# Relationship between phylogenetic groups of *Escherichia coli* and Pathogenicity among Isolates from chickens with Colibacillosis and healthy chickens

Toshiyuki Murase ,[*,†,1] and Hiroichi Ozaki[*,†]

[*]*Laboratory of Veterinary Microbiology, Faculty of Agriculture, Tottori University, 4-101 Koyama, Tottori 680-8553, Japan; and* [†]*The Avian Zoonosis Research Center, Faculty of Agriculture, Tottori University, 4-101 Koyama, Tottori 680-8553, Japan*

**ABSTRACT** Avian pathogenic *Escherichia coli* (**APEC**) is closely related to extraintestinal pathogenic *E. coli*, which are frequently assigned to specific phylogenetic groups (phylogroups). Therefore, we investigated the association between phylogroups of *E. coli* isolates and those recovered from commercial broiler and layer chickens with colibacillosis. We used 104 *E. coli* isolates from chickens with colibacillosis (hereafter referred to as "colibacillosis-related isolates"), 56 *E. coli* isolates obtained from fecal samples of clinically healthy broiler chickens, and 58 isolates obtained from environmental samples of layer chicken housing facilities where clinically healthy layer chickens were reared (hereafter referred to as "healthy chicken-related isolates"). The prevalence of phylogroup F among colibacillosis-related isolates was significantly ($P < 0.05$) higher than that among healthy chicken-related isolates, while phylogroups A and B1 were more frequently distributed in healthy chicken-related isolates. Fifty-seven (87%) of 65 colibacillosis-related isolates belonging to phylogroup F were defined as APEC based on the presence of virulence-associated genes according to a previously established criterion. In contrast, none of the healthy chicken-related isolates were defined as APEC. As evidenced by the chicken embryo lethality assay, 87 of the 92 healthy chicken-related isolates tested had embryo lethality rates of <30% and were considered avirulent, whereas 59 of the 104 colibacillosis-related isolates were considered virulent. Nonetheless, among isolates exhibiting embryo lethality rates of <30%, the mean lethality rate of embryos inoculated with colibacillosis-related isolates was significantly higher than that of embryos inoculated with healthy chicken-related isolates. These observations suggest that phylogroup F predicts colibacillosis among *E. coli* strains with virulence-associated genes.

**Key words:** chicken, colibacillosis, *Escherichia coli*, phylogroup, virulence

2022 Poultry Science 101:102007
https://doi.org/10.1016/j.psj.2022.102007

## INTRODUCTION

Colibacillosis in chickens is caused by avian pathogenic *Escherichia coli* (APEC), which leads to extraintestinal infections, including localized and systemic diseases responsible for significant economic losses to the poultry industry (Nolan et al., 2013). Poor environmental conditions in housing facilities, including high dust levels or low temperature, infectious bronchitis virus, and previous mycoplasma infections, predispose birds to *E. coli* infections (Guabiraba and Schouler, 2015). In addition, *E. coli* can play a role as a primary pathogen. Isolates from cases of pericarditis and perihepatitis in laying hens originated from an outbreak strain (Someya et al., 2007) based on pulsed-field gel electrophoresis (**PFGE**) analysis (Tenover et al., 1995). The strain was virulent in a chicken embryo lethality assay and caused mortality in experimentally infected 11-day-old chickens (Ozaki et al., 2018). Additionally, *E. coli* isolates obtained from colibacillosis cases in broiler chickens in 4 farms belonging to one poultry integration company had identical PFGE patterns (Ozaki et al., 2017). However, in this study (Ozaki et al., 2017), different combinations of virulence-associated genes were identified among isolates. Other previous studies also demonstrated the diversity in virulence-associated genes harbored by APEC isolates (Guabiraba and Schouler, 2015), although detecting certain combinations of genes can



Received April 21, 2022.
Accepted June 7, 2022.

[1]Corresponding author: murase@tottori-u.ac.jp

predict APEC strains (Johnson et al., 2008; Bonnet et al., 2009; Schouler et al., 2012; Mitchell et al., 2015; Stromberg et al., 2017).

APEC is closely related to extraintestinal pathogenic *E. coli* (**ExPEC**), including strains obtained from urinary tract infections, neonatal meningitis, and sepsis in humans, sharing virulence-associated and antimicrobial resistance genes found in the ExPEC isolates. Moreover, the phylogenetic analysis based on the original (Clermont et al., 2000) and revised (Clermont et al., 2013) Clermont schemes demonstrated that ExPEC from human cases were frequently assigned to specific phylogenetic groups (hereafter referred to as "phylogroups"), including B2 and D (Picard et al., 1999; Johnson and Stell, 2000; Logue et al., 2017). APEC isolates obtained from diseased poultry occasionally belong to the phylogenetic group D in Thailand (Thomrongsuwannakij et al., 2022) and groups A and D in Japan (Asai et al., 2011; Ozaki et al., 2017), based on the original Clermont scheme. Recently, several studies focused on avian colibacillosis strains belonging to phylogroups B2 and F based on the revised Clermont scheme due to their zoonotic potential as an etiologic agent of extraintestinal infections in humans (Jeong et al., 2021; Wang et al., 2021; Zhuge et al., 2021).

