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Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada

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Abstract

We conducted an observational study to estimate prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in poultry. Eighty-one broiler chicken and 59 turkey flocks selected among flocks slaughtered in the province of Quebec, Canada, were included in the study. Flock status was evaluated by culturing pooled caecal contents from about 30 birds per flock. Exposure to potential risk factors was evaluated with a questionnaire. Odds ratios were computed using multivariable logistic regression.

The prevalence of *Salmonella*-positive flocks was 50% (95% CI: 37, 64) for chickens and 54% (95% CI: 39, 70) for turkeys, respectively. Odds of *Salmonella* colonization were 2.6 times greater for chicken flocks which failed to lock the chicken house permanently. In turkeys, odds of *Salmonella* colonization were 4.8–7.7 times greater for flocks which failed to be raised by $\leq$2 producers with no other visitors allowed onto the premises, or origin from a hatchery.

The prevalence of *Campylobacter*-positive flocks was 35% (95% CI: 22, 49) for chickens and 46% (95% CI: 30, 62) for turkeys. Odds of colonization were 4.1 times higher for chicken flocks raised on farms with professional rodent control and 5.2 times higher for flocks with manure heap $>$200 m from the poultry house, and also increased with the number of birds raised per year on the farm and with the age at slaughter. For turkeys, odds of *Campylobacter* flock colonization were 3.2 times greater in flocks having a manure heap at $\leq$200 m from poultry house and 4.2 times greater in flocks drinking unchlorinated water.

Keywords: *Campylobacter*; *Salmonella*; Risk factors; Prevalence; Turkey; Chicken; Canada

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1. Introduction

Salmonella spp. and *Campylobacter* spp. are two of the most important food-borne zoonotic pathogens (Allos, 2001; Schlundt et al., 2004). There is evidence that poultry products are one of the most important source of human infection for both organisms (Corry and Atabay, 2001; Hald et al., 2004; Schlundt et al., 2004).

Contamination of poultry carcasses with *Salmonella* or *Campylobacter* seems to be mostly linked to flock contamination during rearing and/or transportation to slaughter (McGarr et al., 1980; Rigby et al., 1980, 1982; Corry et al., 2002; Heyndrickx et al., 2002; Berndtson et al., 1996a; Rivoal et al., 1999). Risk factors for flock colonization by *Salmonella* include season, hatchery of origin, feedmills and various hygienic measures (Renwick et al., 1992; Angen et al., 1996; Rose et al., 1999; Skov et al., 1999; Cardinale et al., 2004b). For *Campylobacter*, several pathways have been suggested to explain flock colonization during rearing, including vertical transmission, contamination from previous flock and exposure to potential sources of the bacterium such as other animals on the farm, insects, rodents, environment, litter and drinking water (Lindblom and Kaijser, 1986; Annan-Prah and Janc, 1988; Pearson et al., 1993; Gregory et al., 1997; Petersen and Wedderkopp, 2001; Hiett et al., 2002a,b; Cardinale et al., 2004a). However, most of the studies were conducted on chicken flocks; little information is available on turkey flocks.

Our objectives were to estimate the prevalence of *Salmonella* and *Campylobacter* colonizations in chicken and turkey flocks slaughtered in Quebec. Potential risk factors for colonization were also studied.

2. Material and methods

2.1. Slaughterhouse selection

From April 24, 2003 to February 23, 2004, chickens were sampled in the four largest commercial processing plants in Quebec. Each plant was visited once during each 4-week period, in a random order (using a random-number generator). For practical follow-up reasons, sampling was usually done on Thursday for the first half of the study, and on Tuesday for the second half. During the same period, turkeys were sampled every week in the only commercial processing plant in Quebec. Sampling was planned for Tuesday, but if not enough flocks were available it was postponed to the following Wednesday or Thursday. The target sample size was fixed at 80 flocks for both chickens and turkeys, calculated to estimate the 95% confidence interval (CI) of colonization prevalence with a maximal error of 10%, assuming a prevalence of 30%.

