

Exacerbating effect of Newcastle disease virus (NDV) infection on sub clinical caecal coccidiosis in broilers vaccinated against NDV

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Abstract: This study reports the effect of experimental infection with NDV on the sub clinical caecal coccidiosis in broiler birds vaccinated against NDV. For this purpose 300 one-day-old broiler chickens were randomly divided into 3 equal groups (G1, G2, and G3) of 100. The birds were placed on floor pens in separated rooms. At 15 days of age G3 was infected intra crop with a dose of 12500 sporulated oocysts of a field strain of caecal *E. species*, which suspected to be *E. tenella* isolated from clinically affected broiler flock with cecal coccidiosis. G1 and G2 were not challenged and remained as control negative for coccidia. Management and nutrition were the same in all groups. All groups of birds were vaccinated at 4th day of age with Infectious Bronchitis (IB) virus vaccine (H120 strain) by eye drop method. At 7th day of age NDV vaccines (Inactivated and Hitchener B1) were given by subcutaneous (SC) and eye drop routes respectively for G2 and G3. The infectious bursal disease virus (IBDV) vaccine and Inactivated H5N2 Avian Influenza (AI) virus vaccine were given at 13th day of age for all groups by eye drop and SC routes respectively. At 17th day of age G2 and G3 were also vaccinated against NDV with Lasota strain vaccine by eye drop while G1 was not vaccinated with any one of NDV vaccines and remained as a blank. The challenging NDV was given for all groups at 25th day of age by intramuscular (IM) injection. The birds of G1 exhibited 100% mortality with obvious PM lesion of NDV infection. The birds of G2 showed torticollis in one bird only. The birds of the G3 started dying with bloody diarrhea a day post challenging with NDV and the clinical signs, postmortem (PM) findings and response to treatment were used to confirm coccidiosis. The rapid onset of the clinical disease and the high mortality rate (36% over a period of 5 days) was considered to have been induced by the challenging NDV administration. The diagnosis of sub clinical coccidiosis and institution of prophylactic anticoccidial therapy would have obviated the clinical disease in the field.

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1. Introduction

Coccidiosis is a disease of almost universal importance in poultry production, caused by intracellular protozoan parasites of the genus *Eimeria*. Coccidiosis in the domestic chickens is an enteric disease. During different stages of *Eimeria* life cycle which is take place in the mucosal layer of the intestine, destruction of the epithelial lining of the intestine takes place and is often accompanied by some degree of inflammation, resulting in local pathological changes (1), dehydration and diarrhea (2), interruption of feeding and digestive processes, blood loss and absorption of many nutrients are reduced (3). There are 9 species of *Eimeria* known to occur in the chicken: *E. tenella*, *E. mitis*, *E. acervulina*, *E. maxima*, *E. necatrix*, *E. praecox*, *E. brunette*, *E. mivati* and *E. hagani* (3). *E. tenella* is the main principle cause of the caecal coccidiosis (3, 4), characterized by accumulation of blood in the caeca and bloody droppings (5, 6). The clinical manifestation of coccidiosis is known to depend on several factors among which are those of the invading parasites, the host and the environment (5, 7). The

initial signs of infection are huddling and anorexia (8). Blood loss and the associated intestinal membrane damage, dehydration, diarrhea and maldigestion are the major causes of death (7, 9). This study performed to clear out the role of NDV infection on subclinical cecal coccidiosis in broilers previously vaccinated with NDV vaccines.

2. Material and Methods

***E. tenella* inoculum:**

The inoculum of sporulated oocysts were prepared at the lab of parasitology, poultry diseases department, faculty of veterinary medicine, Cairo University. The inoculum's challenging dose is 12500 sporulated oocysts / ml / bird which given by oral route.

Viruses and vaccines:

1. Newcastle disease virus (NDV) challenge strain

A velogenic viscerotropic strain of Newcastle disease virus (VVNDV) characterized by Sheble and Reda, 1976 (10), was obtained from

Newcastle Vaccine Research Dept., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The challenge dose was $10^{6.8}$ EID₅₀ / ml / bird and given by IM injection. The vilogenicity of the virus was monitored by injecting non-vaccinated susceptible 10 birds with a dose of $10^{6.8}$ EID₅₀ / ml / bird intramuscularly which resulted in 100% mortality.

2. IB virus vaccine

Nobilis IB 120, live freeze-dried virus vaccine against Infectious Bronchitis serotype Massachusetts (strain H120) was used.

3. NDV vaccines

NDV Vaccine (Hitchener B1 strain) (Schering-Plough Animal Health, Millsboro, Delaware, USA), LaSota NDV vaccine (B1 type, LaSota strain) (For Dodge Animal Health, Iowa 50501, USA) and Nobilis ND Broiler Inactivated vaccine for the immunization of chickens against NDV were used.

4. IBDV vaccine

For this purpose, a freeze-dried live vaccine Nobilis Gumboro 228E (Intervet International, BV Boxmeer-Holland) grown on embryonated eggs having at least $2.0 \log^{10}$ EID₅₀ was used.

5. AI vaccine

Inactivated H5N2 AI vaccine (Intervet International, BV Boxmeer-Holland) was used.

Chickens:

Three hundreds day old broiler chicks were kindly supplied from Ommat Poultry Company.

Experimental design

Chickens:

A total of 300 one-day-old broiler chickens were randomly divided into 3 equal groups (G1, G2, and G3) of 100.

