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## PREVALENCE OF SHIGA TOXIN–PRODUCING *ESCHERICHIA COLI* AND *SALMONELLA ENTERICA* IN ROCK PIGEONS CAPTURED IN FORT COLLINS, COLORADO

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**ABSTRACT:** The potential role of rock pigeons (*Columba livia*) in the epidemiology of shiga toxin–producing *Escherichia coli* (STEC) and *Salmonella enterica* is unclear. Our objective was to determine the prevalence of STEC and *S. enterica* in pigeons at urban and dairy settings as a function of season. Prevalence of STEC and *S. enterica* was estimated by bacteriologic culture of cloacal swabs collected from pigeons trapped at urban and dairy locations in and around Fort Collins, Colorado from January to November 2003. Presumptive *E. coli* isolates were tested for the presence of virulence genes SLT-1, SLT-2, *eae*, *hlyA*, K1, CNF-1, CNF-2, and LT using polymerase chain reaction. Shiga toxins were not isolated from any of 406 samples from pigeons, but virulence genes typically associated with disease in humans were identified in isolates from 7.9% (95% CI: 5.5% to 10.9%) of captured pigeons. *S. enterica* were detected in 3.2% of 277 samples from pigeons, with all positive samples originating from dairy locations (nine of 106 [8.5%]; 95% CI: 4.0–15.5%). The results suggest that although pigeons may acquire *S. enterica* from cattle and play a role in recirculation and persistence of the microorganism at dairies, pigeons are not important carriers of STEC.

**Key words:** Cattle, *Escherichia coli*, pigeons, prevalence, *Salmonella enterica*, STEC.

### INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella enterica* are ubiquitous microorganisms known to cause infection of varying severity in humans and animals. These gram-negative bacteria are able to survive under adverse environmental conditions for extended periods of time (Huston et al., 2002), thus creating many opportunities for exposure and infection. Healthy individuals can become infected, but the young, elderly, and those with a concurrent infection are most vulnerable (Pelzer, 1989).

Infection in humans with either STEC or *S. enterica* generally occurs after consumption of contaminated food or contact with an infected animal or human (Pelzer, 1989). Although outbreaks attributed to contaminated nonanimal products are important sources of human infection,

fecal contamination of meat by infected cattle is the most common source of STEC infection (DebRoy and Maddox, 2001) and a major cause of *S. enterica* infection in humans (Wells et al., 2001).

Shortly after *E. coli* O157 and other STEC strains were recognized in 1982 as important human pathogens (Riley et al., 1983), cattle were recognized as the primary reservoir (Wells et al., 1991). Infected cattle, via contaminated meat and dairy products, elevate the risk that these food-borne pathogens will enter the human food chain (Wells et al., 2001). Food deprivation and transportation to the slaughterhouse stress the cattle's immune systems and induce fecal shedding of the bacteria. Contamination then occurs through interchange of fecal matter between carcasses. The most effective method of preventing entry of bacteria into the human food chain is by minimizing prevalence in the herd.

Identification of the sources of infection in cattle is an important step towards decreasing the prevalence of these pathogens and thus decreasing the risk of infection of humans and cattle. Wild birds are a potential source of contamination that deserve further scrutiny because of their mobility and close association with livestock operations.

Although birds have been identified as carriers of both STEC (Wallace et al., 1997; Dell’Omo et al., 1998) and *S. enterica* (Sambyal and Sharma, 1972), it is unknown whether they become infected when exposed to infected cattle, or whether they transmit STEC and *S. enterica* to cattle and could therefore be responsible for increasing the prevalence of these microorganisms. Peridomestic birds such as pigeons and house sparrows may recirculate STEC and *S. enterica* within the dairy or may introduce the bacteria from neighboring dairies. Thus, an initial step in assessing the effect that birds have on cattle health is to quantify the prevalence of STEC and *S. enterica* in peridomestic birds.

Our objective was to determine the prevalence of STEC and *S. enterica* in pigeons at urban and dairy settings as a function of season. We hypothesized that pigeons at dairies would have higher prevalence of both STEC and *S. enterica* because of the known presence and persistence of these microorganisms in dairy environments (Wells et al., 2001). We also hypothesized that an increase in prevalence would be observed during summer months, when prevalence is typically higher in dairy cattle (Herriott et al., 1998; Van Donkersgoed et al., 1999). Pigeons were selected because they live in close association with cattle and because they are known carriers of STEC (Morabito et al., 2001) and *S. enterica* (Scholtens and Caroli, 1971).

