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Persistence of cellulitis-associated *Escherichia coli* DNA fingerprints in successive broiler chicken flocks

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Abstract

Avian cellulitis in broiler chickens is primarily caused by *Escherichia coli*. Previous research found that the *E. coli* isolates of cellulitis origin were unique to each ranch, suggesting that these *E. coli* were endemic within the ranch environment. To test the hypothesis that the *E. coli* associated with cellulitis are endemic in the litter of the broiler house, we designed a study to determine whether *E. coli* DNA fingerprints associated with cellulitis persist over successive flocks that are grown in the same house. In addition, we assessed the impact of different cleaning and disinfection strategies on this persistence. Two broiler houses were followed on each of five farms over 3–4 flocks. A total of 353 *E. coli* isolates from cellulitis lesions were analyzed in this study, and 314 of these isolates (89%) were DNA fingerprinted by PFGE. In each ranch, there were several DNA fingerprint patterns that were present over successive flocks, regardless of the cleaning and disinfection strategy utilized. Isolates persisted as long as 191 days, implying that these *E. coli* are capable of persisting in the broiler house environment for long periods of time. In addition, these *E. coli* isolates were associated with cellulitis lesions in successive flocks. Thus, the isolates of *E. coli*

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that are associated with cellulitis in broiler chickens appear to be endemic in the litter environment of the broiler house.

Keywords: Chicken-Bacteria; Cellulitis; *Escherichia coli*; Pulsed-field gel electrophoresis; Molecular epidemiology

1. Introduction

Escherichia coli can cause a variety of infections in poultry, including colibacillosis, airsacculitis, swollen head syndrome, synovitis, and cellulitis (Sojka and Carnaghan, 1961; Gross, 1994). In contrast to these other conditions, cellulitis is not associated with clinical illness and does not seem to affect the growth of the bird (Elfadil et al., 1996a). Cellulitis in broiler chickens is characterized by a diffuse inflammatory reaction in the subcutaneous tissue that results in the complete or partial condemnation of the carcass at processing (Messier et al., 1993; Elfadil et al., 1996b). The lesion is initiated by a break in the integument, in some cases due to a scratch from another bird, followed by bacterial contamination. Lactose-positive *E. coli* are the predominant organisms isolated from these lesions, and numerous investigators have causally linked the presence of *E. coli* with cellulitis (Peighambari et al., 1995a; Gomis et al., 1997; Norton et al., 1997; Jeffrey et al., 1999).

Several studies have characterized the *E. coli* associated with cellulitis in a flock and compared these isolates to other pathogenic *E. coli* in poultry, to environmental *E. coli* in the poultry house, and to cellulitis isolates collected on different farms. Ngeleka et al. (1996) typed cellulitis-associated *E. coli* by multilocus enzyme electrophoresis as well as by various virulence factors and found that many of the cellulitis *E. coli* clones were identical to other pathogenic avian *E. coli* isolates. Elfadil et al. (1996a) used a biotyping system and found that all of the cellulitis *E. coli* isolates from a flock were represented in the samples of *E. coli* isolated from the litter of the same broiler house. The researchers concluded that the *E. coli* associated with cellulitis were likely environmentally derived. Peighambari et al. (1995b) used a combination of a biotyping system, drug-susceptibility patterns, and plasmid profiles to characterize *E. coli* from cellulitis lesions and from broiler feces. In this study, there were no differences between the *E. coli* from cellulitis lesions and feces on a farm, but farms had their own unique populations of *E. coli* associated with cellulitis. The researchers speculated that the populations of *E. coli* strains that caused cellulitis were endemic on the farms and were not disseminated from a source common to the farms in the study. Because several studies have shown that a scratch is necessary for the development of a cellulitis lesion (Macklin et al., 1999; Norton et al., 1999), the inoculated pathogen is probably surviving in the litter environment.

In a previous study, we used pulsed-field gel electrophoresis (PFGE) to assess the genetic variability of cellulitis-associated *E. coli* and, by sampling multiple houses on multiple farms, we were able to assess the genetic heterogeneity of these *E. coli* across a spatial scale (Singer et al., 1999a). Although isolates across houses on a farm had a high degree of genetic similarity, isolates from different premises were genetically distinct.

This heterogeneity across premises suggested that isolates were not disseminated from a source common to these facilities, but rather that the *E. coli* isolates existed as an endemic population in the broiler house environment. If the broiler house environment has an endemic population of *E. coli* isolates, there may be a persistence of these isolates over successive flocks. Therefore, the objectives of this study were to (1) determine whether *E. coli* DNA fingerprints associated with cellulitis persisted over successive flocks grown in the same house, and (2) assess the impact different cleaning and disinfection strategies had on this persistence. We hypothesized that the population of *E. coli* isolates associated with cellulitis lesions would persist within a house over time as long as the litter was reused, but that the population of *E. coli* would change when the litter of the house was completely changed and the house thoroughly disinfected.