Control of avian colibacillosis and preventive measures can be achieved with early detection of APEC isolates by monitoring farms using phylogenetic analyses. In the present study, the virulence of *E. coli* isolates was assessed by detecting virulence-associated genes (Bonnet et al., 2009; Mitchell et al., 2015; Stromberg et al., 2017) and performing an embryo lethality assay (Wooley et al., 2000). Strains were isolated from chickens with colibacillosis, fecal samples of healthy chickens, and environmental samples from multiple farms. All isolates were classified into phylogroups (Clermont et al., 2013) to elucidate the association between colibacillosis isolates and each phylogroup.

## MATERIALS AND METHODS

### Bacterial Isolates

A total of 104 *E. coli* isolates obtained from laying chickens and broiler chickens with colibacillosis were used. Isolates from laying chickens were obtained from a farm (farm 1) between December 2005 and March 2006, and in February 2009. The isolates from broiler chickens were obtained from 6 farms (farms 2−7) between October 2009 and January 2011. Emaciated or unhealthy birds were diagnosed according to the following criteria: gross lesions typical of colibacillosis, including pericarditis, perihepatitis, and salpingitis postmortem; isolation of *E. coli* in pure culture from lesion samples. Some of the above isolates were also used in our previous studies (Someya et al., 2007; Ozaki and Murase, 2009; Ozaki et al., 2017). Fifty-six *E. coli* isolates from healthy birds were obtained from fecal samples of broiler chickens collected between October 2007 and January 2008 from four farms (farms 8−11) (Ozaki et al., 2011). Fifty-eight isolates were obtained from environmental samples from farm 1 between July 2012 and June 2017, when laying hens were clinically healthy (Koyama et al., 2020).

### Pulsed-Field Gel Electrophoresis

*Xba*I-digested PFGE patterns of all isolates were analyzed as previously described (Ozaki et al., 2011). Banding pattern analysis was performed with GelComparII version 6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium). Cluster analysis of the fingerprints obtained was conducted through a similarity matrix calculation using the Dice coefficient, followed by dendrogram construction using the unweighted pair group method with arithmetic mean (**UPGMA**) as the algorithm with optimization and tolerance set at 1%. Isolates were assigned to genetically related clusters using the 90% strain similarity threshold and distinguished numerically.

### Phylogenetic Analysis

Phylogroups of *E. coli* isolates were determined using multiplex PCR targeting genes *arpA, chuA, yjaA*, and *trpA*, and DNA fragment TspE4.C2, as previously described (Clermont et al., 2013). PCR profiles obtained in the present study and phylogroup assignment are shown in Supplementary Table 1.

### Detection of Virulence-Associated Genes

The presence of 10 genes (*kpsMT* II, *iss, tsh, sfa, foc, papA, papC, papEF, iutA*, and *fyuA*) in *E. coli* isolates was determined by PCR (Johnson and Stell, 2000; Ewers et al., 2004). The previously published criterion for APEC (Bonnet et al., 2009; Mitchell et al., 2015; Stromberg et al., 2017) was the detection of 4 or more of the following 5 genes/groups: (1) *kpsMT* II (conferring group II capsule synthesis); (2) *iss* (complement resistance); (3) *tsh* (hemagglutinin); (4) one of the 5 genes *sfa, foc, papA, papC*, and *papEF* (adhesins); (5) one of the 2 genes *iutA* and *fyuA* (siderophores).

### Embryo Lethality Assay

The embryo lethality assay for all 104 isolates from chickens with colibacillosis (hereafter referred to as "colibacillosis-related isolates") was performed as previously described (Nolan et al., 1992), with slight modifications (Ozaki et al., 2018). Briefly, 100 to 300 colony-forming units (**CFU**) in 0.1 mL of phosphate-buffered saline (**PBS**) were inoculated into the allantoic cavity of twelve 12-day-old embryonated eggs. The eggs were candled daily, and deaths were recorded for 2 d postinoculation. A total of 114 isolates were obtained from healthy chickens and environmental samples (hereafter referred to as "healthy chicken-related isolates"). PFGE analysis revealed 98 pulsotypes among the isolates (see Results). Six isolates from environmental samples from layer

housing facilities with different pulsotypes were unfortunately lost during maintenance; thus, 92 pulsotypes remained available for the embryo lethality assay. One isolate was randomly selected from those isolates with each of the 92 pulsotypes obtained, consisting of 46 isolates from healthy broilers and 46 isolates from environmental samples from layer housing facilities; these selected isolates were used for the embryo lethality assay. The pathogenicity was determined based on mortality rates 2 d postinoculation using the criteria previously established by Wooley et al. (2000), wherein an isolate causing a mortality rate of >30% is considered virulent.