2.2. Flock selection

At each visit to the slaughterhouses, two flocks located in different production sites (i.e. farm address) were selected whenever possible. A flock was defined as a group of birds from the same hatchery raised in a broiler house during the same period of time. Only one flock raised per production site was allowed for selection in the study. Producers were selected among those known to be shipping broiler-chicken, broiler-turkey, or heavy-turkey flocks to slaughter on the sampling day, with slaughtering of the flock planned between 7:00 and 14:00. Before sampling, producers were asked if they agreed to participate to the study, which involved filling out a

questionnaire and permission to access carcass condemnation and transportation data. If only one pair of two consecutives flocks was available for selection for a sampling day, these two flocks were selected. Otherwise, the first two flocks for which producers agree to participate were selected.

2.3. Bird selection

If broilers from the same flock were brought to slaughter in more than one truck, selection was restricted whenever possible to one randomly selected truck. A systematic sampling method was used for selection, with a target sample size of 30 birds per flock. Birds were selected after the evisceration procedure at a 45–60-s interval; the interval was determined according to number of chickens in the truck to evenly distribute selection within the truck. Sampling was started when the second hand of the slaughterhouse clock reach the zero following the beginning of flock evisceration. If target sample size was not reached after sampling one truck, the following one was sampled whenever possible. For heavy turkeys, sampling was generally distributed over two trucks due to the lower speed of the processing line and the lower number of birds per truck.

2.4. Questionnaire

A questionnaire related to husbandry practices was sent to the person in charge of the flock within 2 days following slaughter. If the questionnaire was not completed and sent back within a 3-week period, weekly phone calls were made until the questionnaire was returned. Questionnaires were reviewed at time of reception. If needed, producers were contacted by phone for any conflicting or missing information. Because this study was part of a broader project for which the questionnaire was built, only questions pertinent to the present study were considered. Most of these questions were checklist, two-choice, or multiple-choice questions, with the exception of questions relative to dates or precise numbers (e.g. number of chickens raised, age at slaughter) which were open questions. Pilot testing of our questionnaire was done by asking one poultry producer, one technical advisor working with poultry producers, and one representative of the Quebec poultry industry to complete the questionnaire and then to comment about the clarity of our questions, any potential difficulties they had in answering them, and the time required to fill it out.

2.5. Bacteriology

After evisceration, intestines from selected birds were placed into individual sterile plastic bags and put on melting ice for a maximum of 8 h prior to culture. For each flock, three pools including caecal content of ~10 birds each were created. Feces from each pool were collected from one caecum of each bird using a sterile cotton swab, put in a sterile stomacher bag and gently manually homogenized. For *Salmonella*, the pooled caecal sample was mixed with 25-ml of buffered peptone water (20 g/L, Difco) until homogenization, and isolation was done as previously described (Arsenault et al., in press). For *Campylobacter*, due to unexpected technical problems, one ml of each pool was frozen in a sterile tube at −70 °C for a maximum of 8 months prior to culture for the first 23 chicken flocks and the first 13 turkey flocks sampled. Pooled caecal content was mixed with 7-ml of sterile PBS until homogenization, and isolation of *Campylobacter* was then done as previously described (Arsenault et al., in press).

2.6. Statistical analysis

The unit of observation was the flock. For prevalence and risk-factor analysis, a flock was considered colonized for a bacterium if at least one of the three pooled caecal samples was positive at bacteriology, and as not colonized otherwise. Prevalence of flock colonization with 95% CI was computed in SAS version 9.1, with the number of broiler houses on the farm as sampling weights to account for the sampling design.

