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Concurrent infections of Newcastle disease virus and *Escherichia coli* in layer chicken and their pathobiology

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Abstract

Newcastle disease is a viral disease which affects avian species and cause heavy economic loss to the poultry farmers. *Escherichia coli* often complicates the viral infections in avian species. Few reports are available on the outbreak of Newcastle disease combined with *Escherichia coli* infection in layer poultry. This outbreak report explaining the coinfection status of both Newcastle disease virus and *Escherichia coli* infections in a layer poultry at the age of 18 and 35 weeks in an organized cage system of poultry rearing in R Sitapura village of Odisha state. Based on the information given by the farm personal an investigation work was carried out. The clinical diagnosis was based on the information's collected from the farm records, clinical signs observed by the investigation team, pathological lesions evidenced by in the postmortem findings and the culture studies for confirmation of *Escherichia coli* and serological studies for the confirmation of Newcastle disease virus. These studies confirmed the concurrent infections of Newcastle disease virus and *Escherichia coli* in the layer poultry in the absence of valid biosecurity measures, and robust vaccination schedule for ND virus where the endemicity is existing for both.

Keywords: Concurrent infection, ND-Colibacillosis, Layer poultry

Introduction

The livestock sector is an important subsector of agriculture in the Indian economy and contribute 12.99% growth rate. The contribution of livestock in total agriculture and allied sector gross value added has increased to 30.23% in 2022-23. India ranks second in egg production and 5th in the meat production in the world (G.O.I CAGR-2014). A significant proportion of farmers involved in the poultry production for their economic status. Animal husbandry sector contributes to about 4.5%-5.5% of the total gross domestic product (GDP) in India. The poultry and livestock production contribute major protein sources to the human population and improving climate change (O.I.E 2017) ^[10]. The contribution of poultry sector to Indian economy is 1.2% of India's GDP. However, the poultry population facing lot of challenges with colibacillosis and Newcastle disease that landed in severe economic loss to the farming community. ND as a viral disease-causing heavy mortality and low egg production. The birds affected with ND commonly seen with gastrointestinal hemorrhage and ulcers. Because of economic values the ND is designated as a reportable disease to OIE (O.I.E 2017) ^[10]. Colibacillosis cause high mortality in poultry in all age group of birds. The major clinical findings observed in the colibacillosis including poor growth, low egg production and poor egg quality. The diagnosis of *E. coli* is based mostly on the culture studies from the infected tissue samples. (Abalaka *et al.* 2017) ^[1] Abalaka. The confirmation of ND virus was carried out using HI test (Sajo *et al.* 2023; Ganesan *et al.* (1992) ^[14, 8] In this outbreak case, the clinical signs exhibited by the infected poultry population, the laboratory studies for the confirmations of ND virus with *E. coli* and P.M findings were carried out.

Case details

Continuous mortality i.e. 302 birds out of 1200 in a layer poultry with cage system of rearing at the age of 18 weeks and again at 35 weeks of age with peak egg production was reported in

a layer poultry farm in R. Sitapura village of Odisha state, India. This farm was running with a flock size of 1200 pullets procured from a local commercial farm and it faced the outbreak of ND and coli septicemia at the age of 18 weeks and at the age of 35 weeks. The birds were fed with commercial feed and untreated bore water. The pullets were said to be vaccinated with Lasota strain by the commercial farmer twice during grower stage. The chicken at pullet stage and the at the layer stage were examined for their clinical manifestations. The birds suffered with, nasal discharge, low intake of feed, high mortalities and morbidities in the pullet stage and in laying stage additional clinical signs with low egg production and leathery eggs. The mortality started at the age of 18 weeks and the PM studies, serological study for ND virus and the culture studies for *E. coli* confirmed the prevalence of both NDV and *E. coli*. The chicken was treated with antibiotics as per antibiogram during pullet stage and were vaccinated with NDV R2b mesogenic strain. The chicken recovered from the infection's and started laying eggs. However, a second outbreak occurred and the etiological agents were again confirmed by clinical signs, P.M findings, culture studies for coli septicemia and H.I studies for NDV.

Clinical studies: Clinical studies on the ailing population of the pullets and the layer status exhibited cough, mucopurulent

nasal discharge, high mortality and morbidity in both pullet and layers, and in addition low egg production in the layer stage.

Pathological examination (PM)

Post mortems were performed both in pullet and layer stages. The examinations revealed the lesions of proventricular hemorrhage (Figure 1), cecal hemorrhage (Figure 2), intestinal ulcers, necrotic duodenal loop (Figure 3), splenitis (Figure 4), mucoid trachea (Figure 5), hydro pericardium (Figure 6), friable liver & cholecystitis (Figure 7), petechial hemorrhages in the heart muscles (Figure 8), fibrin deposits in the heart (Figure 9) and in liver (Figure 10), necrotic cecal tonsil (Figure 11), congested comb and brain (Figure 12), egg yolk/salpingo-peritonitis (Figure 13), pedunculated ovarian follicles/salpingitis (Figure 14).

