

Epidemiological investigation of risk factors for campylobacter colonization in Norwegian broiler flocks

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SUMMARY

An epidemiological investigation was conducted to identify risk factors related to hygiene and husbandry practices which determine the introduction of *Campylobacter* spp. into broiler chicken flocks. All 176 broiler farms in an area in southeastern Norway participated in the study. Each farm was represented by one flock selected at random during a one-year period. The flocks were examined for campylobacter colonization at slaughter, and the flock managers were subsequently interviewed about hygiene and husbandry practices. *Campylobacter* spp. were recovered from 32 (18%) of the flocks. The proportion of colonized flocks varied geographically and seasonally with a peak in the autumn. The following variables were found to be independently associated with an increased risk of campylobacter colonization using logistic regression analysis: (i) feeding the broilers undisinfected water (odds ratio (OR) = 3.42, $P = 0.045$), (ii) tending other poultry prior to entering the broiler house (OR = 6.43, $P = 0.007$), (iii) tending pigs before entering the house (OR = 4.86, $P = 0.037$), (iv) geographic region (Hedmark versus Østfold county) (OR = 2.91, $P = 0.023$), (v) season (autumn versus other seasons) (OR = 3.43, $P = 0.008$). Presence of rats on the farm was associated with an increased risk, but this factor did not reach statistical significance (OR = 3.96, $P = 0.083$). Preventive measures should include disinfection of drinking water and strict hygienic routines when the farm workers enter the rearing room. The results indicate that disinfection of drinking water is the preventive measure most likely to have the greatest impact on the prevalence of campylobacter among broiler chicken flocks in the study area (population attributable fraction = 0.53).

INTRODUCTION

In many countries, poultry production has become increasingly specialized and integrated in an industry based on intensive production systems. Successful

disease control has been a necessary corollary to the efficiency of such systems. Poultry are, nevertheless, recognized as asymptomatic carriers of several important human pathogens, including salmonella and campylobacter. Thermo-tolerant campylobacter bacteria (*Campylobacter jejuni* and *Campylobacter coli*) are among the most common causes of diarrhoeal illness in humans in the industrialized world [1, 2]. The bacteria are isolated frequently from poultry as well as from poultry products [3, 4], and epidemiological studies have incriminated consumption or handling of poultry products as major risk factors for campylobacter infection [5–9]. Thus, colonization control of campylobacter in poultry is of considerable public health importance.

Theoretically, prevention of campylobacter colonization in poultry may be effected in a number of different ways including hygienic measures [10–12], vaccination [13, 14], treatment with competitive exclusion microflora [15], and genetic control (selection of resistant chicken lines) [16]. Previous studies have indicated that it may be possible to maintain broiler flocks campylobacter-free up to the processing age by introduction of hygienic measures in the rearing houses [10–12, 17, 18]. Vertical transmission from colonized breeder flocks to progeny is considered unlikely [10, 18–21]. Most studies designed to identify sources of campylobacter colonization in broiler flocks have been limited to bacteriological surveys [10, 18, 22–25]. Although a number of possible routes of introduction have been identified, a better understanding of the epidemiology of campylobacter in broiler flocks is essential in order to provide a basis for a more specific control strategy.

In Norway, campylobacter infections are a cause of considerable morbidity in humans [26, 27]. A case-control study concluded that control of human campylobacteriosis should include measures that maintain a low prevalence of colonization in broiler chicken flocks [7]. We have conducted an epidemiological investigation in order to: (i) identify factors related to hygiene and husbandry practices on the broiler farm which are likely to have the greatest impact on the presence of campylobacter, and (ii) determine the prevalence of campylobacter colonized flocks in the study area.

MATERIALS AND METHODS

Description of the study

The study was conducted in southeastern Norway during the 1-year period April 1990 to March 1991. All 176 broiler farms which delivered broiler chickens to three poultry processing plants in the counties of Hedmark and Østfold, participated in the study. Each farm was represented by one flock which was selected at random as follows: to minimize seasonal bias, the study period was divided into five 10-week intervals (excluding Easter and Christmas); for each interval and each processing plant, one fifth of the flocks processed during that period at the plant in question were randomly selected, excluding flocks from farms already represented. The flocks were examined for presence of campylobacter at slaughter; from each flock, a cloacal swab was obtained from 26–28 chickens from different transport crates, before scalding and defeathering.