Risk-factor analysis was done using flock colonization status as the dependant variable. We built four multiple-logistic regression models: one for each combination of bacterium and bird species. We limited our study to factors that were previously described in the literature as potential risk factor for the introduction of *Salmonella* or *Campylobacter* in poultry flocks, and for which information was available from the questionnaire. For categorical risk factors, the number of categories by variables limited to ensure category frequencies of $>10\%$ whenever possible. For continuous variables, the assumption of linearity of logit was first evaluated by estimating a simple logistic regression including the variable categorized, and plotting the log odds. In the case of departure from the linearity assumption, variables were dichotomised with the rounded-off median as the cutoff. Following exploratory analysis, all variables related to the presence of visitors (other than producers or employees) during rearing and biosecurity measures taken by those visitors (wearing clean coveralls, treatment of boots, hand washing, visit to other broiler houses, visit order relative to the age of birds) were excluded from modelling and a new variable was created: ''Number and type of person entering the broiler house during rearing''. In fact, each specific biosecurity measure taken by visitors was first put into three categories relative to their implementation (yes, no, no visitors), and were seen to be strongly associated with the presence of visitors. Moreover, none of the biosecurity measures taken by visitors was significantly associated with flock status in exploratory analysis restricted to flocks having visitors (all $P \geq 0.12$).

All variables were first tested using chi-square tests with exact computation (categorical variables) or simple logistic regression (continuous variables) performed in SAS 9.1. Only factors associated ($P < 0.20$) with flock colonization status were considered for modeling. Among selected variables ($P < 0.20$, χ^2 test), all bilateral relationships were evaluated using chi-square tests to evaluate the potential presence of collinearity. Multiple logistic regression models were built using a backward-elimination procedure based on the Wald test, using $P > 0.05$ as criterion of elimination. Variables were only removed if they did not affect coefficients of other variables included in the model by $>30\%$. During the process of model selection, only observations with no missing values for variables included in the fitted model were considered. Interactions were not tested due to paucity of data. For final models including only categorical explanatory variables, goodness-of-fit of the final model was assessed using the Hosmer and Lemeshow test. As a final step for models on *Campylobacter* colonization, condition of samples at time of culture (fresh, frozen) was tested into the final models to evaluate statistical significance and impact on other coefficients.

3. Results and discussion

3.1. Selection of flocks and birds

For chickens, 121 producers were selected for inclusion in the study; 104 agreed, 10 were not reached and 7 refused to participate. A total of 82 flocks were sampled, but caecal samples from

one flock for *Salmonella* and from another one for *Campylobacter* were lost in bacteriology, resulting in a sample size of 81 flocks. All these flocks were raised in the province of Quebec. Absence of sampling for the remaining available flocks was due to technical reasons (for example, snowstorm or last-minute changes in the slaughterhouse schedule). All questionnaires sent to producers were retrieved.

For turkeys, 83 producers were initially available for the study; 71 agreed, 6 were not reached and 6 refused to participate. A total of 60 turkey flocks were sampled, but one producer did not return his questionnaire and the caecal samples from another flock could not be cultured for *Campylobacter*, resulting in a sample size of 59 and 58 turkey flocks for the study of *Salmonella* and *Campylobacter*, respectively. Flocks originated from the province of Quebec, except for five which were from the provinces of New Brunswick or Nova Scotia, Canada.

The mean and standard deviation of the total number of birds included in the three pools of caecal content for each flock was 28 ± 3.1 for chickens and 29 ± 2.3 for turkeys.

3.2. Methodological issues

We used a convenience sample of flocks (in that our visit days and the plants were fixed), but these five plants slaughtered ~77% of the total number of commercial broiler chickens produced in Quebec and 94% of commercial turkeys. It seems unlikely that restriction of sampling to a weekday, based solely on practical considerations, biased our results. Producers were very compliant, further limiting any selection bias. We, therefore, believe that our sample is representative of commercial chicken and turkey flocks slaughtered in Quebec during the studied period.