### Statistical Analysis

Fisher's exact test was used to compare the prevalence of phylogenetic groups of *E. coli* isolates related to colibacillosis versus healthy chickens and compare that in APEC versus non-APEC isolates. Fisher's exact test was also used to compare the prevalence of virulence-associated genes in virulent versus avirulent *E. coli* isolates determined by the embryo lethality assay. Welch's *t*-test was used to compare the mean mortality rates associated with colibacillosis-related versus healthy chicken-related isolates with low lethality (mortality rates of <30%). Differences were considered statistically significant at $P < 0.05$.

## RESULTS

### PFGE and Phylogenetic Analysis

A total of 104 *E. coli* isolates obtained from chickens with colibacillosis were classified into 37 pulsotypes based on PFGE analysis of *Xba*I-digested chromosomal DNA (Table 1 and Supplementary Figure 1). Several isolates from diseased birds in the same housing facilities had indistinguishable PFGE patterns and were assigned to the same pulsotypes. Therefore, 48 and 56 isolates from layer chickens and broiler chickens with colibacillosis were respectively classified into 11 and 26 pulsotypes. In contrast, most isolates from healthy chickens and environmental samples had different PFGE patterns, and a total of 114 healthy chicken-related isolates were classified into 98 pulsotypes.

The distribution of phylogroups among the *E. coli* isolates tested is shown in Table 1. As multiple isolates were classified into identical pulsotypes, especially for colibacillosis-related isolates, and isolates with an identical pulsotype were assigned to a single phylogroup, the prevalence of *E. coli* phylogroups was compared based on the number of pulsotypes. The prevalence of phylogroup F among colibacillosis-related isolates was significantly higher than that among healthy chicken-related isolates ($P < 0.05$). In contrast, phylogroups A and B1 were more frequently distributed in healthy chicken-related isolates than in colibacillosis-related isolates ($P < 0.05$). Twelve isolates with colibacillosis were assigned to phylogroup B2, although these isolates were classified into only 2 pulsotypes.

### Detection of Virulence-Associated Genes

Forty-two isolates positive for the *iss* gene accounted for 88% of the 48 *E. coli* isolates from laying chickens with colibacillosis, followed by *kpsMT* II (37, 77%), *iutA* (27, 56%), *tsh* (24, 50%), *papC* (27, 56%), and *papEF* (17, 35%) (Supplementary Table 2). The *iutA* gene was the most common virulence-associated gene present in all 56 isolates from broiler chickens with colibacillosis (100%), followed by *iss* (55, 98%), *tsh* (39, 70%), *papC* (35, 63%), and *papEF* (30, 54%). In contrast, the *kpsMT* II gene was only detected in 5 isolates (8.9%) among those from broiler chickens with colibacillosis. In 48 *E. coli* isolates from environmental samples of chicken housing facilities where healthy laying hens were reared, the prevalence rates of the virulence-associated gene tested were less than 9%, with those of *iutA* and *iss* genes being 8.6% and 6.8%, respectively. In 56 isolates from fecal samples of healthy broiler chickens, the *iss* gene was detected in 16 isolates (29%), followed by *papEF* (8, 14%), *papC* (7, 13%), and *iutA* (7, 13%).

Twenty-five of the 48 isolates from laying chickens with colibacillosis and 36 of the 56 isolates from broiler chickens with colibacillosis were defined as APEC

**Table 1.** Number of colibacillosis- and healthy chicken-related *E. coli* isolates and pulsotypes belonging to each phylogroup.

| | Colibacillosis-related *E. coli* | | | | Healthy chicken-related *E. coli* | | | |
|---|---|---|---|---|---|---|---|---|
| Property | Layer | | Broiler | | Layer | | Broiler | |
| Isolation date | Dec 2005 to Mar 2006, and Feb 2009 | | Oct 2009 to Jan 2011 | | Jul 2012 to Jun 2017 | | Oct 2007 to Jan 2008 | |
| Origin | Lesions | | Lesions | | Chicken houses[1] | | Feces | |
| Phylogroup | | | | | | | | |
| A[a] | 0 | (0)[2] | 3 | (3) | 13 | (13) | 27 | (23) |
| B1[a] | 2 | (2) | 10 | (5) | 34 | (31) | 21 | (17) |
| B2 | 12 | (2) | 0 | (0) | 1 | (1) | 0 | (0) |
| C | 0 | (0) | 1 | (1) | 6 | (4) | 0 | (0) |
| D | 1 | (1) | 4 | (3) | 4 | (3) | 1 | (1) |
| E | 5 | (1) | 1 | (1) | 0 | (0) | 3 | (2) |
| F[a] | 28 | (5) | 37 | (13) | 0 | (0) | 4 | (3) |
| Total | 48 | (11) | 56 | (26) | 58 | (52) | 56 | (46) |