2. Materials and methods

2.1. Sampling of broilers and E. coli

Two houses on each of five farms were enrolled in this prospective study conducted from June to December, 1998. Rice hulls were used as litter (bedding) in all of the houses. During the study, each house was cleaned in two different ways after the flock had been processed. In the first method, houses received partial cleanouts (PART). This method involved the removal of the densely-packed caked litter usually found adjacent to the feed and water lines. The remaining used litter was then moved to the back 1/2 to 2/3 of the house. New rice hulls were placed in the cleaned front portion of the house. Following an interval of 8–19 days, broiler chicks were placed and brooded on these new rice hulls. When the birds were 8–21 days of age, they were given access to the entire house and, thus, contacted the reused litter. The second method of cleaning involved a complete cleanout (COMP) of the house. All of the used litter was completely removed and, while empty, the house was thoroughly cleaned, disinfected with a quaternary ammonium compound, and then treated with formaldehyde. New rice hulls were added to the entire house, and the broiler chicks were placed and brooded as described above.

Details of the sampling for each house in the study are shown in Table 1. The first flock from each house served as the baseline population (BASE). These flocks had been raised in houses that had received partial cleanouts. After the removal of the birds from these first flocks, the second flocks were placed in houses that again received partial cleanouts (PART 1). Two of the farms had additional flocks with partial litter changes (PART 2). Finally, the last flocks in this study were all grown in houses that had a complete cleanout (COMP). Therefore, all houses were followed for 3–4 flocks, which included a baseline (BASE), at least one partial litter change (PART), and a complete litter change (COMP).

Birds with suspected cellulitis lesions in each flock were collected as available from the processing line prior to evisceration so that the lesions would be intact and uncontaminated. Between 8 and 16 broilers were sampled per house. This number depended on the incidence of cellulitis in the flock as well as variability in the morphologic appearance of the lesions. Each cellulitis lesion was cultured onto MacConkey and blood agar plates. A single, isolated lactose-positive colony was then

Table 1
Sampling scheme used in this study, showing the number of *E. coli* isolates obtained from each flock, and the number of these isolates that were successfully DNA fingerprinted using pulsed-field gel electrophoresis within parentheses[a]

Farm	House	BASE	PART 1	PART 2	COMP	Total
A	1	12 (12)	2 (2)	9 (7)	11 (11)	34 (32)
	2	13 (13)	0 (0)	10 (10)	13 (10)	36 (33)
B	1	10 (5)	12 (10)	12 (9)	12 (12)	46 (36)
	2	8 (3)	10 (7)	11 (11)	10 (8)	39 (29)
C	1	12 (12)	10 (9)	N/C[b]	14 (13)	36 (34)
	2	12 (12)	10 (10)	N/C	16 (16)	38 (38)
D	1	12 (12)	8 (8)	N/C	10 (10)	29 (30)
	2	9 (7)	9 (8)	N/C	11 (10)	29 (25)
E	1	12 (12)	12 (10)	N/C	11 (9)	35 (31)
	2	12 (12)	9 (8)	N/C	10 (5)	31 (25)
Total		111 (99)	82 (72)	42 (37)	118 (106)	353 (314)

[a] The flocks from each house were sampled at baseline (BASE), after the first partial cleanout (PART 1), the second partial cleanout (PART 2), and complete cleanout (COMP). The total number of isolates is also shown (Total).
[b] Not collected.

randomly selected from the MacConkey plate (Singer et al., 2000) and identified as *E. coli* by standard biochemical techniques (MacFaddin, 1980). Assuming that our selection of carcasses approximated a random sampling, the selection of 8–16 *E. coli* isolates per flock provided between 83 and 97% probability of detecting an isolate that was present in 20% of the cellulitis lesions. In a related study, we found that our carcass sampling strategy and bacterial colony selection of *E. coli* did not deviate significantly from a random sampling (Singer et al., 1999b).

