Farm and Management Variables Linked to Fecal Shedding of *Campylobacter* and *Salmonella* in Commercial Squab Production

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ABSTRACT A cross-sectional study was performed to determine the relationship of farm variables and management practices to fecal shedding of *Campylobacter* or *Salmonella* on commercial squab (young pigeon) farms. A detailed survey provided information on biosecurity, cleaning and disinfection, bird health, vector control, and loft and pen. Twenty pigeons on each of 12 farms were cultured before and after the producers completed a voluntary quality assurance training program (QAP), based on principles of hazard analysis critical control point (HACCP). The prevalence of positive samples for *Salmonella* and *C. jejuni* was 1/480 (0.21%) and 19/480 (3.96%), respectively. *Campylobacter* was present on one farm during both visits; three farms during the first visit, and three farms during the second visit. Analysis by fixed-effects logistic regression showed the probability of having a positive *C. jejuni* culture was increased by not using dry manure in the nesting material, not cleaning shipping crates, cleaning landing boards, and by increased frequency of chemical disinfection of water. Having a positive parent and higher numbers of squab per pen (density) were also associated with higher odds of being positive for *C. jejuni*. Factors not associated with a positive *C. jejuni* culture included, other avian species on the farm, type of shipping crate, covered drinkers, fly problems, bird age, level of nest box within the loft, and QAP training. Prevalence of food safety pathogens was extremely low on the squab facilities tested as compared with reports from commercial broiler or turkey flocks. This observation suggests that one or more farm variables or management practices were effectively reducing infection, or possibly a species-related difference existed in carriage rates and shedding of pathogens. These results emphasize critical control points for food safety pathogens may vary widely, and the formulation of effective QAP programs are dependent on science-based knowledge of diverse animal production systems.

(*Key words*: food safety, pigeon, squab, *Campylobacter jejuni*, *Salmonella*)

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INTRODUCTION

Campylobacter jejuni and *Salmonella* are common in commercially reared poultry flocks and are often associated with food borne illness in humans (Gast, 1997; Shane, 1997). Current regulatory efforts by the Food Safety Inspection Service (FSIS) are concentrated on implementing Hazard Analysis Critical Control Point (HACCP) systems for food safety at postharvest facilities, such as poultry processing plants (USDA, FSIS, 1996). In addition, there is considerable governmental discussion about extending regulatory, HACCP-type programs to the farm as the site where primary microbial infection of food animals occurs. In theory, management practices identified as on-farm critical control points for food borne pathogens could be modified to reduce the number of bacteria entering the processing (slaughter) facility (i.e., preharvest food safety). Many animal production commodities have established quality assurance programs (QAP) to address food safety and quality (Kla and Tollefson, 1999). In California, this strategy of preharvest food safety has been embraced by the commercial egg and meat poultry industries through voluntary adoption of QAP (Ernst, 1999). These programs were recently expanded to include training for squab and game bird producers. The majority of information in the QAP training modules is integral to modern husbandry practices for commercial chickens and turkeys. Training programs contain modules on HACCP principles, biosecurity, cleaning and disinfection, rodent and insect control, and record keeping (Ernst, 1999). This study was designed to document specific management practices within the squab industry as they existed prior

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Abbreviation Key: CE = competitive exclusion; HACCP = hazard analysis critical control point; QAP = quality assurance plan.

to the implementation of the QAP and to measure the pre-QAP and post-QAP prevalence of *Campylobacter* and *Salmonella* in squab and adult pigeons. In addition, we evaluated which management practices or farm variables were linked to an increased or reduced prevalence of *Campylobacter* and *Salmonella* infection in an effort to identify potential critical control points for modifying the occurrence of these pathogens.

MATERIALS AND METHODS

Study Population

Twelve farms participated in this project. All participants were members of a single cooperative and were asked to volunteer for the study by the manager. Farms were visited once before and once after completing the QAP training program. The first visits occurred in April and May, and the second visits between June and August. A detailed survey was completed at each visit. Survey questions addressed the following major areas: 1) Biosecurity—number of bird species on a farm, movement of birds and people on and off the farm, quarantine procedures, size and capacity of facility, marketing procedures, use of protective clothing, and foot baths; 2) cleaning and disinfection procedures—for lofts, nests, landing boards, water, drinkers, crates, and manure handling; 3) bird health—diet, nesting material, common illnesses, causes of mortality, mortality rates, culling practices, use of diagnostic facilities, veterinary care, disposal of mortality, use of therapeutic drugs, and housing; 4) vector control—fly, mosquito, and rodent control procedures; types of products used; and record keeping; and 5) loft information—number of pens, number of nest boxes per pen, number of birds per pen, floor type, and ages and sexes of birds sampled.