[a]Prevalence of pulsotypes significantly ($P < 0.05$) different between colibacillosis- and healthy chicken-related isolates.
[1]*E. coli* isolates were obtained from environmental samples from chicken housing facilities, while the chickens were clinically healthy.
[2]The number of pulsotypes is indicated in parentheses.

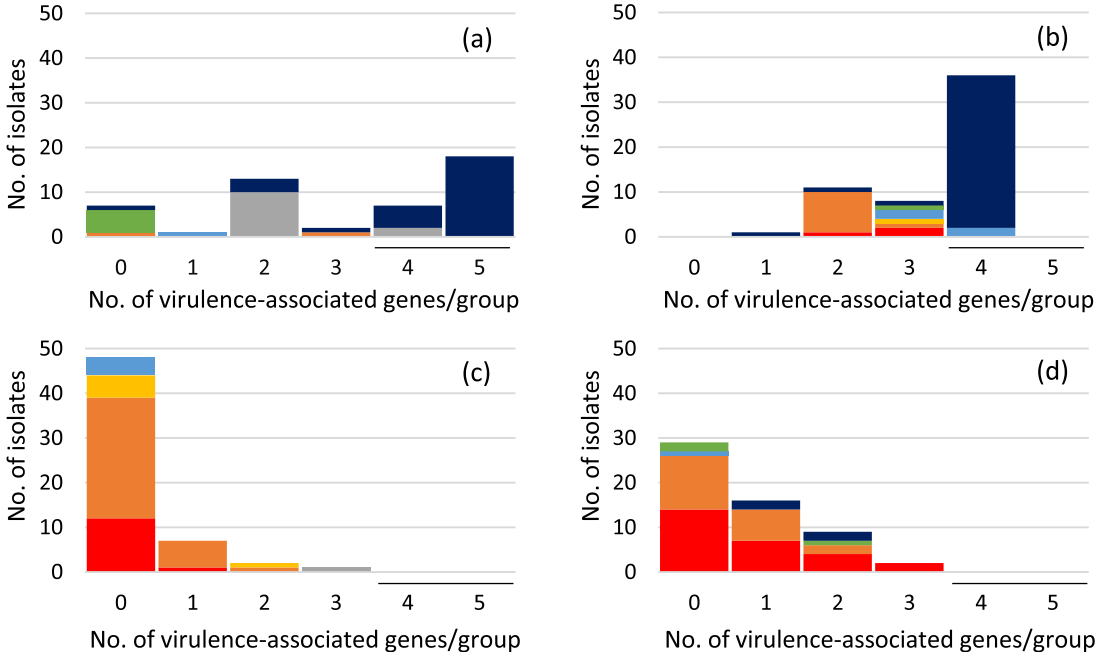


**Figure 1.** Prevalence of virulence-associated genes among *E. coli* isolates from layer chickens with colibacillosis (a), broiler chickens with colibacillosis (b), environmental samples of layer housing facilities obtained while the layer chickens were clinically healthy (c), and healthy broiler chickens (d). Numbers on the x-axis represent those detected among the following 5 genes/groups: (1) *kpsMT* II; (2) *iss*; (3) *tsh*; (4) one of the 5 genes *sfa, foc, papA, papC*, and *papEF*; and (5) one of the 2 genes *iutA* and *fyuA*. Isolates defined as avian pathogenic *Escherichia coli* (see Materials and Methods) are indicated by horizontal lines. Phylogroup: A, red; B1, orange; B2, gray; C, yellow; D, cyan; E, green; F, dark blue.

according to the previously described criterion (Bonnet et al., 2009; Mitchell et al., 2015; Stromberg et al., 2017), based on the presence of virulence-associated genes (Figure 1 and Supplementary Table 2). Fifty-seven (87%) of 65 colibacillosis-related isolates belonging to phylogroup F were defined as APEC. In contrast, none of the healthy chicken-related isolates were defined as APEC because all the isolates had 3 or fewer virulence-associated genes.