2.2. Pulsed-field gel electrophoresis (PFGE) of E. coli from broilers

Each isolate was DNA fingerprinted using PFGE. For extraction of genomic DNA, we utilized the CHEF Bacterial Genomic DNA Plug Kit (BioRad Laboratories, Hercules, CA) as per manufacturer's instructions. Briefly, 62.5 μl of an overnight brain–heart infusion broth culture were centrifuged and washed with TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The cells were resuspended in a cell suspension buffer (BioRad), and an equal volume of molten 2% low-melting-point agarose was added. The combination was mixed and pipetted into the plug mold. Solidified plugs were then treated with a lysozyme solution (BioRad) for 1 h at 37°C followed by digestion in a proteinase K solution (BioRad) at 50°C overnight. The plugs were then washed four times in 1X wash buffer (BioRad), the second of which contained 1 mM PMSF. Plugs were then washed in 0.1X wash buffer for 1 h followed by 1X restriction enzyme buffer for 1 h. The plugs were digested with 20 U of restriction endonucleases *Not*I and *Sfi*I in separate digestions (New England BioLabs, Beverly, MA) at 37°C overnight.

PFGE was performed with a 1.2% agarose gel on a CHEF III apparatus (BioRad, Hercules, CA) in 0.5X TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.3) at 14°C and 200 V. A linearly ramped switching time of 5 to 50 s applied over 22 h was used for *Not*I and *Sfi*I. Three lanes of bacteriophage lambda ladder (BioRad Laboratories, Hercules, CA) consisting of concatemers of 48.5 kb fragments were included on every 15-lane gel as molecular size standards.

After PFGE, the gel was stained with ethidium bromide (0.2 μg/ml) and digitized into a computer using the Gel Doc 1000 (BioRad) and the software program Molecular Analyst v. 1.4.1 (BioRad). The computerized image was analyzed with Molecular Analyst Fingerprinting Plus software v. 1.12 (BioRad). Only PFGE fragments larger than 50 kb were evaluated in order to eliminate the potential influence of large plasmids (Peighambari et al., 1995b).

Genetic similarity between each pair of isolates was assessed with the Dice coefficient of similarity (Dice, 1945). The similarity between pairs of isolates was calculated by: $S_D=2n_{AB}/(n_A+n_B)$, where n_{AB} is the number of bands common to isolates *A* and *B*, n_A the total number of bands for isolate *A*, and n_B the total number of bands for isolate *B*. Dendrograms were created with an unweighted-pair group method using average linkages (UPGMA) clustering. This allowed the similarity structure of isolates to be assessed quantitatively and graphically. Relationships were established for each farm separately. However, the entire database of isolates was also assessed for identical isolates across all farms and time periods.

3. Results

A total of 353 *E. coli* isolates from cellulitis lesions were analyzed in this study (Table 1). We were able to DNA fingerprint 314 of the 353 isolates (89%) by PFGE (Table 1 and Fig. 1). Thirty-nine of the isolates (11%) did not yield bands after repeated attempts. These isolates produced lanes with smeared DNA. Based on the results of other studies, this condition is likely due to the degradation of the DNA during PFGE by endogenous endonucleases (Izumiya et al., 1997).

Using the DNA fingerprint data, a dendrogram based on the Dice similarity coefficients was constructed for each farm in the study (Figs. 2 and 3). There was a considerable diversity of DNA fingerprint patterns within each farm (between 3 and 8 different patterns per flock). However, there were typically one to three fingerprint patterns within each flock that were present in at least two of the lesions from that flock. Houses on a farm shared identical populations of *E. coli* DNA fingerprints.

On each farm, there were several DNA fingerprint patterns that were present over successive flocks. Some of these fingerprints persisted between the BASE and PART flocks. Some DNA fingerprints were present in both the PART and COMP flocks. A small number (between 1 and 3) of the *E. coli* DNA fingerprint patterns were observed in the BASE, PART and COMP flocks of a farm (Figs. 2, 3).

Finally, we analyzed all 314 DNA fingerprints in a single dendrogram in order to assess the degree of similarity in DNA fingerprint patterns across farms. There were seven DNA fingerprint patterns that appeared to be closely related across farms (Table 2). These