## MATERIALS AND METHODS

### Study sites

Pigeons were trapped within the city of Fort Collins, Colorado, and nearby dairies. Fort

Collins is located in northern Colorado along the foothills on the eastern slope of the Rocky Mountains, at an elevation of 1,525 m above sea level. Precipitation averages about 36.8 cm per year and average monthly temperature varies from  $-2.8^{\circ}\text{C}$  in January to  $21.7^{\circ}\text{C}$  in July. South Dairy ( $40^{\circ}40'\text{N}$ ,  $105^{\circ}105'\text{W}$ ) consisted of about 4,500 cattle located on 14.2 ha. East Dairy ( $41^{\circ}40'\text{N}$ ,  $105^{\circ}105'\text{W}$ ) consisted of about 3,000 cattle located on 17 ha. Dairy sites were chosen based on location, presence of pigeon populations, and landowner willingness to participate. The urban bridge sites were located on the north and south ends of the city, respectively, and were used by pigeons as roosting sites. The urban building sampling sites were located in the south central, north central, and outskirts of the downtown area, respectively.

### Trapping methods

At the dairies and building sites, pigeons were trapped using walk-in traps (91.4 by 61.0 by 26.7 cm) baited with mixed grain during three 8-wk periods (January to March, June to August, and September to November 2003). A single trap was placed on a roof or similar structure at each location. Two decoy pigeons testing negative for STEC and *S. enterica* were placed in each trap with food and water, which were replenished daily. At the two urban bridge locations, roosting pigeons were captured by hand at night with the aid of head lamps and hand nets.

At South Dairy, the managers periodically shoot pigeons to limit the number of pigeons at the dairy, but during the study this practice was limited to the third trapping period. These birds were sampled within 6 hr of death. Research was conducted according to animal care and use guidelines in place at Colorado State University (approval number 02-293A-01) and the National Wildlife Research Center (protocol number QA-957).

### Sample collection and transport

Pigeons were removed from the traps daily and new birds were individually marked with a numbered leg band and color corresponding to location. This prevented duplicate sampling and enabled detection of changes in disease status over time. Cloacal swabs were collected using the BBL CultureSwab collection and transport system (Becton, Dickinson, and Company, Sparks, Maryland, USA) that contained Amies Liquid Medium, Liquid Stuart Medium and Cary-Blair Transport Medium. After sampling, pigeons were released at the site of capture. Specimens were transported at

ambient temperature and processed within 2 hr of collection, at the National Wildlife Research Center, Fort Collins, Colorado.

A cloacal swab was collected from each pigeon during the first trapping period (January to March 2003), and samples were screened for STEC only. Two cloacal swabs were collected from each pigeon during the second (June to August 2003) and third trapping periods (September to November 2003). One swab was used to screen for STEC and the other to screen for *S. enterica*.

#### Environmental sample collection

Ten pens were randomly selected at each dairy and three samples of each type (water from water troughs, feed from feed bunks, and cow manure from pen floor) were collected from each pen. Manure samples were collected using the BBL CultureSwab. Water samples were collected by dipping a sterile 125 ml security-snap BacT Bottle (Nalge Nunc International, Hanover Park, Illinois, USA) into the water trough, filling the bottom of the bottle, and snapping the cap shut. Feed samples were collected by inverting a sterile whirl-pak (Nasco, Modesto, California, USA) and using it as a glove to pick up a small amount of feed, after which the bag was turned right side out and sealed to prevent cross-contamination. Samples were labeled and transported to the laboratory at ambient temperature and were processed there within 6 hr of collection.

#### Isolation and identification of *E. coli*

Specimens were enriched in 3 ml of tryptic soy broth (TSB) and incubated for 18–24 hr at 37 C. After enrichment, 750 µl of the culture was transferred to a cryovial containing 750 µl of glycerol and stored in an ultracold freezer at –80 C for future testing and archiving. From the remaining enriched culture, a sample was streaked onto a MacConkey agar plate and incubated for 18–24 hr at 37 C. All morphologically distinct colonies that were dark pink (i.e., strong lactose fermenters) were streaked onto blood agar plates and incubated for 18–24 hr at 37 C. Isolated colonies were confirmed as *E. coli*-positive using three biochemical tests: indole (+), oxidase (–) and potassium hydroxide (+) (MacFaddin, 2000). Each isolate identified as *E. coli*-positive was enriched in TSB as above, and 750 µl of culture was transferred to a cryovial containing 750 µl of glycerol and stored at –80 C.