## *Embryo Lethality Assay Results*

The embryo lethality assay revealed that the *E. coli* isolates tested caused mortality rates of 0% to 92% for embryonated eggs 2 d postinoculation. Thirty-three of the 48 *E. coli* isolates from layer chickens with colibacillosis caused a mortality rate above 30% and were considered virulent according to the criteria described by Wooley et al. (2000) (Figure 2A). Twenty-three of the 33 virulent isolates belonged to phylogroup F, while none of the virulent isolates belonged to phylogroups A and B1. Of 56 isolates from broiler chickens with colibacillosis, 26 were virulent, including 18 isolates from phylogroup F (Figure 2B). Forty-five of the 46 isolates from environmental samples of housing facilities where healthy laying hens were reared were not considered virulent (Figure 2C). The remaining isolate was virulent and belonged to phylogroup B2. Forty-two of the 46 isolates from healthy broiler chickens were not virulent based on the embryo lethality assay (Figure 2D). Three of the remaining four virulent isolates belonged to phylogroup A, while the other belonged to phylogroup F.

Isolates exhibiting low lethality (mortality rate of <30%) from layer chickens and broiler chickens with colibacillosis had mortality rates of 16.7 ± 10.0 (%) and 15.8 ± 8.6, respectively. These rates were significantly higher than those of embryos inoculated with isolates from environmental samples (3.7 ± 6.1) or healthy broiler fecal samples (5.4 ± 8.6). In total, 63% of the healthy chicken-related isolates did not cause mortality, while 85% of the colibacillosis-related isolates caused embryo lethality.

## *Relationship Between the Presence of Virulence-Associated Genes and the Embryo Lethality Assay Results*

Eighty-seven (95%) of the 92 non-APEC *E. coli* isolates from environmental samples and healthy chickens were avirulent according to the embryo lethality assay (Table 2). Among 43 total non-APEC isolates from cases with colibacillosis, only 26 isolates (60%) were avirulent. Twenty-three of 25 APEC isolates from layer chickens with colibacillosis were virulent according to the embryo lethality assay. In contrast, approximately half the APEC isolates from broiler chickens with colibacillosis were virulent. The same phylogroups were found in both virulent and avirulent isolates. The prevalence of virulence-associated genes between virulent and avirulent isolates was identical (Supplementary Table 3).

## DISCUSSION

The distribution of phylogroups significantly differed between colibacillosis-related and healthy chicken-

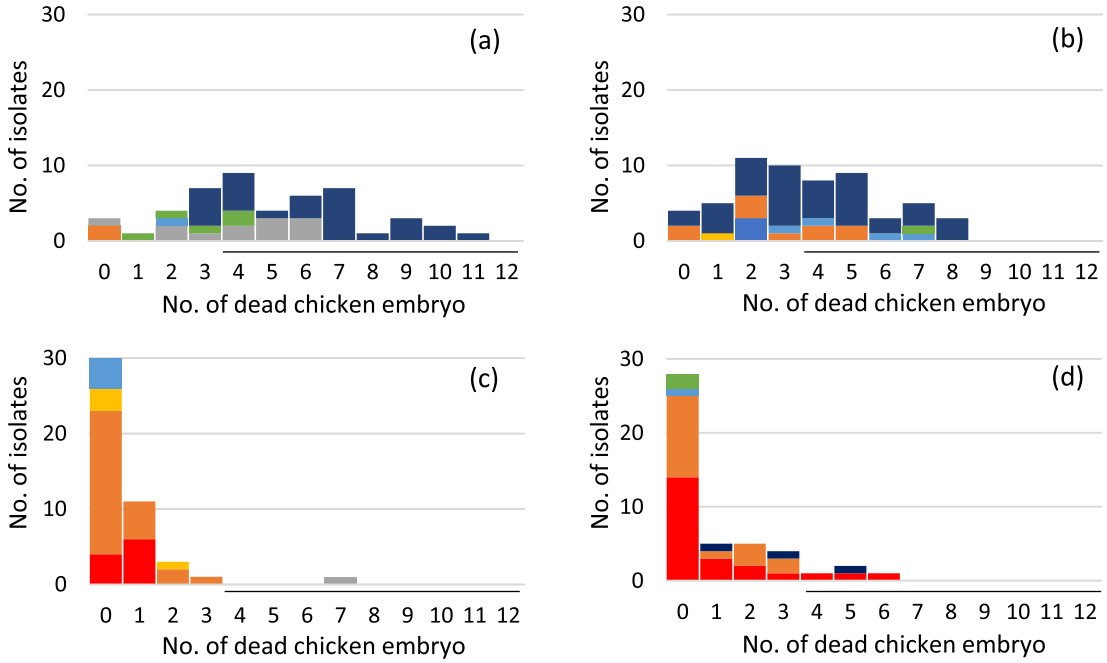


**Figure 2.** Embryo lethality assay inoculated with *E. coli* isolates from layer chickens with colibacillosis (a), broiler chickens with colibacillosis (b), environmental samples of layer housing facilities obtained while the layer chickens were clinically healthy (c), and healthy broiler chickens (d). The number of dead chicken embryos 2 d postinoculation is indicated on the x-axis. Isolates considered virulent (see Materials and Methods) are indicated by horizontal lines. Phylogroup: A, red; B1, orange; B2, gray; C, yellow; D, cyan; E, green; F, dark blue.