Caecal contents were used to evaluate flock status, which has been reported to provide the best sensitivity for *Salmonella* presence in the intestinal tracts of both chickens and turkeys (Brownell et al., 1969; Fanelli et al., 1971; Snoeyenbos et al., 1982; Barrow et al., 1988; Xu et al., 1988), and to harbour the highest number of *Campylobacter* in colonized chickens and turkeys (Wallace et al., 1997, 1998; Achen et al., 1998). Use of pooled samples, including a mixture of individual samples with different bacteriological status, might lead to a reduction in culture sensitivity. However, at least for *Campylobacter* isolation, this bias seems unlikely because in most studies on commercial chicken or turkey flocks, most of birds were reported as intestinal carriers at time of slaughter (Prescott and Gellner, 1984; Smitherman et al., 1984; Lindblom and Kaijser, 1986; Shane, 1992; Jacobs-Reitsma et al., 1995; Berndtson et al., 1996b; Gregory et al., 1997; Evans and Sayers, 2000; Shreeve et al., 2000; Smith et al., 2004).

Risk factors we considered are presented in Tables 1 and 2. No evidence of confounding was seen between variables excluded from the models and those included. In both chicken and turkey final models for *Campylobacter*, the addition of an indicator variable for freezing of samples (yes, no) had minor impact (<5%) on the estimates of other variables and this variable was not statistically significant ($P > 0.61$). Many risk factors were tested relative to the sample size. This could have led us to offer spuriously associated variables to the model. Risk factors selected in final models were interpreted as being associated with bacterium introduction within the flocks; however, we cannot exclude the possibility those risk factors were selected because of an influence on within-flock prevalence, through their impact on colonization detection.

Table 1
Descriptive statistics of variables used in a risk factor study on *Salmonella* and *Campylobacter* prevalences in Quebec, Canada, 2003–2004 (81 chicken and 59 turkey flocks)

Chickens					Turkeys				
Level	*Salmonella*		*Campylobacter*		Level	*Salmonella*		*Campylobacter*	
	n	%+[a]	*n*	%+[b]		*n*	%+[a]	*N*	%+[b]
Generalities									
Age at slaughter (days)									
≤40	33	52	N/A[c]		<90	33	48	33	42
>40	48	52			≥90	26	58	25	52
Hatchery of origin									
Others	21	57	21	24	Others	8	75[d]	8	63
A	29	41	29	28	A	28	32	28	50
B	20	50	20	35	B	23	70	22	36
C	11	73	11	18					
Poultry house									
Number of birds raised per year on the farm, including all poultry houses of the address									
≤300,000	42	48	N/A[c]		≤100,000	32	53	N/A[c]	
>300,000	39	56			>100,000	27	52		
Number of birds in the poultry house									
≤18,000	42	62[d]	43	28	≤5,000	32	38[d]	31	52
>18,000	39	43	38	26	>5,000	27	70	27	41
Number of years since the construction or last major renovation of poultry house									
<5	17	65	17	29	<5	11	55	11	45
5–20	33	52	34	29	5–20	24	54	23	43
>20	31	45	30	23	>20	24	50	24	50
Transfer of turkeys during grow out to other pen(s) where chickens were initially raised with keeping of the same litter									
Yes	Not applicable				Yes	20	60	20	45
No					No	39	49	38	47
Cleaning and disinfection									
Poultry-house washing and disinfection before placement									
Disinfection	52	46	51	24	Disinfection	34	47	33	42
Washing only	6	67	6	33	Washing only	9	67	9	56
None	23	61	24	33	None	16	56	16	50

Table 1 (*Continued*)