All presumptive *E. coli* isolates, including environmental samples and samples collected from pigeons, were tested for the presence of

shiga toxins using the Premier EHEC enzyme-linked immunosorbent assay (ELISA) test kit (Meridian Bioscience, Inc., Cincinnati, Ohio) according to the manufacturer's instructions. Three hundred eighty-one presumptive *E. coli* isolates from pigeons were recultured by inoculation onto nutrient agar slants and submitted to the Gastroenteric Disease Center at Pennsylvania State University for serogrouping and toxin assays. Isolates were tested for the presence of virulence genes for SLT-1, SLT-2, *eae*, *hlyA*, K1, CNF-1, CNF-2, and LT, using polymerase chain reaction (Wittwer et al., 1994; DebRoy and Maddox, 2001). Briefly, isolates were screened in a presumptive assay using antisera reactive with 167 O-serogroups (Orskov et al., 1977), and positive reactions were confirmed by microtitration assays (DebRoy et al., 2004). Positive (established strains from American Type Culture Collection or other known sources) and negative (*E. coli* K-12) controls were included for each assay. Because of budgetary constraints, serogrouping and toxin testing were not performed on the environmental isolates. Consequently, no information was available regarding the presence of virulence genes or correspondence of serogroups between pigeon and environmental samples.

#### Isolation and identification of *Salmonella*

Specimens were selectively enriched in tetrathionate brilliant green broth with 200 µl of iodine and incubated at 42 C. At 24 hr, 100 µl of culture was transferred to 10 ml of Rappaport-Vassiliadis broth and incubated for 18–24 hr at 37 C. Cultures were then inoculated onto two agar plates selective for *Salmonella* growth (brilliant green agar with novobiocin and xylose-lysine-tergitol-4 agar), and incubated for 18–24 hr at 37 C, and then at room temperature for an additional 24 hr. Five morphologically distinct colonies with typical characteristics of *S. enterica* organisms were selected from either of the two agar plates, inoculated onto triple sugar iron agar slant and lysine iron agar slant, and incubated at 37 C for 18–24 hr to identify reactions typical of *S. enterica*. Each culture was then streaked onto blood agar and incubated for 18–24 hr at 37 C. Then, the isolate was inoculated in 3 ml of TSB and incubated overnight at 37 C. All presumptive positive isolates were also tested with API 20E (BioMerieux, Durham, North Carolina, USA), a series of biochemical tests, to identify the bacterial strain.

Presumptive positive isolates were thawed, plated onto blood agar plates, and incubated at 37 C overnight. Nine pigeon and 21 environ-

mental isolates were submitted to Colorado State University Diagnostic Lab, Fort Collins, Colorado, for confirmation and serogrouping. Each isolate was tested by slide agglutination with antisera specific for *Salmonella* O groups A, B, C<sub>1</sub>, C<sub>2</sub>, D, and E. Serotyping of isolates was performed at the National Veterinary Services Laboratory in Ames, Iowa, using standard methods.

#### Isolation and identification of environmental samples

For the isolation of *E. coli* and *S. enterica*, cow manure samples collected from the pen floor were processed following the same isolation procedure used for the pigeon samples. Feed and water samples were pre-enriched in buffered peptone water (BPW) and incubated at 37 C for 6 hr. One gram of feed was added to 10 ml of BPW and agitated in a stomacher for 60 sec at normal speed. To process the water samples, 1 ml of water was added to 10 ml of BPW. After incubation, the culturing methods described for the samples from pigeons were used.

#### Data analysis

We used 95% confidence intervals based on the normal approximation to the binomial distribution to characterize prevalence estimates (MINITAB Statistical Software Release 13.32, Minitab Inc., State College, Pennsylvania, USA). Prevalence was estimated by counting each pigeon testing positive once, regardless of the number of isolates. Prevalence estimates for environmental samples were calculated similarly. A z-statistic was calculated to determine the probability for differences in prevalence estimates between urban and dairy locations over time.

