±0 C
G4	3322.2±214.7 **A**	1600±212 D	232±43.1 D	155±22.1 C	0±0 C
G5	3555.5±255.2 **A**	2032±209 C	987±87.2 C	233±30.2 C	0±0 C
LSD	332.28	366.5	505.36	736	1054.23

Number of samples: 5 from each group.

Immunity against Avian Influenza.

Maternal immunity against AIV by ELISA test showed low immune response with mean value (3452±543.4). The highest antibody titre was given by first group followed by second group as compared to unimmunized groups which found no evidence of an AIV-specific immune response.

Table 2 Antibody titer (Means ± SE) against Avian Influenza in broiler chickens in different periods by ELISA Test

Periods	7 days	14 days	21 days	28 days	35 days
Groups	ND antibody titre Means ± Stander error				
G1	1988.1±321 A	2055.6±210.8 A	2879.6±564.2 A	3674.8±587 A	5773.6±676 A
G2	1944.3±243 A	1758.7±166.7 B	2243.7±321 B	2932.2±411 B	4608.3±221.6 B
G3	1899.2±213.9 A	1232±378.1 C	233.5±97.8 D	0±0 C	0±0 C
G4	1965.3±311.6 A	1139±335 C	879.7±77.3 C	0±0 C	0±0 C
G5	2177.2±436.7 A	1345±211 C	869.8±45.9 C	0±0 C	0±0 C
LSD	411.31	243.8	477.6	534.87	644.77

Number of samples: 5 from each group.

Chicken Interferon γ (IFN-γ)

Among 7-day-old, 21-day-old, and 35-day-old chicks, there were statistically significant differences in IFN-titer between all groups ($P< 0.05$). Highest antibody titre was given by first group followed by second group as compared with unimmunized groups.

Table 3 Interferon γ titre (Means ± SE) in serum of broiler chickens in different periods by ELISA test

Periods	7 days	21 days	35 days
Groups	Interferon titre Means ± Stander error		
G1	45.6±2.1 **A**	53.4±11.8 A	55.4±12.3 A
G2	38.2±4.2 **B**	41.8±9.7 B	43.1±10.2 B
G3	30.7±1.9 **C**	33.3±6.1 C	22.5±9.2 C
G4	40.5±3.6 **B**	46.6±7.8 B	30.7±7.3 C
G5	7.1±0.7 **D**	5.4±0.6 D	3.3±0.9 D
LSD	4.6	6.78	11.06

Number of samples: 5 from each group.

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Weight gain and Final weight

The average body weight of all groups at one point in the study was 45 grams. The highest body weight was recorded in fifth group and followed by third and second groups. Significantly backwardness has been evident in treated groups especially infected with S. Pollorum in comparison with control group.

Table 4. Weight gain and final weight (gram) of different groups in different periods (Mean ± SE)

Groups	7 days	14 days	21 days	28 days	35 days	Final weigh
G1	122.3±0.19**B**	231.8±0.42B	305.5±0.65C	518.6±1.2C	756.2±21.1B	1979.4±211C
G2	125.8±0.2**B**	255±0.33B	386.4±0.54B	611±3.2B	889.4±26A	2312.6±322B
G3	140.4±0.16A**B**	277±0.22B	396.1±0.47B	720±5B	803.7±25A	2382±342AB
G4	135.3±0.12**B**	266.7±0.54B	311±0.34C	548.6±6.2C	760±32B	2066.6±415C
G5	158.9±0.22**A**	333.1±0.34A	478.5±0.87A	723.3±5.2A	875.3±78.3A	2614.1±287A

Number of samples: 5 from each group.

Feed conversion ratio and feed conversion efficiency

Feed conversion efficiency measurements were found to be in agreement with measured feed conversion ratios. When comparing feed conversion efficiency across all groups, there was a significant difference at the ($P< 0.05$). level, with the (fifth),(third), (second), (fourth), (first), groups achieving respectively,

Table 5 Results of feed consumption, feed conversion ratio and feed conversion efficiency of different groups (Mean ± SE).

Groups	final weigh	Feed consumption	FCR	FCE
G1	1979.4±211 C	3980±76.8 A	2.01±0.11 A	0.49±0.03 B
G2	2312.6±322 B	4256±10.3 B	1.84±0.4 B	0.54±0.04 AB
G3	2382.2±342 AB	4290±21.7 C	1.8±0.7 B	0.55±0.05 AB
G4	2066.6±415 C	3960±21.2 C	1.91±0.007 A	0.52±0.01 B
G5	2614.1±287 A	4460±34.6 C	1.7±0.007 C	0.58±0.03 A

Number of samples: 5 from each group.

Discussion

The findings are consistent with those of (Hamal et al. 2006), who found that multiple vaccinations with high titres had a cumulative effect on Ab production. This may bring us back to how avian cells responded to the Salmonella infection by expressing specific immune mediators from infected cells. Lymphocytes can illustrate the function of cell-mediated immune responses by employing a particular antigen. It has been shown that employing attenuated Salmonella to create recombinant live vaccines can considerably boost the immunological effect of the exogenous gene. The La Sota vaccination can offer cross-protection against various NDV genotypes. (Ke Ding et al., 2018. Kaiser and Staheli, 2008). Chickens injected with Salmonella produced antibodies at a slower rate than those infected orally. Chickens that were 10 days old

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shed more S. pullorum and produced more antibodies than chickens that were 42 weeks old. There is proof that some antibiotics, such as enrofloxacin, may negatively impact hens' humoral defensive mechanisms (Laxminarayan and Heymann, 2012). S. Pullorum causes a greater Th2-like compared to the Th1-type response, which is typically connected to S. Typhimurium or S. Enteritidis Activated macrophages are the source of the inflammatory cytokine IL-18, which is IFN- inducing (Th1 immunological mechanism). When compared to G2 and G3, the immunized groups showed IFN- (Th1 cytokines) at 14, and 21 DPI. Most, but not all, of the hens given the live NDV vaccine in younger birds produce IFN after recall stimulation. (Tang et al., 2018, Couper et al., 2008). By the age of 4 D, chicks infected with S. pullorum lose the balance of their intestinal flora. Pathogenic Salmonella colonization is reduced by Lactobacillus. L. plantarum Zhang-LL and L. (Caicai Chen et al., 2020. Higgins et al., 2008). L. plantarum Zhang-LL and L. paracasei KL1both exhibit high resistance to the unfavorable gastrointestinal environment. After exposure to S. Pullorum infection, chickens showed decreased levels of protein and steroid synthesis in the jejunum. Salmonella-infected chicks also showed lower levels of arginine, methionine, glycine, and tryptophan. Inflammation-related steroid synthesis is a crucial component of a negative feedback loop (Sui et al., 2016. Ross et al. 1955; Antunes et al., 2011).

Conclusion

This research S. Pullorum infection increased the vaccinal immunity (IgG and IFN- γ) of vaccinated group against Newcastle and influenza. Treatment with ciprofloxacin and colistin significantly improved body weight and weight gain for those who had not received treatment.

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