Chickens					Turkeys				
Level	*Salmonella*		*Campylobacter*		Level	*Salmonella*		*Campylobacter*	
	n	%+[a]	*n*	%+[b]		*n*	%+[a]	*N*	%+[b]
Fumigation of poultry house with formaldehyde and potassium permanganate before placement									
No	73	51	73	25	No	53	55	52	50
Yes	8	63	8	50	Yes	6	33	6	17
Vermin control									
Visual detection of darkling beetles by producers during rearing of the lot									
No	58	50	58	24	No	39	56	38	40[d]
Yes	23	57	23	35	Yes	20	45	20	60
Type of rodent control during rearing									
Professional	36	56	37	41[d]	Professional	28	61	27	37[d]
None/home type	45	49	44	16	None/home type	31	45	31	55
Efficiency of fly control during rearing according to the producer[e]									
Excellent	71	54	71	30	Excellent	44	53	44	41
Average/inadequate	10	40	10	10	Average/inadequate	14	50	14	64
Biosecurity									
Presence of animal species other than poultry (cattle, sheep, goats, horses and/or pigs) on the farm									
Yes	11	36	10	10	Yes	11	45	11	55
No	70	54	71	30	No	48	54	47	45
Distance between poultry house and the nearest manure heap (m)									
≤200	27	44	27	11[d]	≤200	26	54	25	64[d]
>200	54	56	54	35	>200	33	52	33	33
Permanent locking of poultry house									
No	47	62[d]	46	24	No	30	50	30	53
Yes	34	38	35	31	Yes	29	55	28	39
Treatment of producers' boots before entrance in poultry house									
Washing/foot bath	14	43	14	29	Washing/foot bath	15	53	15	53
Single use cover boot	30	47	31	26	Single use cover boot	17	59	17	35
No treatment	37	59	36	28	No treatment	27	48	26	50

Hand washing by producer(s) before entrance in poultry house									
No	58	53	58	28	No	43	51	42	41[d]
Yes	23	48	23	26	Yes	16	56	16	63
Number and type of persons (producers, visitors) entering in the poultry house during rearing									
$\leq$2 producers, no visitors	23	65	23	22	$\leq$2 producers, no visitors	16	19[d]	16	31[d]
$\leq$2 producers, with visitors	43	44	43	30	$\leq$2 producers, with visitors	26	69	25	44
>2 producers $\pm$ visitors	15	53	15	27	>2 producers $\pm$ visitors	17	59	17	65
Feeding and watering									
Feed mill									
Others	53	49	53	23[d]	Others	37	51	36	39[d]
B	7	86	7	14	A	9	33	9	78
D	14	50	14	50	B	7	57	7	71
E	7	43	7	29	C	6	83	6	17
Texture of feed[e]									
Pellet	66	50	65	26	Pellet	57	53	56	46
Meal	14	64	15	33	Meal	2	50	2	50
Poultry-house water source									
Aqueduct	26	50	26	42[d]	Aqueduct	17	53	16	44
Deep well	44	48	44	20	Deep well	31	55	31	42
Surface well	11	73	11	18	Surface well	11	45	11	64
Chlorination of drinking water									
No	19	63	19	16	No	27	48	27	59[d]
Yes	62	48	62	31	Yes	32	56	31	36
Addition of acetic acid to drinking water									
No	68	53	68	28	No	44	48	43	47
Yes	13	46	13	23	Yes	15	67	15	47

[a] % of *Salmonella*-positive flocks.
[b] % of *Campylobacter*-positive flocks.
[c] Not applicable since tested as continuous variable.
[d] $P \leq 0.20$ in univariable analysis (χ^2 test with exact computation for the whole variable).
[e] Missing values were present.

Table 2
Descriptive statistics of continuous variables used in a risk factor study on *Campylobacter* prevalence in Quebec, Canada, 2003–004

Variables	Chickens			Turkeys		
	n	Range	Median	*n*	Range	Median
Age at slaughter (days)	81	36, 45	41	–	–	–
Ten-thousands of birds raised per year on the farm, including all poultry houses of the address	80	4.7, 189	29	58	0.4, 570	9