## RESULTS

### STEC in pigeons

*E. coli* were detected in 326 of 406 pigeons (80.3%) sampled at seven locations between January and November 2003. Trapping efforts were less successful at dairy sites ( $n=139$ ) than at urban sites ( $n=267$ ) because of the greater availability of feed at dairy sites. Some samples yielded multiple colonies of distinct morphology and color and others yielded no growth; 381 presumptive *E. coli* isolates were submitted for serogrouping and for toxin testing.

No shiga toxins were identified in any of the isolates. However, virulence genes including *eae*, CNF-1, K1, and *hlyA* were detected in isolates from 7.9% of 406 pigeons (Table 1). Prevalence of virulence attributes was similar ( $z=0.38$ ,  $P=0.705$ ) between the urban (8.2%,  $n=267$ ) and dairy sites (7.2%,  $n=139$ ). *E. coli* isolates were identified with virulence characteristics during each of the 3 trapping periods, but the prevalence was lower during winter (2.3%,  $n=129$ ) than summer (10.1%,  $n=159$ ) or fall (11.0%,  $n=118$ ),  $P=0.005$  and  $P=0.006$ , respectively.

To ensure that capture method did not influence prevalence, we compared methods and prevalence estimates for urban and dairy locations within and between trapping periods. During the first capture period, prevalence for virulence genes was similar ( $z=0.74$ ,  $P=0.457$ ) for pigeons captured in walk-in baited traps (5%,  $n=43$ ), and pigeons captured with hand nets (2%,  $n=53$ ). Prevalence of virulence genes was similar ( $z=0.46$ ,  $P=0.644$ ) between pigeons trapped at dairy sites with walk-in baited traps during the second period (9%,  $n=57$ ), and pigeons shot at dairies during the third period (6%,  $n=47$ ).

Environmental isolates were screened for STEC during all three trapping periods at South Dairy and during the second and third trapping periods at East Dairy. STEC was not recovered from manure ( $n=90$ ), water ( $n=90$ ) or feed samples ( $n=90$ ) collected at South Dairy. At East Dairy, shiga toxins were detected in 4 of 60 (7%) manure samples, but they were not recovered from any feed ( $n=60$ ) or water ( $n=60$ ) samples.

### *S. enterica* in pigeons

*S. enterica* were isolated from samples obtained from the environment and pigeons at dairies but not from any pigeon samples collected at urban sites (Table 2). All positive samples recovered from pigeons were collected on different days from different pigeons, except for two



TABLE 1. Virulence attributes of *E. coli* detected in samples from pigeons ( $n=406$ ) in Fort Collins, Colorado from January to November 2003.

| Location | Trapping period | O-type            | <i>eae</i> <sup>a</sup> | CNF-1 <sup>b</sup> | K1 <sup>c</sup> | <i>hlyA</i> <sup>d</sup> |
|----------|-----------------|-------------------|-------------------------|--------------------|-----------------|--------------------------|
| Urban    | Winter          | — <sup>e</sup>    | +                       |                    |                 |                          |
| Urban    | Winter          | 117               |                         |                    |                 | +                        |
| Urban    | Winter          | 63                | +                       |                    |                 |                          |
| Dairy    | Summer          | —                 | +                       |                    |                 |                          |
| Urban    | Summer          | —                 | +                       |                    |                 |                          |
| Urban    | Summer          | 128               | +                       |                    |                 |                          |
| Urban    | Summer          | 128               | +                       |                    |                 |                          |
| Dairy    | Summer          | 128               | +                       |                    |                 |                          |
| Dairy    | Summer          | 128               | +                       |                    |                 |                          |
| Urban    | Summer          | 128               | +                       |                    |                 |                          |
| Dairy    | Summer          | 128               | +                       |                    |                 |                          |
| Dairy    | Summer          | 128               | +                       |                    |                 |                          |
| Urban    | Summer          | 128               | +                       |                    |                 |                          |
| Urban    | Summer          | 135w <sup>f</sup> | +                       |                    |                 |                          |
| Urban    | Summer          | 149               | +                       |                    |                 |                          |
| Dairy    | Summer          | 15                | +                       |                    |                 |                          |
| Urban    | Summer          | 25w               |                         | +                  | +               |                          |
| Urban    | Summer          | 88                |                         | +                  |                 |                          |
| Dairy    | Summer          | 96                | +                       |                    |                 |                          |
| Dairy    | Fall            | 120               |                         |                    |                 | +                        |
| Urban    | Fall            | 128               | +                       |                    |                 |                          |
| Urban    | Fall            | 128               | +                       |                    |                 |                          |
| Urban    | Fall            | 128               | +                       |                    |                 |                          |
| Urban    | Fall            | 145               | +                       |                    |                 |                          |
| Urban    | Fall            | 145               | +                       |                    |                 |                          |
| Urban    | Fall            | 145               | +                       |                    |                 |                          |
| Dairy    | Fall            | 43                | +                       |                    |                 |                          |
| Dairy    | Fall            | 91                |                         |                    |                 | +                        |
| Urban    | Fall            | 96                | +                       |                    |                 |                          |
| Urban    | Fall            | 96                | +                       |                    |                 |                          |
| Urban    | Fall            | 96                | +                       |                    |                 |                          |
| Urban    | Fall            | 96                | +                       |                    |                 |                          |