Bacteriological examination

Cultural isolation studies were carried out by using the homogenized *E. coli* infected tissue materials i.e. trachea, spleen and intestinal contents in MacConkey's agar media and stained with Gram's staining method. Biochemical confirmation studies carried out with catalase, triple sugar iron, Indole, oxidase and citrate tests as per the methods of Olutiola *et al.* (1991) [11].



Fig 1: Proventricular hemorrhages observed in layer chicken affected with Newcastle disease and *Escherichia coli* co-infection.



Fig 2: Cecal hemorrhages showing severe congestion and bleeding in the intestinal tract of infected birds

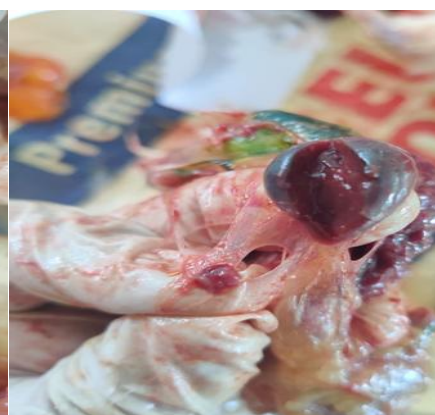


Fig 3: Necrotic duodenal loop with ulcerative lesions indicating severe enteritis associated with concurrent infection



Fig 4: Enlarged and congested spleen (splenitis) suggestive of systemic infection



Fig 5: Mucoid trachea showing excessive mucus accumulation due to respiratory involvement



Fig 6: Hydropericardium characterized by accumulation of fluid in the pericardial sac



Fig 7: Friable liver with cholecystitis indicating hepatic involvement in colisepticemia.

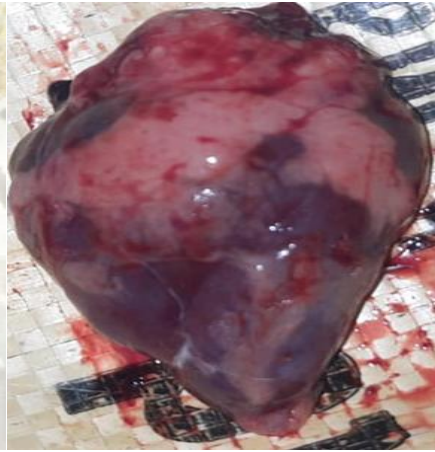


Fig 8: Petechial hemorrhages on heart muscles demonstrating vascular damage



Fig 9: Fibrinous deposits on the heart surface consistent with fibrinous pericarditis.

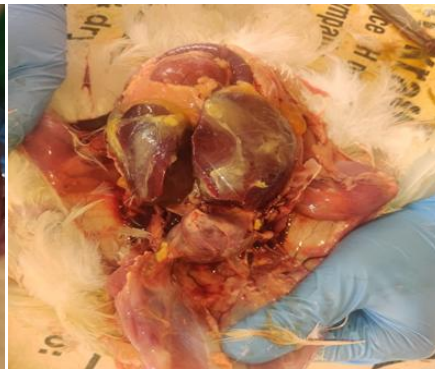


Fig 10: Fibrin deposition on the liver surface indicating severe inflammatory response.



Fig 11: Necrotic cecal tonsils showing advanced intestinal pathology



Fig 12: Congested comb and brain reflecting systemic circulatory disturbances



Fig 13: Egg yolk peritonitis (salpingo-peritonitis) observed in affected layer birds



Fig 14: Pedunculated ovarian follicles with salpingitis indicating reproductive tract involvement

Serological test: Evaluation of the NDV H.I titre values by Hemagglutination inhibition test.

The HI test was performed both in pullet and the in layer stage as per the procedures of World Organization for Animal Health (WOAH, 2021)^[15].

Treatment

Based on the clinical history, P.M findings, bacterial culture and serology, the outbreak was diagnosed as Newcastle disease virus with *Escherichia coli* as a concurrent infection in the layer poultry. The birds were treated with antibiotic as per antibiogram studies and the birds recovered from the clinical infections after a period of 4 weeks post treatment period during the first outbreak. The second outbreak which occurred in these birds at the age of 35 weeks were again

confirmed for the above infections with the clinical signs, P.M findings, bacterial culture and HI tests for NDV.

Discussion

Newcastle disease and *Escherichia coli* combined infection were reported for their challenging approach in the poultry farming practices (Abalaka *et al.* 2017, 2021)^[1] Abalaka. Badau *et al.* 2024^[4]; Abalaka *et al.* (2017)^[1] reported the failure of protection chain by various diseases like Infectious bursal disease (IBD) and Avian influenza (HPAI). Qamar-un-Nisa *et al.* (2018)^[13] reported the concurrent infection of ND virus with *Escherichia coli* infection in broilers, where the infection of *E. coli* enhanced the degree of severity of clinical signs, gross lesions and death rates i.e.25% which is in agreement with the present case studies. Bitrus *et al.* (2025)^[5]