3.3. Salmonella

3.3.1. Prevalence

Prevalence of *Salmonella* spp. positive chicken flocks was 50% (95% CI: 37, 64). This is very similar to results of a prevalence survey conducted in 1989–90, in which 65% of chicken flocks sampled in Quebec were positive to *Salmonella* according to cultures of floor litter (Renwick et al., 1992). In the province of Ontario 18% of chicken flocks were found to have intestinal carriers of *Salmonella* (Prescott and Gellner, 1984), but this prevalence might have been underestimated due to the limitation of the sample size to only 10 birds per flock. Various estimates of *Salmonella* flock colonization has been reported in other countries, with 13% (Skov et al., 1999) in Denmark, 27% in the Netherlands (Jacobs-Reitsma et al., 1994) and 70% in France (Rose et al., 1999). Comparison of these prevalence estimates is not straightforward due to differences in sampling methods and flock characteristics.

In turkeys, the prevalence of *Salmonella*-positive flocks was 54% (95% CI: 39, 70), which is similar to the one found in chickens. A previous Canadian study reported a prevalence of positive turkey flocks according to environmental samples of 86.7% (Irwin et al., 1994). However, the bacterium might be present in the poultry-house environment without being detected in the birds' feces (Heyndrickx et al., 2002).

3.3.2. Risk factors

In chickens, only the permanent locking of broiler house was associated with a reduced risk of colonization in chickens (Table 3). This variable is likely to represent a surrogate variable for the quality of biosecurity measures implemented by producers. Good hygienic barriers reduce the risk of *Salmonella* colonization in breeders flock (Henken et al., 1992).

In turkeys, flocks with two persons or less taking care of the birds and with no visitor entering the poultry house during rearing were at lesser risk of *Salmonella* colonization (Table 3). The relative contribution of producers versus visitors in the risk of *Salmonella* colonization could not be assessed due to limitation in the study sample size. Our results are in line with results of a study conducted in the Netherlands, in which more consultant visits in the poultry house increased the risk of *Salmonella enteritidis* colonization in broiler-breeder farms, whereas farm yard disinfection decreased the risk. The portable material that visitors might bring could also be a potential risk factor for the horizontal transmission of *Salmonella* (Heyndrickx et al., 2002).

Hatcheries were associated with *Salmonella* turkey flock colonization, as previously reported for chickens (Bhatia and McNabb, 1980; Lahellec and Colin, 1985; Angen et al., 1996; Chriél

Table 3
Final logistic-regression models for risk factors for *Salmonella* colonization in chicken and turkey flocks slaughtered in Quebec, Canada, 2003–2004

Variables	Odds ratio	
	Estimate	95% CI
Chicken flocks ($n = 81$)[a]		
Permanent locking of poultry house		
No	2.6	1.1, 6.5
Yes	1.0	
Turkey flocks ($n = 59$)[b]		
Number and type of persons entering in the poultry house during rearing		
>2 producers, ±visitors	7.7	1.5, 38.4
≤2 producers, with visitors	7.5	1.4, 41.8
≤2 producers, no visitors	1.0	
Hatchery of origin		
Others	5.1	0.7, 38.6
B	4.8	1.3, 17.9
A	1.0	

[a] Intercept = −0.001; model likelihood-ratio $\chi^2 = 4.4$, d.f. = 1, $P = 0.04$.
[b] Intercept = 0.18; model likelihood-ratio $\chi^2 = 17.7$, d.f. = 4, $P = 0.001$; Hosmer-and-Lemeshow goodness-of-fit test: $\chi^2 = 5.5$, d.f. = 5, $P = 0.36$.

et al., 1999; Skov et al., 1999). Vertical transmission of *Salmonella* has experimentally been demonstrated in chickens (Keller et al., 1995), and horizontal transmission in hatcheries has also been suggested (Angen et al., 1996; Christensen et al., 1997; Skov et al., 1999). In Canada, a previous study reported the presence of *Salmonella* in commercial chicken hatcheries in agreement with our findings (McGarr et al., 1980). Nevertheless, we cannot exclude that the hatchery effect only represents a geographical variation in the distribution of colonization flocks, because flocks supplied by a same hatchery tend to be clustered within space in Quebec.