<sup>a</sup> Gene responsible for attaching and effacing lesions.

<sup>b</sup> Cytotoxic necrotizing factor type 1 (toxin).

<sup>c</sup> K1 capsular antigen.

<sup>d</sup> Alpha hemolysin.

<sup>e</sup> O-type not available.

<sup>f</sup> Represents a weak reaction with the antisera.

pigeons that were identified with *Salmonella* Typhimurium var. Copenhagen. *Salmonella* serotypes Newport, Senftenberg, and Montevideo were isolated from pigeons and from at least one type of environmental sample. *Salmonella* Typhimurium var. Copenhagen was recovered only from pigeons, whereas *Salmonella* Bredeney, *Salmonella* Cerro, *Salmonella* Mbandaka, and *Salmonella* Anatum were recovered only from environmental sam-

ples. There was no seasonal difference in prevalence of *Salmonella* in samples collected from pigeons,  $P=0.91$ : 3%,  $n=159$  (June to August 2003); and 3%,  $n=118$  (September to November 2003). A difference was observed ( $z=3.14$ ,  $P=0.002$ ) between pigeons trapped at urban (0%,  $n=171$ ) and dairy locations (9%,  $n=106$ ).

At South Dairy, during the fall and summer, *S. enterica* were recovered from

TABLE 2. Serotypes of *Salmonella enterica* detected in samples collected from pigeons and the environment in Fort Collins, Colorado, from January to November 2003.

| Source of sample | Total no. of samples | No. positive (%) | No. isolates of each serotype |                |                |                |                |                |                 |                |
|------------------|----------------------|------------------|-------------------------------|----------------|----------------|----------------|----------------|----------------|-----------------|----------------|
|                  |                      |                  | N <sup>a</sup>                | S <sup>b</sup> | T <sup>c</sup> | B <sup>d</sup> | C <sup>e</sup> | M <sup>f</sup> | Mb <sup>g</sup> | A <sup>h</sup> |
| Pigeons          | 277                  | 9 (3%)           | 4                             | 1              | 3              |                |                | 1              |                 |                |
| Urban            | 171                  | 0 (0%)           |                               |                |                |                |                |                |                 |                |
| Dairy            | 106                  | 9 (9%)           | 4                             | 1              | 3              |                |                | 1              |                 |                |
| Environmental    |                      |                  |                               |                |                |                |                |                |                 |                |
| Manure           | 120                  | 8 (7%)           | 2                             | 2              |                |                | 1              | 3              |                 |                |
| Water            | 120                  | 2 (2%)           |                               | 1              |                |                |                | 1              |                 |                |
| Feed             | 120                  | 11 (9%)          |                               | 5              |                | 2              |                | 2              | 1               | 1              |

<sup>a</sup> Newport.<sup>b</sup> Senftenberg.<sup>c</sup> Typhimurium var. Copenhagen.<sup>d</sup> Bredeney.<sup>e</sup> Cerro.<sup>f</sup> Montevideo.<sup>g</sup> Mbandaka.<sup>h</sup> Anatum.

