# Research Note: Application of an *Escherichia coli* spray challenge model for neonatal broiler chickens

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**ABSTRACT** Avian pathogenic *Escherichia coli* (***E. coli***) is an opportunistic pathogen often introduced to neonatal chicks during the hatching process. This commensal bacterium, particularly as a pioneer colonizer of the gastrointestinal tract, can have substantial implications in the rearing of poultry because of reduced flock performance. In order to mimic the effects of the natural bacterial bloom present during the hatch, a seeder challenge model was developed to expose neonatal chicks to virulent *E. coli*. On day 20 of embryogenesis, selected early hatched chicks (n = 18/hatcher) were briefly removed and sprayed challenged with saline (vehicle) or *E. coli* at $1 \times 10^7$ colony-forming unit (**CFU**)/chick (exp 1) and $2.5 \times 10^7$ CFU/chick (exp 2). These challenged chicks were returned to the hatcher to serve as seeders to transmit the pathogen to the indirect challenged or contact chicks (n = 195/hatcher). For two 7-d experiments, the efficacy of transmission was evaluated via enteric bacterial recovery, body weight gain (**BWG**), and mortality. For exp 1 and exp 2, significantly ($P < 0.0001$) more gram-negative bacteria were recovered from the seeder and contact gastrointestinal samples than the negative control samples on day of hatch. In addition, there was a reduction ($P < 0.05$) in 7-d BWG and significantly ($P < 0.0001$) higher mortality in the contact-challenged chicks than the negative control chicks in both exp 1 and exp 2. These data suggest that this challenge model could be used to evaluate different methods of controlling the bacterial bloom that occurs in the hatching environment.

**Key words:** *Escherichia coli*, challenge, broiler, hatchery, model

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## INTRODUCTION

During the hatching process, humidity and temperature increase, yielding the ideal environment for bacterial and fungal growth. Microorganisms, both pathogenic and apathogenic, are horizontally transmitted throughout the hatching cabinet (Heyndrickx et al., 2002). Pathogens can be vertically transmitted from an infected hen at oviposition and then later transferred horizontally during hatch (Berchieri et al., 2001). Bacteria present at incubation can penetrate the eggshell (Lock et al., 1992; Berrang et al., 1999), resulting in the colonization and horizontal transfer of microorganisms. During hatch, chicks may be exposed to hours of heat stress, increasing the possibility of being colonized by a pathogen (Lara and Rostagno, 2013). While there are many microorganisms present in hatch cabinets, *Escherichia coli* (*E. coli*) is one of the most prevalent (Graham et al., 2018). *E. coli* is gram-negative and includes both commensal and pathogenic strains that serves as a pioneer colonizer of the gastrointestinal tract (**GIT**) of chicks (Lu et al., 2003). It is often observed under stress or coinfection in chickens; therefore, playing a significant role in chick quality and health (Reid et al., 1960). The infection of an avian pathogenic *E. coli* (**APEC**) can result in septicemia, omphalitis, and high mortality in commercial broiler houses (Kendler and Harry, 1967). Pathogens with tropisms for the GIT have also been found to be transmitted via the respiratory route (Kallapura et al., 2014). Owing to APEC having a tropism for both the respiratory tract and the GIT (Barnes and Gross, 1997), respiratory transmission during hatch is a concern. Moreover, APEC isolates have been described as resulting in substantial economic losses for the industry (Kabir, 2010).

Seeder challenge models have previously been used in food-producing animals to evaluate the horizontal transmission of pathogens (Lechtenberg et al., 1994; Michiels et al., 2012; Graham et al., 2019). A seeder model may be used to simulate commercial hatching conditions where the entire hatch cabinet may become contaminated by



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a very low number of initial infected eggs or chicks (Gross and Seigal, 1997). As chicks hatch, the high temperature and increased humidity serve as ideal environment conditions to promote the natural amplification of microbes, also known as the "bloom," and these pathogens horizontally spread throughout the hatching cabinet. The purpose of the presented study was to evaluate the effect of a virulent *E. coli* spray challenge seeder model on early performance parameters.