related isolates. Additionally, the presence of virulence-associated genes correlated with the phylogroups in the present study. The prevalence rate of phylogroup F in colibacillosis-related *E. coli* isolates was significantly higher than that in healthy chicken-related isolates. Furthermore, 87% of colibacillosis-related isolates within phylogroup F were defined as APEC according to the presence of virulence-associated genes. These observations indicate that isolates within phylogroup F are likely to be highly virulent to chickens and may be the primary causative agents of colibacillosis. Moreover, it is likely that isolates with a single pulsotype caused multiple infections as only 37 pulsotypes were observed for 104 *E. coli* isolates from chickens with colibacillosis. In contrast, most of the healthy chicken-related isolates were found to be distinct from each other using PFGE analysis. Additionally, all healthy chicken-related isolates were defined as non-APEC, indicating that these isolates originated from fecal commensal *E. coli*. Moreover, phylogroups A and B1 were significantly associated with healthy chicken-related isolates. Among 452 isolates of *E. coli* recovered from the lesions of poultry with colibacillosis in the United States, phylogroup C was the most common (125 isolates), followed by F (87), B1 (84), B2 (69), and A (46). Nevertheless, these isolates originated from chickens as well as other birds, including geese and ducks, suffering from perihepatitis and other colibacillosis syndromes (Logue et al, 2017). Phylogroups A and B1 have been identified as sister groups (Lecointre et al., 1998). Logue et al. (2017) suggested that *E. coli* isolates assigned to phylogroups A and B1 from the feces of apparently healthy poultry and from colibacillosis lesions have a low pathogenic potential based on virulence genotyping.

All healthy chicken-related isolates were defined as non-APEC based on the presence of virulence-associated genes and the previously published criterion (Bonnet et al., 2009; Mitchell et al., 2015; Stromberg et al., 2017), highlighting the validity of the criterion. However, 43 of the 104 colibacillosis-related isolates were also defined as non-APEC. One possible reason is that infections with these *E. coli* isolates were facilitated by damage to the respiratory mucosa due to previous viral or mycoplasma infections and poor hygienic conditions

**Table 2.** Relation between the presence of virulence-associated genes and the embryo lethality assay results of *E. coli* isolates tested.

| | Colibacillosis-related *E. coli* | | Healthy chicken-related *E. coli* | |
|---|---|---|---|---|
| Category[1] | Layer | Broiler | Layer | Broiler |
| APEC | | | | |
| Virulent | 23 (B2, 2; F, 21)[2] | 19 (D, 1; F, 18) | 0 | 0 |
| Avirulent | 2 (F, 2) | 17 (D, 1; F, 16) | 0 | 0 |
| Non-APEC | | | | |
| Virulent | 10 (B2, 6; E, 2; F, 2) | 7 (B1, 4; D, 2; E, 1) | 1 (B2, 1) | 4 (A, 3; F, 1) |
| Avirulent | 13 (B1, 2; B2, 4; D, 1; E, 3; F, 3) | 13 (A, 3; B1, 6; C, 1; F, 3) | 45 (A, 10; B1, 27; C, 3; D, 4) | 42 (A, 20; B1, 17; D, 1; E, 2; F, 2) |

[1]APEC and non-APEC are determined based on the presence of virulence-associated genes. Virulent and avirulent represent the embryo lethality assay results.

[2]The number of isolates with each of the phylogroups is indicated in parentheses.

of the housing facilities (Guabiraba and Schouler, 2015). Another reason may be the presence of unknown colibacillosis virulence traits in *E. coli* strains. Among the virulence-associated genes tested the *kpsMT* II gene conferring group II capsule synthesis had a high prevalence rate (77%) in isolates from layer chickens with colibacillosis despite the 8.9% rate in isolates from broiler chickens with colibacillosis. In previous studies, the prevalence rate of the *kpsMT* II gene among APEC isolates varied from 23% to 86% (Johnson et al., 2008; Bonnet et al., 2009; Mitchell et al., 2015). Johnson et al. (2008) also demonstrated significant differences in distribution among APEC isolates of the 3 pathotypes. Further investigation into the pathological aspects of the APEC isolates obtained in the present study is warranted to clarify the role of this gene.