12% of 60 feed samples, 7% of 60 manure samples, and 2% of 60 water samples. During fall, serotypes Senftenberg (five of seven, 71%) and Bredeney (two of seven, 29%) were isolated from feed samples. Senftenberg was isolated from one of two (50%) of the positive manure samples during summer months, whereas Newport (one of four, 25%), and Cerro (one of four, 25%), were isolated during the fall. *Salmonella* Senftenberg was isolated from a water sample during the fall trapping period. At East Dairy, *S. enterica* were recovered from 7% of 60 manure samples, 7% of 60 feed samples, and 2% of 60 water samples. Serotypes Montevideo and Newport were isolated from the manure samples during summer. *Salmonella* Montevideo and *Salmonella* Anatum were identified in feed samples during the fall, but *Salmonella* Mbandaka was the only serotype detected in the summer months. *Salmonella* Montevideo was also isolated from a water sample during summer.

## DISCUSSION

### *E. coli*

Little information is available regarding serogroups of *E. coli* and virulence mar-

kers in birds. The STEC serogroup, O86, has been identified as the cause of disease or mortality in birds (Foster et al., 1998), but other serogroups including O157 (Shere et al., 1998), O153, O136 (Makino et al., 2000), O15, O18, O25, O45, O75, O152 (Morabito et al., 2001), and O128 (Schmidt et al., 2000) have been isolated from asymptomatic birds. Despite ambiguous evidence that the above serogroups cause disease in their avian carriers, there is concern that these *E. coli* serogroups are a risk to humans and cattle, and that birds are agents of transport and transmission. The *eae* gene, which is associated with STEC serogroups such as O5, O128, O145, and O157 in human infections, was the most commonly detected virulence factor in the pigeons we sampled. This gene is necessary but not sufficient for the production of attaching and effacing lesions in humans and cattle (Phillips et al., 2000). Although it is troubling that we detected serogroups in pigeons known to cause disease in humans and cattle, we were unable to identify the variant of shiga toxin with the methods we used. Other studies have identified the variant of shiga toxin in pigeons as Stx2f

(Schmidt et al., 2000); this variant is typically not found in human or cattle isolates.

With a modest sampling effort, we identified a number of serogroups in samples isolated from pigeons that have been associated with STEC disease in humans or cattle (e.g., O1, O5, O8, O45, O88, O91, O103, O105, O117, O128, O145, O157, O165, O168, and O172). However, we classified these *E. coli* isolates as nonpathogenic because we did not identify associated virulence genes in the isolates. Because we did not test for all human and cattle virulence markers, it is possible that we underestimated the prevalence of pathogenic *E. coli* carried by pigeons. The low sensitivity of the ELISA that we used to detect shiga toxins may be another explanation for the low prevalence we detected. The apparent absence of STEC in the environment may have been because of inadequate sensitivity of the culture method, which prevented detection of STEC at low prevalence.

The highest prevalence of virulence factors associated with *E. coli* was observed in pigeons during the summer and fall trapping periods, indicating that the risk of transmission is greater during these seasons than during the winter. This observation is consistent with other studies that have also documented a rise in prevalence during warmer months (Hancock et al., 1994; Mechie et al., 1997).

Although direct transmission of pathogenic forms of *E. coli* from pigeons to humans is unlikely, pigeons may have an affect on the prevalence of pathogenic bacteria in cattle. Higher prevalence in the herd increases the risk that infected cattle will be sent to the slaughterhouse because they may impact cattle health and productivity. As a consequence, these pathogens may enter the human food chain via contaminated bovine products.

### ***Salmonella***

*Salmonella* Senftenberg was isolated from a pigeon sample during the summer

and from a water trough where pigeons were observed loafing before sample collection during the fall at South Dairy, suggesting that cross-contamination may be occurring. We also isolated *Salmonella* Newport from both pigeon and environmental samples collected from South Dairy. Our data are consistent with other studies that have implicated birds as disseminators of *Salmonella* at dairies (Kirk et al., 2002), and identified an association between *Salmonella* serotypes in environmental and bird samples (Čížek et al., 1994). *Salmonella* Senftenberg and *Salmonella* Newport also have both been isolated from human and animal sources (CDC, 2004), and Newport is an important cause of infection in cattle (Clegg et al., 1983).