## EXPERIMENTAL DESIGN

### E. coli *Culture and Challenge*

A virulent, non–lactose-fermenting serotype O2 *E. coli*, previously associated with colisepticemia and mortality in both chickens and turkeys, was selected for these experiments (Huff et al., 2002, 2003). In these studies, 500 μL of *E. coli* was removed from a frozen aliquot and added to 50 mL of tryptic soy broth (tryptic soy broth, cat. no. 90000-378; VWR, Suwanee, GA). The culture was incubated at 37°C for 18 h. After incubation, bacterial cells were washed with sterile 0.9% saline by centrifugation at 1,800 × *g* for 15 min and resuspended in saline. The wash procedure was completed 3 times. *E. coli* colony-forming units (**CFU**) enumeration was determined by the shake plate method on MacConkey agar (MacConkey Agar, cat. no. 89429–342; VWR) to determine the estimate stock concentration and then cells were held overnight for approximately 16 h at 4°C (Sanders, 2012). The culture was then diluted to the desired CFU concentration for spray challenge (day 20 of embryogenesis, n = 18 selected from early hatched chicks at 20% pip). *E. coli* challenge dose (CFU/mL) was confirmed as described above and reported in each experiment. Each seeder chick was removed from the hatching environment, spayed on its chest and back using a calibrated hand pump sprayer to deliver approximately 0.5 mL of inoculum at each location, and then immediately returned to the hatching environment to potentially horizontally transmit the pathogen.

### *Enumeration of Bacteria*

For both experiments, whole gut samples (ventriculus to cecum) were aseptically removed and collected into sterile tissue collection bags. Samples were weighed, homogenized, and 1:4 wt/vol dilutions were made using sterile 0.9% saline. Ten-fold serial dilutions of each sample, n = 12 samples from each group, were made in sterile 96-well bacti-flat bottom plates, and the serially diluted samples were plated on culture media. Evaluation of the total number of presumptive lactic acid bacteria (**LAB**) was completed on De Man, Rogosa, and Sharpe agar (Difco Lactobacilli MRS Agar, cat. no. 90004–084; VWR), as well as enumeration of presumptive gram-negative bacteria, specifically with colonies with lactose-negative morphology (Challenge strain is non-lactose fermenting.), on MacConkey agar (MacConkey Agar, cat. no. 89429–342; VWR). All plates were incubated at 37°C for 18 h, and bacterial counts were expressed as $Log_{10}$ CFU/g of sample.

### *Development of an* E. coli *Spray Challenge Model for Neonatal Broiler Chickens*

The objective of both experiments 1 and 2 was to evaluate the horizontal transmission of the pathogen by measuring the bacterial colonization in the GIT at day-of-hatch (**DOH**) in both seeder and contact chicks of both treatments by measuring bacterial colonization at day 7 and evaluating the challenge's impact on performance. Mortality was recorded throughout the 7-d trial period in each experiment. In each trial, embryonated Ross 308 broiler hatching eggs were candled at d 18 of incubation and placed into separate hatchers (G.Q.F. Manufacturing 1602 N Hova-Bator Incubator with a circulating air fan kit) at random. Hatcher units were housed in separate facilities to prevent possible contamination between treatments during the hatch. On day 20 of embryogenesis, at 20% pip, seeder chicks (n = 18 seeders/hatcher or 8.45%) were inoculated with 1 mL of *E. coli* or 1 mL of 0.9% sterile saline (vehicle) per chick via spray. On day 21, dry chicks were removed from the hatchers, hatchability was recorded, and select chicks (n = 12 per group) were euthanized to evaluate presumptive LAB and gram-negative bacteria as previously described. The confirmed seeder challenge dose was 1 × 107 CFU/mL/chick for exp 1 and 2.5 × 107 CFU/mL/chick for exp 2. In both experiments, negative control chicks were weighed and allocated into 8 pens (n = 20/pen), and the contact-challenged chicks were weighed and allocated into 16 pens (n = 20/pen). Weight allocation on DOH was performed to normalize

**Table 1.** Presumptive non–lactose-fermenting gram-negative and lactic acid bacteria (LAB) recovered from gastrointestinal tract at day-of-hatch (exp 1 & exp 2).

| | Gram-negative bacteria[1] ($Log_{10}$) | | LAB ($Log_{10}$) | |
|---|---|---|---|---|
| Treatment | Exp 1 | Exp 2 | Exp 1 | Exp 2 |
| Negative control | $0.00 \pm 0.00^{c}$ | $0.00 \pm 0.00^{c}$ | $0.00 \pm 0.00^{b}$ | $0.25 \pm 0.25^{b}$ |
| Spray challenged seeder chicks | $8.30 \pm 0.11^{a}$ | $7.78 \pm 0.45^{a}$ | $6.53 \pm 0.92^{a}$ | $4.74 \pm 0.82^{a}$ |
| Spray challenged contact chicks | $4.09 \pm 0.71^{b}$ | $5.19 \pm 0.79^{b}$ | $4.17 \pm 0.85^{a}$ | $3.87 \pm 0.95^{a}$ |

The lowercase superscript letters indicate significant differences between treatments per column ($P < 0.05$).