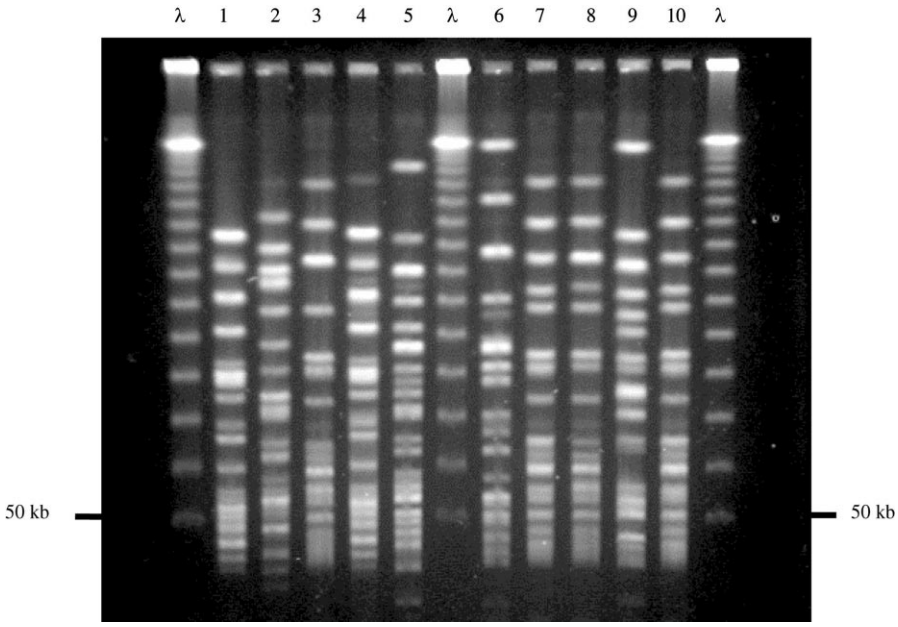

Fig. 1. Pulsed-field gel of DNA obtained from cellulitis *E. coli* that was digested with *Sfi*I. Lanes with λ represent bacteriophage lambda DNA molecular size markers. Only bands >50 kb were considered in the analysis. Isolates 1 and 4 have identical fingerprints. Isolates 7, 8 and 10 have identical fingerprints and are closely related to Isolate 3.

Table 2
Tabulation of the DNA fingerprint patterns obtained by pulsed-field gel electrophoresis that were similar (S_D>90%) across different farms[a]

Fingerprint	Farm	Flocks
1	A	BASE, PART 2, COMP
	B	COMP
	E	BASE, PART, COMP
2	C	BASE, COMP
	D	BASE, PART, COMP
3	B	PART 2
	E	BASE
4	D	BASE, COMP
	E	PART
5	A	PART 2
	D	BASE, PART
6	A	BASE
	B	BASE
	E	COMP
7	D	COMP
	E	COMP

[a] For each of the similar DNA fingerprints, the farms and flocks that shared this similar pattern are shown.

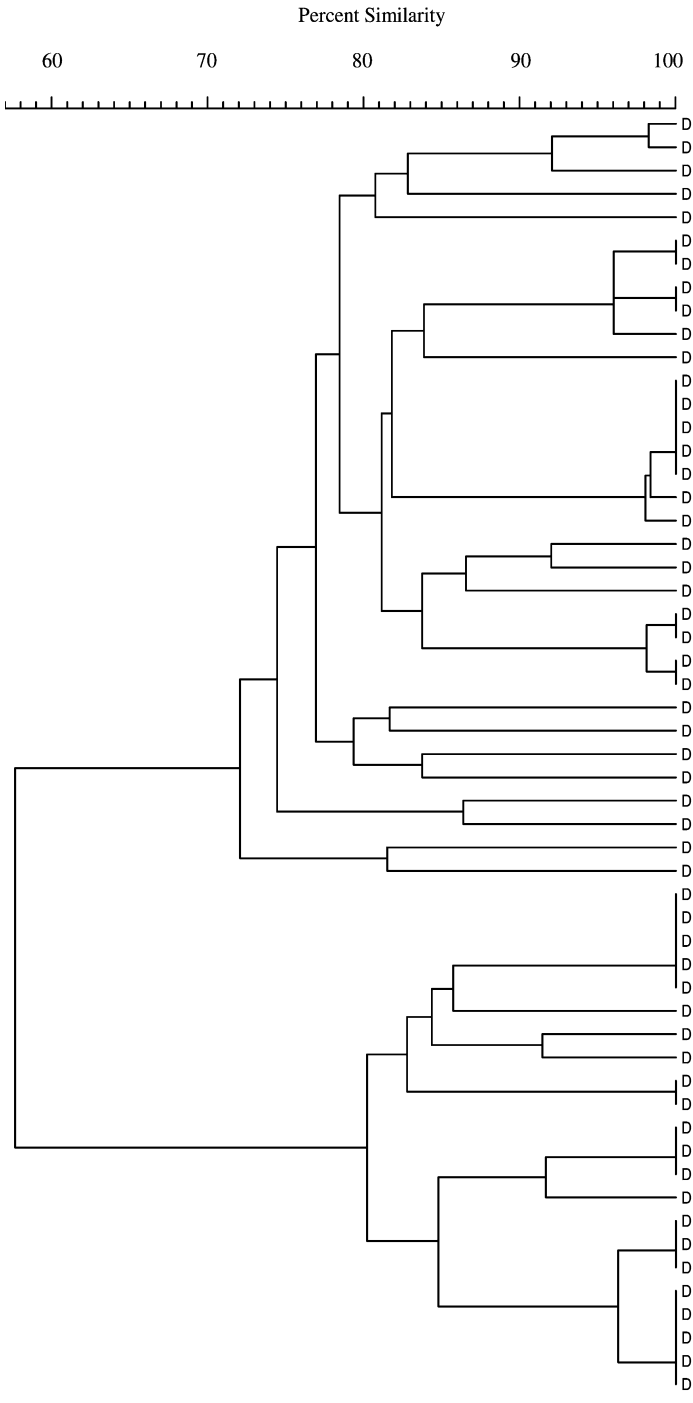

Fig. 2. Dendrogram of *E. coli* isolates from cellulitis lesions of farm D. Relationships are based on the Dice coefficient of similarity. The label for each isolate denotes the Farm, House and Flock from which the isolate was obtained (see Table 1).