Situation of the experiment:

All management and nutrition were the same and all groups were kept on litter floor pens in separate rooms and fed on a commercial ration *ad-libitum* up to 42 days of age (end of experiment).

Chickens of all groups were vaccinated with Infectious Bronchitis (IB) virus vaccine (H120 strain) at 4th day of age by eye drop method. Inactivated H5N2 AI vaccine and IBDV vaccine were given by subcutaneous and eye drop routes respectively at 13th day of age to all birds.

Birds of G2 and G3 only were vaccinated against NDV with Hitchener B1 vaccine at 7th day of age and LaSota vaccine at 17th day of age by eye drop instillation, while inactivated NDV vaccine was given by SC route for G2 and G3 at 7th day of age.

Chickens of G3 only were orally treated with low dose of sporulated oocysts of *E. tenella* (12500) at 15th day of age (11).

The VVND challenging virus was given in a dose of $10^{6.8}$ EID₅₀ / ml / bird by IM injection for all groups at 25th day of age and birds were kept under close observation for further 2 weeks for documentation of clinical signs, mortality and PM lesions.

3. Results and Discussion

In G3, a day after the administration of challenging NDV, 2 birds died (Day 26). The deaths were increased to be 30 birds by day 27, then 3, 2 and 1 died at day 28, 29 and 30 respectively. At the point of the peak of mortality the majority of the surviving birds were seen huddling along the sides of the pen, diarrhetic (bloody) (Fig. 1, 2) and anorectic. All the dead birds were in very good body condition. PM examination revealed caeca heavily distended with blood (Fig. 3) and without affection of the small intestines (Fig. 4) and with numerous necrotic patches in caecal wall (Fig. 5). The dissected caecum was severely inflamed with thickened wall, sloughing of its mucosa and had pronounced bloody caecal chores (Fig.6). The diagnosis of caecal coccidiosis was purely based on the clinical signs and post mortem lesions which were, to a large extent, pathognomonic (3). One bird of the G2 showed nervous signs (torticollis) without mortality. The blank control group (G1) exhibited 100% mortality with obvious PM lesions of Newcastle disease (ND) Fig. (7, 8, 9 and 10).

Table -1- Vaccination program of all Groups

Age in days	Vaccine	Group vaccinated	Route of application
4	IB	All birds	Eye Drop
7	ND Hitchener-B1 Inactivated	G2 and G3	Eye Drop SC
13	IBD AI	All birds	Eye Drop SC
17	ND (LaSota)	G2 and G3	Eye Drop



Fig. 1: Bloody dropping.



Fig.2: Bloody dropping.



Fig. 3: Caeca distended with blood.



Fig.4: Caeca distended with blood without affection of the small intestines.



Fig. 5: Caeca distended with blood with numerous necrotic patches in caecal wall.



Fig. 6: Dissected caeca was severely inflamed showing sloughed mucosa, with thickened wall, and had pronounced caecal chores.



Fig. Chicken, Coccidiosis, NDV, Subc; inical Coccidiosis, Coccidiosis



Fig. 8: Necrosis and hemorrhage in intestinal lymphoid aggregates evident from the serosal surface (Fig. 7) and mucosal surface (Fig. 8).



Fig. 9: Peritonitis with fibrin deposition



Fig. 10: Hemorrhage in the mucosa of proventriculus.

Alexander (12) reported that *E. tenella* has a pre patent period of 123 hours. As the birds of G3 had sub clinical dose of *E. tenella* infection (12500 sporulated oocysts) and the birds did not exhibit any clinical disease through 10 days after challenging with such dose, also the birds appeared healthy on the day of NDV challenging and a mortality rate of 2% and 30% were recorded on days 1 and 2 respectively post NDV administration while the total mortality rate reached 36% over a period of 5 days, and all clinical signs and PM lesions of dead birds were greatly suggestive to caecal coccidiosis without any

evidence of ND lesions, so the acute onset of the clinical disease (caecal coccidiosis) and its high mortality rate have been precipitated by the stress of challenging NDV. Since the incubatory and latent disease conditions (coccidiasis) are known to be quickened and aggravated by vaccination (5), so that in case of disease out breaks (field exposure to NDV) in the farm with higher levels of infectious dose, and involvement of more virulent strains of NDV (here simulated by challenging with VVNDV strain) a more severe, sudden and acute clinical disease (caecal coccidiosis) is expected. ND and IB viruses have been noted to aggravate the symptoms of coccidiosis (13, 14). Stress-inducing factors are known to alter the clinical manifestation of coccidiosis (5, 7, and 15). Treatment of the birds with anticoccidial drugs resulting in improvement of the survivor birds. Prompt treatment with anticoccidial of proven efficacy and record fast action may have reasonably mitigated the clinical picture (16). Similar condition would likely not have occurred in the field had sub clinical coccidiosis (coccidiasis) been diagnosed and counteracted.

Conclusion

This study reports an increased mortality to caecal coccidiosis infection precipitated by the experimental infection with NDV in broiler chickens vaccinated with NDV vaccines (Hitchener B1, Lasota and Inactivated vaccines).

Coccidiosis has remained the most important poultry disease threatening the productivity of poultry industry. Although there is dearth of accurate data to quantify the economic impact, records of losses due to the disease (clinical and sub clinical) and the cost of applied control measures appear appreciable and reprehensible. Prompt diagnosis of the condition and recourse to non-drug based control measures, may, in the long run, prove very rewarding.

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