CONCURRENT EXPERIMENTAL INFECTION OF *E. COLI* AND NEWCASTLE DISEASE VIRUS IN TURKEYS.

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ABSTRACT

One thousand six hundred day old poultts were randomly divided into four pens of 400 poultts each. Twenty-five percent of the birds in pens 3 and 4 were inoculated orally, with *Eschericia coli* (*E. coli*) 0111ab. Ten percent of the poultts in pen 2 were inoculated, intratracheally with 10^9 EID₅₀ of Newcastle disease virus (NDV), A1.315/12 and 10 percent of the poultts in pen 3 (previously exposed to *E. coli* 0111ab) were also inoculated with NDV.

A total of 19 *E. coli* 0111ab recoveries, was made out of 200 birds that were exposed to *E. coli* in pens 3 and 4. Sampling of the liver was superior to sampling of either the caecal junction or the yolk sac in *E. coli* recoveries.

Airsacculitis was first observed in birds dying at 3 weeks of age, but the severity of leisions was maximum at 7 to 10 weeks of age. High mortality and morbidity observed in th commercial farms in epizootics of concurrent infections of *E. coli* and NDV, were not observed in this study.

Key words: — Poults, Newcastle disease, *Eschericia coli*, airsacculitis.

INTRODUCTION

In turkeys, airsacculitis has reached such proportions in many parts of the world, that it has become a major problem to poultrymen, meat inspectors and health officials. About three decades ago, groups of workers (Fahey, 1955; Biddle and Cover, 1957) incriminated *Escherichia coli* as the most significant

organism associated with airsacculitis. Gross (1958) clarified the relationship of certain viruses (infections bronchitis and Newcastle disease virus) with development of airsacculitis in birds. The most common serotypes isolated from *E. coli* out-breaks in turkeys and chickens are 01, 02, 078 and occasionally 0111ab.

E. coli has been isolated from the yolk sac of stunted turkeys (Schmidt, 1983),

and is believed to be associated with stunted birds in both turkeys and chickens. The presence of yolk sac infection causes incomplete absorption of the yolk material.

The objectives of this experiment were as follows:

- (a) To study the pathogenicity of *E. coli* strain 0111ab.
- (b) To study the pathogenicity of mild lentogenic strain of Newcastle disease virus (NDV, AI. 315#12).
- (c) To study the effect of combination of *E. coli* 0111ab and NDV, AI.315 # 12 in turkey.

MATERIALS AND METHODS

One thousand six hundred Wrolstad males, small white day-old poults were randomly distributed into 4 pens. Each pen accommodated 400 poults. The poults were fed a commercial diet, which was free of antibiotics.

E. coli Exposure

At a day of age, 25 percent of the poults (100) in pens 3 and 4 were inoculated orally with 4×10^8 cells of *E. coli* in 0.2ml of the broth suspension. All the inoculated birds were wingbanded for identification. Pen 1 poults served as non-infected controls.

Newcastle Disease Exposure

At one month of age, pre-NDV exposure, forty birds from each pen were bled to determine the status of NDV in the pens before the study commenced. After this operation, 10 percent of the birds in pens 2 and 3 (40 in each pen) were intratracheally exposed to lentogenic strain of NDV 10^9 ELD₅₀ per bird.

All the inoculated birds were wingbanded. In pens 2 and 3, the unexposed birds were maintained as contacts. Table 1 summarizes the experimental design in pens 2-4. In these pens, free intermixing of exposed and unexposed contacts were permitted, group identity being maintained by wing bands. Immediately after the NDV inoculation, the pattern of movement of personnel into and out of the pens was controlled, in an attempt to reduce the spread of the infections from one pen to another by the caretaker.

E. coli Isolation

The cloacal swabs of the experimental birds were cultured for *E. coli*. The samples were placed in 5ml of negative broth tubes and were incubated at 37 °C and streaked onto Eosinmethylene blue (EMB) plates. A presumptive diagnosis of *E. coli* infection was made, if the majority of the colonies was characteristically dark with metallic sheen on EMB agar and brick red on MacConkey agar plates. A definite diagnosis of *E. coli* was then established by transferring suspect colonies of the organism from EMB agar onto TSI, Simmon citrate and Tryptic soy agar slants. Antigenic identification of the isolates was made.

Ventilation of Experimental Building

The mean temperature of the experimental building from the 4th week of the start to the end of the experiment was 20 °C. During the first four weeks, Quartz brooder lamps were used in each pen for supplemental heat. The starting temperature around the brooder was 32.2°C to 35°C in the four pens. The measurements of ammonia concentrations in the atmosphere was obtained

Table 1.

E. COLI AND NEWCASTLE DISEASE VIRUS INOCULATIONS

Date	*Pen 1	Pen 2	Pen 3	Pen 4
Day-old	—	—	<i>E. coli</i> 25% of poults (100 poults)	<i>E. coli</i> 25% of poults (100 poults)
4 Weeks	—	NDV 10% of poults (40 poults)	NDV 10% of poults (40 poults)	

*Pen 1, Poults were non-infected controls

with a gas detector on the first day of the experiment. Subsequent readings were obtained daily throughout the duration of the study.