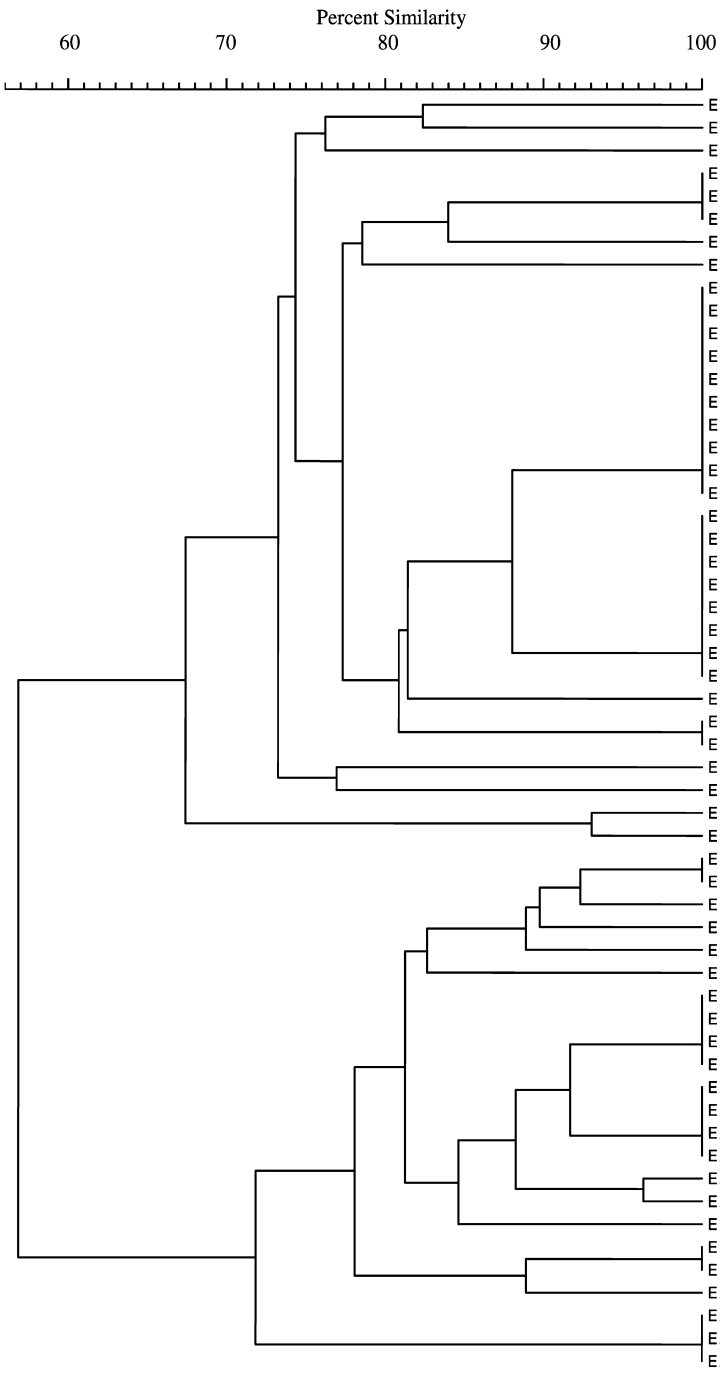

Fig. 3. Dendrogram of *E. coli* isolates from cellulitis lesions of farm E. Relationships are based on the Dice coefficient of similarity. The label for each isolate denotes the Farm, House and Flock from which the isolate was obtained (see Table 1).

isolates had a Dice coefficient of at least 90%. However, none of the DNA fingerprints across farms were identical.

4. Discussion

Previous studies (Peighambari et al., 1995b; Singer et al., 1999a) found that the population of *E. coli* associated with cellulitis was unique to each farm, and therefore, these *E. coli* were probably being maintained as an endemic population on each farm. However, to appropriately assess the notion of endemicity or persistence, several study design criteria must be met. First, an appropriate number of isolates must be collected per farm in order to provide adequate power for finding specific isolates if they are in fact present. Second, an identification system must be used that provides a high degree of resolution and thus can distinguish closely related isolates. Finally, persistence implies a stability in the environment over time and, therefore, farms must be followed over successive flocks in order to determine if isolates are truly endemic.