Microbiology

Five squab or adult pigeons (two parent-squab combinations plus an additional squab or adult pigeon) from four pens (n = 20 pigeons per farm) were cultured for *Campylobacter* and *Salmonella* on two occasions. Culture results were tracked for individual pigeons within the loft. Each fecal culture was obtained by cloacal swab, similar to previously reported techniques (Annan-Prah and Janc, 1988; Hood et al., 1988; Pearson et al., 1996; Pokamunski et al., 1986) using a sterile culturette.[2] Swabs were immediately rubbed onto CAMPY plates[3] and then inserted into the culturette transport media. The CAMPY plates were streaked for isolation using sterile sticks within 2 h of the initial plating, placed into a microaerophilic chamber with a positive control culture, and transported to the laboratory. *Campylobacter* cultures were inspected for growth after 2 d of incubation at 41 C. For *Salmonella* cultures, the sterile culturettes were placed into selenite broth[3] and incubated for 18 to 24 h at 37 C and then streaked onto xylose lysine desoxycholate agar[3] and Hektoen agar.[3] The identities of suspect colonies were confirmed using standard biochemical tests and procedures for *Campylobacter jejuni* (Quinn et al., 1990) and *Salmonella spp.* (FDA, 1995).

Statistical Analysis

Variables were initially screened for significance with Fischer's exact test on a 2×2 table design (Epi Info, 1994). Variables with probabilities $P > 0.1$ were not tested further until a final model was constructed. The association between the each of the remaining host factors (e.g., age, sex) or management practices (e.g., water disinfection) and the probability of cloacal shedding of *C. jejuni* were tested using exact logistic regression (Hirji et al., 1989; Mehta and Patel, 1996). A forward-stepping algorithm was used, with P value of ≤ 0.10 for inclusion of the term in the model. The exact conditional scores test was performed for each factor in order to test the null hypothesis that all regression coefficients were simultaneously zero (odds ratio = 1) for the specified term (Hirji et al., 1989; Mehta and Patel, 1996). Once the final model was constructed, we retested each term to reaffirm its level of significance given the inclusion of all the other terms in the model. Nonsignificant terms (variables with probabilities $P > 0.1$) were also retested to reaffirm their nonsignificance, using the exact conditional scores test to estimate P values.

RESULTS

The size of the breeding flock on a farm ranged from 500 to 2,500 pairs, with a mean of 1,053 pairs. Five farms reported being at maximum capacity, whereas seven farms were in the process of expanding their flocks. The number of breeding pairs in a pen ranged from 5 to 30, with a mean of 15 pairs. The number of squabs shipped to market annually averaged 12,350 for the 12 farms. Nine farms had been raising squab for >5 yr and three for <5 yr. Other avian species on squab farms (number of farms) included doves (2), chickens (6), ducks (2), pheasant (1), geese, (1) lovebirds (1), finches (1), and parrots (2). Other animal species on farms included cats (10), dogs (11), horses (3), goats (1), and sheep (1).

A total of 480 squabs and pigeons was cultured on 12 farms. Only one sample (0.21%) was positive for *Salmonella spp.*, and 19/480 (3.96%) were positive for *C. jejuni*. No other species of *Campylobacter* were identified. Three farms were positive for *C. jejuni* on the first visit (one or more positive birds, n = 20), and three farms were positive on the second visit; one farm was positive on both visits (Table 1). On farms positive for *C. jejuni*, the number of positive birds per farm (4 pens × 5 swabs per pen = 20) ranged from one to five. The number of positive birds per pen (n = 5) ranged from one to three (Table 1). Given

[2]Product no. 4360210, Becton Dickinson & Co., Cockeysville, MD 21030.

[3]Product no. 110122, Remel, Inc., Lenexa, KS 66215.