*Salmonella* Typhimurium var. Copenhagen was recovered from pigeons but not from environmental samples in our study. This finding has important implications because Typhimurium var. Copenhagen is a significant cause of food poisoning and enteric fever in humans, and is responsible for the majority of the cases of salmonellosis in cattle (Daniels et al., 1993). Although this serotype was not isolated from environmental samples, we collected samples on only 1 day during each trapping period, suggesting that it may have been present in the environment but was not detected.

A similar association of serotypes in pigeon and environmental samples was observed at East Dairy. Montevideo, a serotype often isolated from dairy cattle with salmonellosis (Galland et al., 2001) and one of the top 20 most frequently reported serotypes in humans (CDC, 2004), was recovered from a pigeon and from all three types of environmental samples. Correspondence of serotypes found in humans, cattle and pigeons indicate that transmission is a concern, and pigeons represent a potential source of contamination to cattle and human health. Although no other serotypes were detected in pigeon and multiple environ-



mental sample types, serotypes frequently implicated as the cause of disease were identified in the environmental samples. *Salmonella* Anatum and *Salmonella* Newport, which were isolated from the feed and manure samples respectively, are two of the most commonly detected serotypes in cattle (Smith and House, 1992; Sorenson et al., 2002). *Salmonella* Mbandaka, which was detected in a feed sample during the summer trapping period, is less frequently reported in cattle (CDC, 2004). At both dairy locations, prevalence of *S. enterica* was similar or higher in feed samples compared to manure samples. This unexpected result may be attributed to the pre-enrichment step used for the feed and water samples, which increases the sensitivity of culture methods and may have artificially inflated the prevalence of *S. enterica* in the feed samples compared to samples from manure.

Prevalence of *S. enterica* in pigeons at our dairy locations was comparable during summer (8.8%; 95% CI: 2.9–19.3%) and fall (8.2%; 95% CI: 2.3–19.6%). Other studies have documented seasonal variation in prevalence with the highest prevalence occurring during summer months (Fedorka-Cray et al., 1998; Kabagambe et al., 2000). We captured pigeons with walk-in baited traps during summer, whereas during the fall the majority of pigeons at dairy locations were sampled immediately after being shot by dairy managers. Although no difference in prevalence was detected between pigeons trapped with walk-in baited traps or shot ( $z=1.23$ ,  $P=0.220$ ), the methods were compared over two trapping periods, which confounds our interpretation of temporal variation.

Samples from pigeons and manure screened during the summer months from South Dairy consisted of similar serotypes, indicating that transmission from birds to cattle may be more likely to occur during the summer months. Although analogous serotypes were found in pigeon and environmental samples at East Dairy

during the fall trapping period, this correspondence is based on only two pigeons trapped during the fall ( $n=8$  during the summer).

Pigeons used as decoys in traps were tested before and after each trapping period to ensure that they would not contaminate other pigeons that entered the trap. Despite being exposed to contaminated pigeons that entered the trap, neither STEC nor *S. enterica* were detected in any of the pigeons used as decoys. This suggests that contamination does not readily occur between pigeons, and that the environment may be a more important source of contamination for pigeons.

The prevalence of both STEC and *S. enterica* that we detected may be lower than the true prevalence because each pigeon was sampled only once and the bacterial microorganisms are shed intermittently, which limits the sensitivity of fecal culture. Also, culture methods underestimate the true prevalence of bacterial shedding (Smith et al., 1994; Fedorka-Cray et al., 1998). The isolation strategy that we used to detect STEC was inappropriate for detecting STEC at low prevalence, further limiting the sensitivity of the method.

Our results indicate that pigeons are potential carriers of *S. enterica* and may amplify transmission of the bacteria to cattle. Because pigeons occupy small home ranges and there is little exchange between adjacent populations in close proximity (Sol and Senar, 1995), pigeons infected with *S. enterica* may represent more important sources of recirculation of these microbes within dairies than of distribution to neighboring dairies. Further supporting this hypothesis is the fact that *S. enterica* were detected only in pigeons trapped at dairy locations, suggesting that pigeons likely acquired the bacteria from the dairy.

Because pigeons were trapped primarily at one dairy location, the pigeons sampled may not be representative of all popula-

tions of pigeons at dairies. Even so, the few samples collected from the second dairy coincide with those collected at the first dairy. The data presented in this paper suggest that pigeons may play a role in the recirculation and persistence of *S. enterica* within dairies.

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