In this study, we followed two houses on each of five farms over 3–4 flocks in order to gauge the possibility of persistence of *E. coli* DNA fingerprints. We used a sample size that provided ample power (>82%) for detecting matches across farms and within a farm over time. We used a DNA fingerprinting technique, PFGE, that provides a high level of resolution for distinguishing *E. coli* (Böhm and Karch, 1992; Singer et al., 1999a). Finally, we timed our study and collection periods so that we could assess the influence of partial litter cleanouts vs. complete litter cleanouts on the persistence of *E. coli* DNA fingerprints.

We had hypothesized that fingerprint patterns would persist as long as the litter was reused, but that the population of cellulitis-associated *E. coli* would change when the litter was completely changed. When the dendrograms for each farm were assessed, identical DNA fingerprints were found between successive flocks, regardless of the type of cleaning and disinfection procedures used. The elapsed time between BASE and COMP samplings in this study ranged from 134 to 191 days. These findings imply that *E. coli* persist in the broiler house environment over successive flocks for long periods of time. In addition, these *E. coli* isolates are associated with cellulitis lesions in successive flocks.

We observed that different houses on a farm had very similar populations of cellulitis-associated *E. coli*, and this finding corroborates the results from our previous study (Singer et al., 1999a). In this current study, we observed a few similar DNA fingerprint patterns across farms (S_D>90%), a finding that is different from our previous study in which no fingerprint patterns were shared across farms (Singer et al., 1999a). Although identical fingerprints were collected from different houses on a farm, no identical fingerprints were observed between farms. This current study utilized a larger sample size than our previous study, which could explain the discrepancy. In our earlier study, we concluded that it would be almost impossible to infer that the DNA fingerprints from houses, farms, and complexes were disseminated from a common source. There were no clear patterns involving the potential origin of these isolates. Because all of the isolates in this current study were derived from cellulitis lesions in broiler chickens, we would not

expect the *E. coli* to be completely dissimilar. The finding of seven common DNA fingerprint patterns across farms does not affect our conclusion that DNA fingerprint patterns persisted over successive flocks and were not likely disseminated from a common source.

Although we analyzed more *E. coli* isolates per flock and more flocks than had previously been studied in relation to cellulitis, sample size still potentially influenced our findings. There were several instances in which a DNA fingerprint was absent in a subsequent flock, but then reappeared in the third flock. A larger sample size may have detected that specific DNA fingerprint in the subsequent flock. The possibility also exists that an isolate persisted in the environment, but was not associated with lesions in the subsequent flock and was, therefore, missed by our sampling method.

Another variable that could have affected our inferences is genetic drift. Many of the DNA fingerprints were observed to be very similar (>90% similarity based on Dice coefficient) to other clusters of DNA fingerprints on that farm. It is extremely difficult to discern if these closely related isolates were initially derived from the same clone. We would expect that, over a long duration in the broiler house environment such as the one used in our study, there would be some genetic drift of the *E. coli* genome. Based on the two enzyme systems that we employed and the number of bands that were produced per isolate, a Dice similarity coefficient of >90% typically corresponded to <4 band differences between a pair of isolates. According to published criteria for assessing relatedness of strains when using PFGE (Tenover et al., 1995, 1997), pairs of isolates with fewer than four band differences could be considered closely related. Thus, we may have underestimated the number of isolates persisting in the broiler house environment.

The presence of identical DNA fingerprint patterns over successive flocks does not necessarily imply with certainty that these isolates persisted. There are other potential explanations for the apparent persistence of *E. coli* DNA fingerprints. It is possible that the observed isolates were reintroduced to each flock and, therefore, were not truly persisting. However, many of the potential disseminating sources, such as feed, litter, and hatchery, were common to the farms in the study. Therefore, we would have expected to observe many identical or extremely similar DNA fingerprints across the farms in the study. There were only seven instances in which farms shared similar isolates (Table 2). On the contrary, houses on a farm usually shared the same population of DNA fingerprints, suggesting the possibility of a mechanical vector such as workers moving isolates from house to house. This is the same situation we observed in a previous study (Singer et al., 1999a). Another scenario that could mimic a persistence of fingerprint patterns would be if the molecular technique lacked adequate resolution for discriminating isolates. In this study, we were focused on avian isolates of *E. coli* that were capable of inducing cellulitis lesions. Within this specific population of isolates, we observed a considerable degree of genetic heterogeneity using PFGE. Therefore, we do not believe that the presence of identical DNA fingerprints observed over successive flocks was due to the inability of our methodology to distinguish differences. The presence of identical DNA fingerprints observed over successive flocks is strong evidence for the persistence of specific *E. coli* clones in the broiler house environment.