According to the embryo lethality assay, most healthy chicken-related isolates (non-APEC) were deemed avirulent. However, correlations between the presence of virulence-associated genes and the embryo lethality assay results were not definitive among colibacillosis-related isolates in the present study. Fujimoto et al. (2021) reported that several *E. coli* isolates from broilers with suspected colibacillosis were considered avirulent based on the embryo lethality assay. Our previous study (Ozaki et al., 2018) demonstrated that an avirulent (mortality rate of 17%) *E. coli* isolate from a layer chicken with colibacillosis caused airsacculitis and pericarditis in experimentally-inoculated chickens. When embryo lethality assay results in the present study were compared among isolates exhibiting low lethality (mortality rate of <30%), colibacillosis-related isolates had higher mortality rates than healthy chicken-related isolates. Chicken embryos were likely affected, to some extent, by colibacillosis-related isolates even though the isolates were considered avirulent.

In summary, phylogroup F and virulence-associated genes were predominant in colibacillosis-related *E. coli* isolates, while most healthy chicken-associated isolates with a low prevalence of virulence-associated genes were assigned to phylogroups A and B1. In the chicken embryo lethality assay, the lethality of chicken embryos seemingly correlated with the origin of the isolates; however, we cannot rule out the possibility that a limited number of farms and the collection time span of different samples in the present study affected the distribution of the phylogroups among *E. coli* isolates. Nevertheless, it is noteworthy that phylogroup F was predominant in both layer and broiler chickens with colibacillosis. Detection of phylogroup F may help predict highly virulent APEC. The zoonotic potential of phylogroup F *E. coli* in the present study may be clarified through further research on multilocus-sequence typing (Zhuge et al., 2021) and antimicrobial resistance (Wang et al., 2021) of the isolates.

## ACKNOWLEDGMENTS

The authors thank Akane Kawahara, Faculty of Agriculture, Tottori University, Tottori, Japan, for technical assistance and invaluable help with data management. This work was supported by JSPS KAKENHI Grant Number 20K06432. We would like to thank Editage (www.editage.com) for English language editing.

## DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102007.

## REFERENCES

Asai, T., K. Masani, C. Sato, M. Hiki, M. Usui, K. Baba, M. Ozawa, K. Harada, H. Aoki, and T. Sawada. 2011. Phylogenetic groups and cephalosporin resistance genes of *Escherichia coli* from diseased food-producing animals in Japan. Acta Vet. Scand. 53:52.

Bonnet, C., F. Diarrassouba, R. Brousseau, L. Masson, E. Topp, and M. S. Diarra. 2009. Pathotype and antibiotic resistance gene distributions of *Escherichia coli* isolates from broiler chickens raised on antimicrobial-supplemented diets. Appl. Environ. Microbiol. 75:6955–6962.

Clermont, O., S. Bonacorsi, and E. Bingen. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. 66:4555–4558.

Clermont, O., J. K. Christenson, E. Denamur, and D. M. Gordon. 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo- groups. Environ. Microbiol. Rep. 5:58–65.

Ewers, C., T. Janssen, S. Kiessling, H. C. Philipp, and L. H. Wieler. 2004. Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. Vet. Microbiol. 104:91–101.

Fujimoto, Y., H. Inoue, T. Kanda, M. Ijiri, and R. Uemura. 2021. Virulence-associated gene profiles of *Escherichia coli* isolated from chickens with colibacillosis in Japan and their correlation with pathogenicity in chicken embryos. Avian Dis. 65:401–405.

Guabiraba, R., and C. Schouler. 2015. Avian colibacillosis: still many black holes. FEMS Microbiol. Lett. 362:fnv118.

Jeong, J., J.-Y. Lee, M.-S. Kang, H.-J. Lee, S.-I. Kang, O.-M. Lee, Y.-K. Kwon, and J.-H. Kim. 2021. Comparative characteristics and zoonotic potential of avian pathogenic *Escherichia coli* (APEC) isolates from chicken and duck in South Korea. Microorganisms 9:946.

Johnson, J. R., and A. L. Stell. 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. 181:261–272.

Johnson, T. J., Y. Wannemuehler, C. Doetkott, S. J. Johnson, S. C. Rosenberger, and L. K. Nolan. 2008. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J. Clin. Microbiol. 46:3987–3996.

Koyama, S., T. Murase, and H. Ozaki. 2020. Research note: longitudinal monitoring of chicken houses in a commercial layer farm for antimicrobial resistance in *Escherichia coli* with special reference to plasmid-mediated quinolone resistance. Poult. Sci. 99:1150–1155.