reported the PM lesions in the outbreak of ND virus with colibacillosis in a 7 weeks old pullets which included respiratory related lesions, cloudy air sacs, splenitis, mottled liver, inflamed cecal mucosa, proventricular hemorrhages, petechial hemorrhage of the intestinal mucosa, lesions in the ovarian follicle, oviduct, peritoneum, abdominal cavities and hydro pericardial syndrome. The HI titre values at 18 weeks of age revealed 0 log 2 being below 4 log 2 titre values which is considered as the minimum protective titre values against the lethal effect of field ND virus. Bitrus *et al.* (2025) [5] suggested these values for minimum protection against field ND virus, which coincides with the finding of this study. The birds were immunized again after this outbreak with R2b mesogenic strain of NDV. However, second outbreak again occurred at the age of 35 weeks with high mortality and the egg production reached 0 level with a greater number of leathery eggs. The serological studies with H.I tests in this 35-week age group of layers indicated a minimum titre value of 9 log 2(1:512) and the maximum titre values of 12 log 2 (1:4096). These studies on the HI titre values were suggested as indicative of field infection titre values of ND virus, since these titre values were unusual in the absence of vaccination program in the laying period. These observations coincide with the findings of Bitrus *et al.* (2025) [5].

Amyleigh Watts and Paul Wigley (2024) [3] reported the P.M finding such as swollen head, air sacculitis, exudate in lungs, nephritis lesions, salpingo-peritonitis, caseous perihepatitis, spotted liver lesions, pericarditis often with fibrinous deposits in the *E. coli* infected poultry population. The above P.M findings coincides with the finding of the present studies. Oshadu *et al.* (2025) [12] reported the clinical signs of retarded growth, low egg yield, poor-quality eggs, nasal discharge, congested combs and wattles, with the PM findings of pericarditis, friable liver, misshapen and pedunculated ovarian follicles, necrotic cecal tonsils, frothy lungs, egg yolk peritonitis, petechial hemorrhages in the papillae of the proventriculus, mucoid trachea, pale spleen, empty crop, and live worms in the intestine. Further the authors reported HI titre values of the infected layer poultry of one year age with ND virus and it ranged between 1:16-1:1024, where the authors considered 1:128 as the minimum protective titre values to avoid drop in egg production against the effect of ND virus. Ganesan *et al.* (1992) [8] reported HI titre values ranged between 0-40 in layer poultry with drop in egg production due to mild form of Newcastle disease and the recovery of the layer birds for restoration of the egg production status by boosting the HI titre values by vaccinating the layer population with RDVK mesogenic vaccine.

In this case study at the age of 18 weeks, mortality started among the pullets. On an average 18-22 pullets were brought for PM studies for a period of 10 days and the scattered mortality reported for a further period of 20 days. After confirmation of the diseases for ND virus and *Escherichia coli* infections, the birds were immunized with mesogenic R2b ND virus strain followed by antibiotic treatment and calcium supplementation therapy successfully. However, a second outbreak occurred at the age of 35 weeks in this group of birds when the birds were in peak egg production. Vaccination failure occurs due to inadequate cold chain facilities and lack of monitoring of vaccine seroconversion in birds and a lack of effective implementation of farm biosecurity measures. (Edward and Ogbonna 2023) [6]. Sajo *et al.* (2023) [14] observed poor seroconversion of HI antibody titre values by performing HI test by ND virus vaccines with

live attenuated vaccines in the birds already vaccinated and reported severe form of ND in the vaccinated birds which shed the virus continuously in the droppings. The endemic status of the NDV could be a result of emergence of new NDV sub types (Alsahami *et al.* 2018) [2]. Badau *et al.* (2024) [4] suggested the lesions in the affected birds i.e. the petechial lesions in the intestine, the proventricular hemorrhages in the papillae and congested cecal mucosa could be due to the acute stage of viscerotropic velogenic virus infection.

In this case the birds were provided with bore water continuously. The bore water studies by culture examination confirmed the *E. coli* infection and it is suggested that the *E. coli* infection exacerbated the field ND virus of velogenic nature. The *E. coli* was confirmed in these episodes by lesions and culture studies and it was attributed as one of the major pathogenic factors for the development of lesions in the ovarian system, pericardium and kidneys. Even though the farm personal was advised to provide sanitized water supply after the first outbreak at the age of 18 weeks, the coli septicemia occurred which could be attributed to sanitary insufficiency by violating the biosecurity norms recommended which included delayed disposal of the droppings, improper feed storage, separation of the infected birds, good personnel hygiene records. Prevention of these diseases not achieved by the failure in comprehensive biosecurity, and regular monitoring of both ND virus and *E. coli* organisms by serology and culture studies. The repeated occurrences of both NDV and coli septicemia suggested a high virulent nature of NDV strain involvement as the area is endemic for ND virus and the disease intensity was precipitated by *E. coli* infections because of the contaminated water source.

Conclusion

The case study revealed the concurrent infection status of ND virus with *Escherichia coli* in the affected chicken in both outbreaks at the age of 18 and 35 weeks. These studies emphasize the importance of disease surveillance, monitoring, vaccine potency, vaccination protocol, biosecurity measures to prevent the outbreak of ND complicated with *E. coli* infections.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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