Not only did the cellulitis-associated *E. coli* in this study persist over successive flocks, but they were associated with cellulitis lesions in successive flocks of broilers. These *E. coli* are likely environmentally derived, and we would expect the broiler house environment to have a diverse population of *E. coli* living in the litter (Elfadil et al., 1996a). Consequently, for these cellulitis-associated *E. coli* to be present in cellulitis lesions over successive flocks, one or both of the two following conditions could exist. First, these *E. coli* could be the most numerous in the environment and, therefore, have the best chance of entering a scratch. Second, these isolates may possess virulence factors that make them particularly adapted for initiating cellulitis lesions (Peighambari et al., 1995b; Ngeleka et al., 1996). Some of the proposed virulence factors that may enhance the ability of an *E. coli* isolate to grow within the subcutaneous tissue of the broiler include aerobactin and other siderophores, fimbrial antigens, cytotoxins, and high molecular-weight plasmids, such as colicin V (Ngeleka et al., 1996).

There are a variety of mechanisms through which these cellulitis-associated *E. coli* may be surviving over successive flocks. When the houses undergo a partial cleanout, the most obvious possible source is the reused poultry litter. However, when the birds are removed from the houses and are not living on the litter, bacterial counts have been shown to decline very rapidly (Davies and Wray, 1996a). In our field studies, this used litter, in the absence of birds, often has bacterial counts below our detection limit of 2×10^3 cfu/g dry matter (data not shown). It is possible, though, for very low numbers of bacteria to persist in a stable resting state in the used litter and, when the next flock begins to live on this used litter, to begin to multiply. There are other mechanisms that could also explain the persistence, especially after a total cleanout. Other potential sources include the dirt floors of the houses or dust in the houses. Bacteria have been shown to persist for long periods in the dust of poultry houses. Even after a complete cleaning and disinfection, viable *E. coli* have been shown to survive in a stable state in poultry house dust for >28 weeks in the absence of birds (Harry, 1964). Mechanical vectors, such as workers and machinery, are other possible mechanisms. Rodents and insects have been shown to be a mechanism for maintaining isolates of bacteria in poultry houses over time (Henzler and Opitz, 1992; Davies and Wray, 1996b; Iwasa et al., 1999). Even though formaldehyde was used in the houses of this study during the total cleanout, this form of disinfection would clearly be ineffective against these mechanisms of persistence.

In this study, we observed populations of cellulitis-associated *E. coli* DNA fingerprints that were typically unique to the farm. This finding suggests an endemicity of cellulitis-associated *E. coli* isolates in the broiler house environment. A key question that now must be addressed relates to the bacterial load of these cellulitis-associated *E. coli* in the litter. If the amount of a specific bacterial clone in the environment is directly related to its ability to induce cellulitis, then efforts to reduce the overall *E. coli* load in the litter should help decrease the incidence of cellulitis. However, if cellulitis-associated *E. coli* isolates in the litter can exist in very low numbers and still induce lesions because of the presence of specific virulence factors, then attempts to decrease the incidence of cellulitis through bacterial control strategies would have to focus on removing these cellulitis-inducing isolates from the environment. Based on the results of this study, the persistence of cellulitis-associated *E. coli* may be difficult to control.

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References

Böhm, H., Karch, H., 1992. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. J. Clin. Microbiol. 30, 2169–2172.

Davies, R.H., Wray, C., 1996a. Persistence of *Salmonella enteritidis* in poultry units and poultry food. Br. Poult. Sci. 37, 589–596.

Davies, R.H., Wray, C., 1996b. Studies of contamination of three broiler breeder houses with *Salmonella enteritidis* before and after cleansing and disinfection. Avian Dis. 40, 626–633.

Dice, L.R., 1945. Measures of the amount of ecological association between species. Ecology 26, 297–302.

Elfadil, A.A., Vaillancourt, J.P., Meek, A.H., Gyles, C.L., 1996a. A prospective study of cellulitis in broiler chickens in southern Ontario. Avian Dis. 40, 677–689.

Elfadil, A.A., Vaillancourt, J.P., Meek, A.H., Julian, R.J., Gyles, C.L., 1996b. Description of cellulitis lesions and associations between cellulitis and other categories of condemnation. Avian Dis. 40, 690–698.

Gomis, S.M., Watts, T., Riddell, C., Potter, A.A., Allan, B.J., 1997. Experimental reproduction of *Escherichia coli* cellulitis and septicemia in broiler chickens. Avian Dis. 41, 234–240.