[1]Data are expressed as mean $log_{10}$ CFU/g ± SE.

**Table 2.** Effect of virulent *E. coli* horizontal transmission on average BWG and 7-d mortality in neonatal broiler chickens (exp 1 & exp 2).

| | 7-d BWG (g)[1] | | Mortality (%)[2] | |
|---|---|---|---|---|
| Treatment | Exp 1 | Exp 2 | Exp 1 | Exp 2 |
| Negative control | 129.52 ± 3.55[a] | 133.02 ± 3.43[a] | 0[y] | 0[y] |
| Spray challenge contact | 115.89 ± 3.07[b] | 119.05 ± 3.06[b] | 10.31[z] | 15.62[z] |

The superscript letters "[y,z]" indicate significant differences between treatments at $P < 0.001$. The superscript letters "[a,b]" indicate significant differences between treatments at $P < 0.05$.

[1]Data are expressed as mean ± SE.

[2]Data are expressed as number of deaths/total (%).

BW and prevent initial treatment effects on BW. Pen BW was determined at placement and on day 7 to determine BWG. Mortality was recorded for the duration of each 7-d trial period. Chicks were provided *ad libitum* access to water and a balanced, unmedicated corn and soybean meal diet meeting the nutritional requirements for broilers recommended by Aviagen (Aviagen, 2019). All experiments and animal handling procedures complied with the University of Arkansas Institutional Animal Care and Use Committee guidelines under permit #18079.

### Statistical Analysis

All data were subjected to one-way analysis of variance at a completely randomized design using the GLM procedure of SAS (SAS Institute, 2002). Data are expressed as mean ± standard error. Significant differences ($P < 0.05$) among means were further separated using Tukey's multiple range test for presumptive LAB and gram-negative bacterial recovery. The pen was the experimental unit for the BW data, and means were separated using Student's *t* test on DOH and day 7. Mortality was compared using the chi-square test of independence to determine the significance threshold ($P < 0.001$) for these studies (Zar, 1984).

## RESULTS AND DISCUSSION

Under commercial conditions, chicks may be exposed to APEC isolates in the hatching cabinet which cause colisepticemia, airsacculitis, and increased early chick mortality resulting in significant economic losses for the poultry industry (Kendler and Harry, 1967). Thus, a laboratory model could be used to evaluate the effects of exposure to APEC isolates during the hatching phase. At day 20 of embryogenesis, seeder chicks were inoculated via a spray with a virulent *E. coli* or saline vehicle and then placed back into the hatching cabinet to horizontally transmit the pathogen.

On DOH, there was a significant increase ($P < 0.0001$) in presumptive gram-negative recovery from GIT samples from the seeders and contact chicks. Results were consistent in both exp 1 and 2, indicating that spraying the inoculum on seeder chicks horizontally transmitted the pathogen during hatch (Table 1). Significant differences ($P < 0.0001$) in LAB were also observed between the challenged groups and negative control (Table 1), indicating that gram-negative bacteria may contribute to colonization by LAB (Wilson et al., 2020). LAB are naturally found in the GIT of animals (Reuben et al., 2019). Some researchers believe LAB plays a role in restoring the natural microflora after an infection (Higgins et al., 2010). This could potentially contribute to the amplification of LAB after challenge as reported in the present experiments. No significant differences were observed between the challenge and negative control groups on day 7 for presumptive gram-negative recovery (data not shown).

The *E. coli* isolate used in these experiments was chosen based on negative impacts on both performance and mortality in previous experiments (Huff et al., 2002, 2003). In exp 1 and 2, differences ($P < 0.02$) in 7-d BWG were observed, indicating that the challenge had a negative impact on performance (Table 2). The 7-d mortality was significantly ($P < 0.001$) higher in the challenge group than that in the negative control group (Table 2). All mortalities were necropsied, and the cause of death was determined to be related to *E. coli* infection.

Spraying select early hatching chicks, also known as seeder chicks, at day 20 of embryogenesis, effectively transmitted the pathogen throughout the hatching cabinet resulting in an increase in gram-negative recovery at DOH, presumptive LAB at DOH, 7-d mortality, and a negative impact on 7-d BWG. In a commercial hatchery, chicks are exposed to gram-negative bacteria that serve as pioneer colonizers of the GIT (Graham et al., 2018). Further research is being conducted to evaluate potential alternatives of pathogen control within the hatch cabinets using seeder challenge models to mimic commercial conditions.

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## DISCLOSURES

The authors declare no conflicts of interest.

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