Beetles can be infected by *Salmonella* (Skov et al., 2004), and according to molecular typing methods, they can be involved in the transmission of the bacterium between two consecutive broiler flocks, even in the presence of all-in all-out procedures (Skov et al., 2004). However, as in our study, others failed to find any association between beetle observation and *Salmonella* flock status (Angen et al., 1996; Chriél et al., 1999; Skov et al., 1999).

As in previous studies conducted in chickens, we found no association between *Salmonella* flock prevalent colonization and variables related to pest control, downtime period, manure disposal and poultry house cleaning and disinfection practices with the exception of one study that found that detergent use for cleaning decreased the risk (Renwick et al., 1992; Fris and Bos, 1995; Angen et al., 1996; Chriél et al., 1999; Skov et al., 1999; Cardinale et al., 2004b).

Feedmills were often reported as potential sources of *Salmonella* for poultry flocks (Jones et al., 1991; Henken et al., 1992; Angen et al., 1996; Hoover et al., 1997; Rose et al., 1999; Chadfield et al., 2001; Corry et al., 2002). In our study, there was no association between feedmills and flock colonization. However, many feedmills were present in the database, and for analysis purposes all those with a low frequency had to be grouped together. It is possible that this lowered the likelihood to find any significant differences. On the other hand, for 83% of chicken and 97% of turkey flocks, feed was pelleted. The heat treatment used in the pelleting process should have reduced the *Salmonella* feed contamination, as previously suggested (Bhatia et al.,

1979; Bhatia and McNabb, 1980; Jones et al., 1991; Veldmam et al., 1995; Rose et al., 1999; Jones and Richardson, 2004).

3.4. Campylobacter

3.4.1. Prevalence

We estimated at 35% (95% CI: 22, 49) the prevalence of *Campylobacter* positive chicken flocks. This is similar to the 47% prevalence reported in the province of Ontario, Canada (Prescott and Gellner, 1984), but lower than the 60% prevalence estimated in Quebec (Nadeau et al., 2002). Freezing of some samples in our study is likely to have reduced the isolation rate of *Campylobacter*, leading to an underestimation of the true prevalence, although the impact of freezing was not detected in statistical models. In France, Denmark, Finland, Norway and the Netherlands, prevalence estimates ranging from 18% to 82% were reported (Aho and Hirn, 1988; Kapperud et al., 1993; Jacobs-Reitsma et al., 1994; Hald et al., 2000; Wedderkopp et al., 2000; Heuer et al., 2001; Refrégier-Petton et al., 2001). Prevalence of *Campylobacter*-positive turkey flocks was 46% (95% CI: 30, 62), which is similar to that we observed in chickens.

3.4.2. Risk factors

For chickens, the age at slaughter was associated with an increase in the risk of prevalent colonization (Table 4), as previously reported (Berndtson et al., 1996c; Bouwknegt et al., 2004;

Table 4
Final logistic-regression models for risk factors for *Campylobacter* colonization in chicken and turkey flocks slaughtered in Quebec, Canada, 2003–2004

Variables	Odds ratio	
	Estimate	95% CI
Chicken ($n = 81$ flocks)[a]		
Age at slaughter (days)		
Continuous	1.4	1.1, 1.9
Number of birds raised per year on the farm, including all poultry houses at the address (by 10,000)		
Continuous	1.02	1.001, 1.03
Type of rodent control during rearing		
Professional	4.1	1.2, 14.3
None/home type	1.0	
Distance between poultry house and the nearest manure heap (m)		
>200	5.2	1.1, 24.1
≤200	1.0	
Turkey ($n = 58$ flocks)[b]		
Distance between poultry house and the nearest manure heap (m)		
≤200	4.2	1.3, 13.3
>200	1.0	
Chlorination of drinking water		
No	3.2	1.0, 10.2
Yes	1	

[a] Intercept = −16.2; model likelihood-ratio $\chi^2 = 21.9$, d.f. = 4, $P < 0.001$.
[b] Intercept = −0.02; model likelihood-ratio $\chi^2 = 9.6$, d.f. = 2, $P < 0.01$; Hosmer-and-Lemeshow goodness-of-fit test: $\chi^2 = 2.8$, d.f. = 2, $P = 0.25$.