Gross, W.G., 1994. Diseases due to *Escherichia coli* in poultry. In: Gyles, C.L. (Ed.), *Escherichia coli* in domestic animals and humans, CAB International, Wallingford, pp. 237–259.

Harry, E.G., 1964. The survival of *Escherichia coli* in the dust of poultry houses. Vet. Rec. 76, 466–470.

Henzler, D.J., Opitz, H.M., 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. Avian Dis. 36, 625–631.

Iwasa, M., Makino, S., Asakura, H., Kobori, H., Morimoto, Y., 1999. Detection of *Escherichia coli* O157:H7 from *Musca domestica* (Diptera: Muscidae) at a cattle farm in Japan. J. Med. Entomol. 36, 108–112.

Izumiya, H., Terajima, J., Wada, A., Inagaki, Y., Itoh, K.I., Tamura, K., Watanabe, H., 1997. Molecular typing of enterohemorrhagic *Escherichia coli* O157:H7 isolates in Japan by using pulsed-field gel electrophoresis. J. Clin. Microbiol. 35, 1675–1680.

Jeffrey, J.S., Chin, R.P., Singer, R.S., 1999. Assessing cellulitis pathogenicity of *Escherichia coli* isolates in broiler chickens assessed by an in vivo inoculation model. Avian Dis. 43, 491–496.

MacFaddin, J.F., 1980. Enterobacteriaceae and other intestinal bacteria. In: MacFaddin, J.F. (Ed.), Biochemical Tests for Identification of Medical Bacteria. Williams & Wilkins, Baltimore, pp. 439–464.

Macklin, K.S., Norton, R.A., McMurtrey, B.L., 1999. Scratches as a component in the pathogenesis of avian cellulitis in broiler chickens exposed to cellulitis origin *Escherichia coli* isolates collected from different regions of the US. Avian Pathol. 28, 573–578.

Messier, S., Quessy, S., Robinson, Y., Devriese, L.A., Hommez, J., Fairbrother, J.M., 1993. Focal dermatitis and cellulitis in broiler chickens: bacteriological and pathological findings. Avian Dis. 37, 839–844.

Ngeleka, M., Kwaga, J.K.P., White, D.G., Whittam, T.S., Riddell, C., Goodhope, R., Potter, A.A., Allan, B., 1996. *Escherichia coli* cellulitis in broiler chickens — clonal relationships among strains and analysis of virulence-associated factors of isolates from diseased birds. Infect. Immun. 64, 3118–3126.

Norton, R.A., Bilgili, S.F., McMurtrey, B.C., 1997. A reproducible model for the induction of avian cellulitis in broiler chickens. Avian Dis. 41, 422–428.

Norton, R.A., Macklin, K.S., McMurtrey, B.L., 1999. Evaluation of scratches as an essential element in the development of avian cellulitis in broiler chickens. Avian Dis. 43, 320–325.

Peighambari, S.M., Julian, R.J., Vaillancourt, J.P., Gyles, C.L., 1995a. *Escherichia coli* cellulitis — experimental infections in broiler chickens. Avian Dis. 39, 125–134.

Peighambari, S.M., Vaillancourt, J.P., Wilson, R.A., Gyles, C.L., 1995b. Characteristics of *Escherichia coli* isolates from avian cellulitis. Avian Dis. 39, 116–124.

Singer, R.S., Jeffrey, J.S., Carpenter, T.E., Cooke, C.L., Chin, R.P., Hirsh, D.C., 1999a. Spatial heterogeneity of *Escherichia coli* isolated from avian cellulitis lesions in broilers. Avian Dis. 43, 756–762.

Singer, R.S., Jeffrey, J.S., Atwill, E.R., Cooke, C.L., Carpenter, T.E., Hirsh, D.C., 1999b. Assessing sampling bias in molecular epidemiologic studies isolates of *Escherichia coli* from avian cellulitis lesions as an example. The 48th Western Poultry Disease Conference, Vancouver, Canada.

Singer, R.S., Johnson, W.O., Jeffrey, J.S., Chin, R.P., Carpenter, T.E., Atwill, E.R., Hirsh, D.C., 2000. A statistical model for assessing bacterial colony selection strategies: a case study of *Escherichia coli* and avian cellulitism. J. Vet. Diagn. Invest. 12, 118–125.

Sojka, W.J., Carnaghan, R.B.A., 1961. *Escherichia coli* infection in poultry. Res. Vet. Sci. 2, 340–352.

Tenover, F.C., Arbeit, R.D., Goering, R.V., 1997. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Infect. Control Hosp. Epidemiol. 18, 426–439.

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