TABLE 1. Prevalence of positive cloacal swabs for *Campylobacter jejuni* on 12 California farms before and after quality assurance program (QAP) training

Farm	Prevalence of *C. jejuni* Prior to QAP	After QAP	Two-sided Fisher's exact test[1]
1	3/17 (15%)	0/20 (0%)	0.23
2	0/20 (0%)	0/20 (0%)	1.0
3	0/20 (0%)	0/20 (0%)	1.0
4	0/20 (0%)	0/20 (0%)	1.0
5	0/20 (0%)	0/20 (0%)	1.0
6	0/20 (0%)	0/20 (0%)	1.0
7	0/20 (0%)	2/20 (10%)	0.49
8	0/20 (0%)	0/20 (0%)	1.0
9	0/20 (0%)	0/20 (0%)	1.0
10	2/20 (10%)	0/20 (0%)	0.49
11	5/20 (25%)	4/20 (20%)	1.0
12	0/20 (0%)	3/20 (15%)	0.23
Total	10/240 (4.2%)	9/240 (3.8%)	1.0

[1]Evaluated whether the prevalence of *C. jejuni* was statistically different after QAP compared with before QAP.

that the overall prevalence of *C. jejuni* shedding was 4.0%, the likelihood of a farm having 4/20 or 5/20 positive birds was 0.006 or 0.0009, respectively. Similarly, the probability of a pen having 2/5 or 3/5 positive birds was 0.014 or 0.0006, respectively. These unlikely outcomes for farm-level and pen-level prevalence of positive cultures suggest that the prevalence of shedding was much higher for some of these farms and pens compared with others.

Farm practices associated with higher odds of *C. jejuni* isolation from squab and adult pigeons included not using manure as a component of nesting material, not cleaning shipping crates upon return from the slaughter facilities, and an increased frequency of chemical disinfection of water (Table 2). At the pen level, cleaning of landing boards and higher numbers of squab per pen were associated with more *C. jejuni*-positive birds. Also, the odds of shedding *C. jejuni* was 28 times higher for squabs reared by an infected parent compared with a squab reared by a noninfected parent (exact conditional score test on 1 df = 38.0, $P = 0.0002$; 90% confidence interval, 5.9 to 140). Management factors that were not associated with *C. jejuni* shedding ($P > 0.1$) included the presence of other animal or avian species on the farm, bird age, level of the nest box within the pen, type of shipping crate used, rodent control program, perceived degree of fly problems, and QAP training. Variables not associated with the probability of *C. jejuni* isolation cannot be considered critical control points for HACCP-type programs for food safety. No farms used disinfectant foot baths between pens, nor was protective clothing (boot covers, rubber boots, hairnets, gloves) worn for entering the bird-rearing area.

DISCUSSION

Biosecurity measures, such as limiting traffic of people and vehicles onto farms and especially into the pen areas, were widely practiced. In contrast, on the farm, foot traffic occurred between pigeon pens and between pigeon pens and areas occupied by different animal species. Not cleaning shipping crates was significantly associated with higher odds of a positive *C. jejuni* isolation. This relationship may reflect a potential for the transmission of contaminated feces from producer to producer and back to the farm at the processing plant, as a result of cross-contamination among crated squabs. Alternatively, routine cleaning of the shipping crates may be linked to some unmeasured variable that reduced *C. jejuni* isolations.

Rapid transmission of *C. jejuni* to negative pen mates by infected chicks has been repeatedly demonstrated (Clark and Bueschkens, 1988; Kazwala et al., 1990; Shanker et al., 1990). We expected that extensive exposure of pigeons to fecal material would increase the odds of positive bacterial cultures within a loft. We documented the level of the nest box that was home to the sampled pigeons in

TABLE 2. Prevalence of and final fixed-effects logistic regression model for management factors associated with cloacal shedding of *Campylobacter jejuni* in California squab and pigeons

Factor	Prevalence of *C. jejuni*	Adjusted odds ratio (90% CI)[1]	Exact conditional scores test
Nesting material			<0.001[2]
Nonmanure materials	19/320 (5.9%)	1.0[3] —	
Manure +/− pine needles	0/160 (0.0%)	0.02[4] (0.0, 0.15)	
Cleaning the landing board			<0.001
Never cleaned	10/420 (2.4%)	1.0 —	
Cleaned	9/60 (15.0%)	7.7 (2.6, 25.4)	
Cleaning shipping crates			0.02
Never cleaned	3/100 (3.0%)	1.0 —	
Cleaned 1 to 4/yr	2/160 (1.3%)	0.1 (0.01, 0.7)	
Cleaned more than 4/yr	14/220 (6.4%)	0.2 (0.05, 1.1)	
Frequency of water disinfection[5]	. . .	1.03 (1.002,1.05)	0.06
Total number of squab per pen[5]	. . .	1.03 (1.002,1.06)	0.06

[1]CI = Confidence interval.