Blood Samples

Four millilitres of blood sample was collected by cardiac puncture or from wing vein and assayed for NDV and *Mycoplasma meleagridis* (Mm) antibodies. Different collections were made on 4th, 6th and 8th week of the experiment. For serological testing, blood samples were allowed to clot overnight and serum was removed, heat-inactivated and tested for specific NDV and Mn antibodies with the procedure of the Haemagglutination-inhibition (HI) test for NDV and serum plate for Mn.

Mortality

Complete mortality records of all the poults assigned to different pens were kept. Attempts were made throughout the experiment to determine the cause(s) of death, isolation and identification of *E. coli* organism present in the organs

cultured were made. Each dead bird was examined grossly at the time it died, with emphasis on the liver, caecal junction and yolk sac. These organs were cultured for *E. coli*.

RESULTS

Pens 3 and 4 were positive for Mn before the birds were one month old. Ten birds (2.5%) tested for Mn in pens 1 and 2 six weeks of age, the serological tests showed that Mn was present in all the birds in pens. The observation supported previous assumption that most commercial poults are infected with Mn. At eight weeks of age birds in pens 1, 2 and 3 were positive for NDV. Pen 1 which housed unexposed controls, picked up NDV (10/40N) but those in pen 4 remained negative.

Mortality

Percent mortality of birds in pens 1, 2, 3 and 4 were 11.5; 11.75, 13.25 and 13.00 respectively. The variations were not significant.

E. coli Recovery

Nineteen *E. coli* 0111ab recoveries were made from a total of 200 birds, which was exposed to *E. coli* in pens 3 and 4.

Three (1.5%) recoveries were made from the caecal junction, five (2.5%) from the yolk sac, and eleven (5.5%) from the liver. The yolk sac was observed at the end of the third week, therefore culturing of this organ was discontinued from that time. Five out of 200 (2.5%) inoculated birds yielded *E. coli* 0111ab at necropsy, while *E. coli* was isolated from 14 out of 600 (2.3%) contact-exposed birds.

Twelve cases of round heart disease were observed at necropsy. The affected hearts were enlarged 2 to 4 times normal size and the surfaces were often haemorrhagic with some of them ruptured at slightest pressure. All the affected birds were runts.

Although a total of 80 birds in pens 2 and 3 was exposed to NDV at 4 weeks of age, very few birds exhibited severe respiratory signs like gasping coughing rales and out-stretched head. Periodic coughing was observed for birds in the two pens. Airsacculitis was observed in birds that died at 3 weeks of age, but the severity of lesions were maximum at 7–10 weeks of age. At the processing plant only 2 birds (from pen 3), which had severe airsacculitis, were condemned. Infected air sacs were thickened and often had a gaseous exudate on the respiratory surface.

Ammonia Concentrations

Ammonia concentrations in pens 1–4 are shown in figure 1. The highest level of ammonia during the study (40ppm) was recorded in Pen 2 on day 50. The reading was taken three hours after the litter was raked by a technician. No sign

of discomfort that could be attributed to ammonia concentrations in the pens was observed. However, from day 55 to the end of the study, ammonia gas was perceived in all the pens.

Weight of Birds and Feed Conversion

The use of weight gain or loss was observed as an unreliable criterion of infection in the experimental groups of poult. Feed consumption data are depicted. (Table 2) along with the mean body weight. Treatment effects on two parameters were not significant in this study.

Feed conversion was defined as the kg of feed consumed per kg of live weight produced at 79 days of age. Since the analysis of variance showed that variations due to the four treatments were not significant it seems conclusive that concurrent infection of turkeys with the strains of *E. coli* and NDV did not have an appreciable effect on feed conversion.

DISCUSSION

The results presented in this experiment were comparable with inconsistencies of *E. coli* infections under field conditions. A high mortality has been frequently observed in epizootics of concurrent infection of *E. coli* and NDV in commercial farms. Efforts failed to mimic this situation under the experimental conditions of this study.

The following possibilities may account for the performance of birds in this experiment.

- (1) It is speculated that *E. coli* 0111ab and NDV AI 315#12 used in the studies were too mild to induce diseases as observed in the field.

Table 2.

**FEED CONSUMPTION BODY WEIGHT AND FEED CONVERSION
OF TURKEY IN PENS! 1-4**

	<i>Feed consumption</i>	<i>Average Weight of birds</i>	<i>No. of birds</i>	<i>Feed Conversion</i>
	(Kg)	(Kg)		
1 - Control	2917.30	3.5	354	2.35
2 - NDV exposed group	2785.00	3.5	337	2.17
3 <i>E. coli</i> + NDV exposed group	2817.70	3.5	337	2.39
4 <i>E. coli</i> exposed group	2894.40	3.6	352	2.38

(2) The common stress factors, which play important roles in the development of outbreaks of colibacillosis in commercial farms, were either absent or mild. The generally recognized stress factors include over-crowding, over-heating, chilling very high ammonia or dust concentration in the air, vaccination and social stress. For example, Anderson *et al.* (1964) reported that ammonia concentration as high as 100ppm, have been recorded in the atmospheres of some commercial turkey houses.

The incidence of round heart disease in this experiment was not associated with *E. coli* or NDV infection. The cause of the disease is not at present completely understood.

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Figure 1. Ammonia Concentrations in the atmosphere for Turkeys in Pens 1-4