Barrios et al., 2006). This could be either related to an increase in the risk of colonization with exposure time, or an increase in the probability of detecting the infection due to a within-flock increase in prevalence of *Campylobacter*-positive birds with time. Odds of colonization also increased with the number of birds raised on the farm, a variable highly correlated with the number of broiler houses on the site. An epidemiological study conducted in the Netherlands reported a similar observation, in which the presence of $\geq$5 broiler houses on the premises increased risk of colonization (Bouwknegt et al., 2004). Birds from other flocks raised in the same production site are likely to act as reservoirs of the bacterium, which can be transmitted between flocks by workers. Rodent, flies or wild birds can also be vectors of the bacterium (Annan-Prah and Janc, 1988; Berndtson et al., 1996b; Gregory et al., 1997; Hiett et al., 2002b).

In chicken flocks, professional rodent control was associated with an increase in the odds of *Campylobacter* colonization. We did not know the criterion used by producers for requesting professional services, but if those reasons were linked to the severity of rodent infestation, it could explain this association. Another hypothesis is that teams of exterminators spread the bacterium. On the other hand, we cannot exclude a residual confounding effect caused by size farm, since professional rodent control was more frequent in large broiler farms.

Presence of a manure heap at <200 m from the broiler house was significantly associated with a decreased risk of prevalent *Campylobacter* flock colonization in chicken flocks. The most likely explanation for this unexpected finding is the presence of a residual confounding effect with farm size, the distance between manure heap and broiler house being strongly and positively associated with the number of birds raised on the farms. Such an association between farm size and manure-heap distance was not seen for turkey flocks, for which the presence of manure heap at <200 m from the broiler house was a risk factor for *Campylobacter* colonization (Table 4). Manure is a potential reservoir of *Campylobacter* (Kelley et al., 1994), and manure disposal outside the farm was associated with a reduced risk of *Campylobacter* colonization in broiler-chicken flocks (Cardinale et al., 2004a). To our knowledge, turkey flocks did not have direct contact with the manure heap; mechanical carriers such as rodents, wild birds, water, flies or people might however have brought the infectious agent from the manure heap to the flock.

In turkeys, drinking-water chlorination was associated with a reduced risk of colonization. Water can be a persistent source of the bacterium on a broiler farm (Pearson et al., 1993) and providing undisinfected water was a risk factor for broiler-flock colonization with *Campylobacter* (Kapperud et al., 1993). We should point out that 66% of flocks using chlorination were located in the same administrative region, and thus we cannot exclude that this variable was only an indicator of some spatial clustering of *Campylobacter* positive.

Many studies reported a reduction in risk of *Campylobacter* flock colonization if specific hygienic measures were taken by people entering the broiler house (van de Giessen et al., 1996; Evans and Sayers, 2000; Hald et al., 2000; Gibbens et al., 2001; Smith et al., 2004). In our study, none of the factors relative to specific hygienic measures taken by producers or visitors was associated with flock colonization. Because our questionnaire did not specify frequency of use or quality of hygienic measures taken by producers, a large discrepancy might have been present in the way producers applied those measures, with some methods being effective and others less so, reducing the likelihood of finding any significant association.

4. Conclusion

Prevalence of *Salmonella*-positive chicken and turkey flocks slaughtered in the province of Quebec, Canada, was estimated at 50% and 54%, respectively, whereas prevalence estimates of

Campylobacter-positive flocks were 35% in chickens and 46% in turkeys. Risk factors for colonization differed between turkey and chicken flocks. Such differences could be related perhaps to age at slaughter, flock management, and relative exposure to potential reservoirs.

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