Lecointre, G., L. Rachdi, P. Darlu, and E. Denamur. 1998. *Escherichia coli* molecular phylogeny using the incongruence length difference test. Mol. Biol. Evol. 15:1685–1695.

Logue, C. M., Y. Wannemuehler, B. A. Nicholson, C. Doetkott, N. L. Barbieri, and L. K. Nolan. 2017. Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal *Escherichia coli* using the (previous and revised) Clermont phylogenetic typing methods and its impact on avian pathogenic *Escherichia coli* (APEC) classification. Front. Microbiol. 8:283.

Mitchell, N. M., J. R. Johnson, B. Johnston, R. Curtiss 3rd, and M. Mellata. 2015. Zoonotic potential of *Escherichia coli* isolates from retail chicken meat products and eggs. Appl. Environ. Microbiol. 81:1177–1187.

Nolan, L. K., H. J. Barnes, J.-P. Vaillancourt, T. Abdul-Aziz, and C. M. Logue. 2013. Colibacillosis.Pages 751−805 in Diseases of Poultry. D. E. Swayne, J. R. Glis-son, L. R. McDougald, L. K. Nolan, D. L. Suarez and V. Nair, eds. 13th ed. Iowa State Press, Iowa.

Nolan, L. K., R. Wooley E., J. Brown, K. Spears R., H. Dickerson W., and M. Dekich. 1992. Comparison of a complement resistance test, a chicken embryo lethality test, and the chicken lethality test for determining virulence of avian *Escherichia coli*. Avian Dis. 36:395–397.

Ozaki, H., H. Esaki, K. Takemoto, A. Ikeda, Y. Nakatani, A. Someya, N. Hirayama, and T. Murase. 2011. Antimicrobial resistance in fecal *Escherichia coli* isolated from growing chickens on commercial broiler farms. Vet. Microbiol. 150:132–139.

Ozaki, H., Y. Matsuoka, E. Nakagawa, and T. Murase. 2017. Characteristics of *Escherichia coli* isolated from broiler chickens with colibacillosis in commercial farms from a common hatchery. Poult. Sci. 96:3717–3724.

Ozaki, H., and T. Murase. 2009. Multiple routes of entry for *Escherichia coli* causing colibacillosis in commercial layer chickens. J. Vet. Med. Sci. 71:1685–1689.

Ozaki, H., K. Yonehara, and T. Murase. 2018. Virulence of *Escherichia coli* isolates obtained from layer chickens with colibacillosis associated with pericarditis, perihepatitis, and salpingitis in experimentally infected chicks and embryonated eggs. Avian Dis 62:233–236.

Picard, B., J. S. Garcia, S. Gouriou, P. Duriez, N. Brahimi, E. Bingen, J. Elion, and E. Denamur. 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect. Immun. 67:546–553.

Schouler, C., B. Schaeffer, A. Brée, A. Mora, G. Dahbi, F. Biet, E. Oswald, J. Mainil, J. Blanco, and M. Moulin-Schouleur. 2012. Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes. J. Clin. Microbiol. 50:1673–1678.

Someya, A., K. Otsuki, and T. Murase. 2007. Characterization of *Escherichia coli* strains obtained from layer chickens affected with colibacillosis in a commercial egg-producing farm. J. Vet. Med. Sci. 69:1009–1014.

Stromberg, Z. R., J. R. Johnson, J. M. Fairbrother, J. Kilbourne, A. Van Goor, R. Curtiss 3rd, and M. Mellata. 2017. Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health. PLoS One. 12: e0180599.

Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33:2233–2239.

Thomrongsuwannakij, T., R. Narinthorn, T. Mahawan, and P. J. Blackall. 2022. Molecular and phenotypic characterization of avian pathogenic *Escherichia coli* isolated from commercial broilers and native chickens. Poult. Sci. 101:101527.

Wang, M., M. Jiang, Z. Wang, R. Chen, X. Zhuge, and J. Dai. 2021. Characterization of antimicrobial resistance in chicken-source phylogroup F *Escherichia coli*: similar populations and resistance spectrums between *E. coli* recovered from chicken colibacillosis tissues and retail raw meats in Eastern China. Poult. Sci. 100: 101370.

Wooley, R. E., P. S. Gibbs, T. P. Brown, and J. J. Maurer. 2000. Chicken embryo lethality assay for determining the virulence of avian *Escherichia coli* isolates. Avian Dis. 44:318–324.

Zhuge, X., Z. Zhou, M. Jiang, Z. Wang, Y. Sun, F. Tang, F. Xue, J. Ren, and J. Dai. 2021. Chicken-source *Escherichia coli* within phylogroup F shares virulence genotypes and is closely related to extraintestinal pathogenic *E. coli* causing human infections. Transbound. Emerg. Dis. 68:880–895.