[2]Tests the null hypothesis that all logistic regression coefficients are simultaneously zero (odds ratios = 1) for the specified term with the other two terms in the model, using the exact conditional scores test due to sparse data and a zero cell.

[3]Referent category for the odds of shedding *C jejuni*.

[4]Median unbiased estimate for odds ratio due to zero numerator.

[5]Frequency of water disinfection and total number of squab per pen were modeled as continuous variables.

our study because we further hypothesized that pigeons closer to the floor of the loft (level 1) would have a higher prevalence of bacteria. We found no significant differences in positive isolation rates at any level. This result may be because although each mated pair claims a particular box for nesting, there is considerable mixing of adult birds in the loft while feeding, drinking, and on the common floor. Management factors aimed at limiting the transmission of pathogens within a farm were rare. Typically there was a minimal barrier of wire that allowed the movement of aerosols and dust between pens. The water continuously flowed from pen to pen within a row. Finally, the squab producers moved from pen to pen in the same footwear on a daily basis. This procedure would tend to promote transmission of *C. jejuni* among pens on a facility as has been reported among houses on broiler farms (Genigeorgis et al., 1986; Stern, 1992). However, our failure to identify *C. jejuni* from the majority of birds within a pen, pens within a farm, or from the same farms on repeat visits in four of six cases conflicts with that assumption.

We found no age association between positive shedding and being an adult, juvenile, or squab pigeon. However, having a parent that was positive for *C. jejuni* greatly increased the odds of being positive as a squab, which may imply transmission from positive birds in intimate contact, either by fecal-oral route or possibly from the crop of the parent to the oral cavity of the squab.

A lower probability of having a positive culture for *C. jejuni* was associated with the presence of manure in the loft and inclusion of manure as part of the nesting material. If we speculate that manure was providing protection against *C. jejuni* infection, this conclusion would be consistent with the principles of competitive exclusion (CE) of pathogenic bacteria from the intestine by normal intestinal flora (see Stavric and D'Aoust, 1993, for review). Competitive exclusion has been successfully demonstrated in gallinaceous poultry. Feeding fecal material from healthy adult chickens has protected susceptible chicks against challenge by pathogenic bacteria such as *Salmonella spp.*, *Escherichia coli*, and *Clostridia*. Against *C. jejuni*, however, the efficacy of CE products for preventing colonization in chicks has been mixed (Stern, 1992, 1994; Schoeni and Wong, 1994; Morishita et al., 1997; Stern, et al., 1988). Also compatible with the CE theory is our finding that more frequent chemical disinfection of water was associated with higher odds of a positive isolation of *C. jejuni*. Water treatment with sodium hypochlorite has the potential to alter the electrolyte balance of birds, induce local buffering within the intestinal lumen (Leeson et al., 1995), and might have influenced *C. jejuni* infection or shed, with subsequent transmission to squabs. Factors related to physiological stress, such as higher numbers of squab in a pen due to a high stocking density of breeders, or a high production level in the breeding pairs, might also have caused a change in susceptibility or shed of *C. jejuni*. Disturbance of the pigeons during cleaning of the landing boards near the nests and physical disruption of the manure itself (cleaning landing boards) might have caused increased exposure to pathogens among pigeons in a loft.

By completing the QAP training, producers gained proficiency on how to document and critically evaluate their management procedures. Changes in management practices or factors related to the prevalence of food safety pathogens before and after QAP training were not disclosed by our comprehensive survey. Additionally, we documented only the use of management practices, such as rodent control, but not their efficacy in this descriptive survey (e.g., an increase in rodent kills). Management practices presented to squab producers during QAP training that are common to commercial chicken and turkey production, such as all-in-all out management, rearing only one species of animal on a farm, continuous chlorination of drinking water, and total clean-out followed by extensive cleaning and disinfection, were not found on squab farms. We do not know what consequence the adoption of these elements of HACCP-based QAP programs designed for commercial chicken and turkey production may have on the prevalence of food safety pathogens on squab farms. Given the low prevalence of *C. jejuni* and *Salmonella* on squab farms, we conclude that broad application of QAP programs across species may not be appropriate in all cases. These results suggest that making a critical assessment (gathering science-based data) of the factors that influence the prevalence of pathogens is an important step in formulating on farm HACCP-type programs intended to reduce food borne pathogens.

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