The farmers were subsequently interviewed using a structured questionnaire which covered: (i) husbandry and hygienic practices during the rearing period and when the house was empty, (ii) animal contacts including presence of other livestock, (iii) general information concerning the flock, housing, equipment, and drinking water supply, and (iv) mortality, disease signs, flock behaviour, feed consumption, and weight at slaughter. The interviews were conducted by four consultants from the Norwegian Poultry and Egg Association who were trained as interviewers. The median interval between the date of slaughter and the interview was 39 days (range 0–298). To avoid bias, the interviewers and the respondents were unaware of the microbiological results at the time of the interview. Details concerning the drinking water supply was provided by the local food hygiene inspection authorities. Information regarding carcass rejection was obtained from the processing plants.

On 147 farms, a drinking water sample was collected from a tap in the production control room outside the chicken room at the time of the interview. Samples were examined for campylobacter and for standard parameters of bacteriological quality of the water (see below).

Bacteriological methods

Culture of swabs of cloacal faeces and water samples were carried out at two food hygiene laboratories. Cloacal swabs were plated out directly onto Preston agar (Oxoid Ltd., Basingstoke, England) in the processing plant immediately after collection. In addition, samples from 104 flocks were subjected to selective enrichment by placing all swabs in 50 ml Preston broth (Oxoid Ltd.). Plates and enrichment cultures were incubated within 30 min of collection at 42–43 °C in a microaerobic atmosphere, which was achieved using gas generating sachets (BR 38; Oxoid Ltd.) in anaerobic jars without catalysts. Plates were read after 24 and 48 h. The enrichment broths were incubated for 24 h, and one loopful was streaked onto Preston agar plates.

Water samples were collected in sterile glass flasks, brought to the laboratories in thermobags, and examined the same day as collected. An aerobic plate count procedure was used to enumerate heterotrophic microorganisms [28]. Enumeration of total and faecal coliforms was done using a membrane filtration technique [28]. Isolation of *Campylobacter* spp. was attempted by passing 100 ml of water through a 0.45 µm membrane filter (Millipore Corp., Bedford, MA, USA) which was then placed in 10 ml Preston broth without selective supplement (SR 117; Oxoid Ltd.) and incubated in microaerobic atmosphere for 2–4 h at 37 °C. After this resuscitation step, the selective supplement was added, and the cultures were then incubated for 24 h at 42–43 °C before subculture onto Preston agar.

Thermotolerant *Campylobacter* spp. were identified on the basis of morphological, cultural, and biochemical parameters [29] as outlined previously [35]. Suspected campylobacter isolates (1–2 per flock) were submitted to the National Institute of Public Health for verification and biotyping [30].

Statistical analyses

Univariate analysis was carried out with the computer program Epi Info (Centers for Disease Control, Atlanta, GA, USA). For dichotomous variables, the significance of differences between groups was assessed using the χ^2 test; Fisher's exact test was used when an expected cell value was less than five. Continuous variables were analysed using either the Student's *t* test or Mann-Whitney's test as appropriate. Multivariate analysis with multiple linear logistic regression was done with the computer program Egret (Statistics and Epidemiology Research Corporation, Seattle, WA, USA). Variables were included in the multivariate analysis if the *P* value was 0.25 or less, were potential confounders, or if they were of theoretical interest regardless of statistical significance. All results are expressed as odds ratios (OR) with 95% confidence intervals (CI) and two-tailed *P* values. Population attributable fractions were calculated according to the method described by Martin and colleagues [31].

RESULTS

Broiler management of tested flocks

All 176 flocks were reared on litter in environmentally controlled houses. The median flock size was 6500 (range 2000–39500). With one exception, all farms were operated on a single age, all-in/all-out basis, and 165 farms had only one broiler house. All farms were supplied with feed of the same composition containing avoparcin (15 mg/kg) and anticoccidial agent. The broilers were slaughtered at a median age of 36 days (range 34–66). After depopulation, old litter was removed, and the pens and equipment were washed (97% of farms), and disinfected (usually with formaldehyde gas) (87%). The median time before restocking was 33 days (range 4–191). Most farms had five cycles of production annually.

Prevalence of campylobacter

Campylobacter spp. were isolated from 32 (18%) of the 176 flocks examined. *C. jejuni* biotype 1 was recovered from 24 flocks, *C. jejuni* biotype 2 from 3, and *C. coli* from 5 flocks. In 8 flocks, campylobacters were isolated from all chickens sampled; in 5 flocks, campylobacters were cultured from 10–25 individuals, while in the remaining 19 flocks < 10 samples were positive. All colonized flocks were detected by direct plating of cloacal swabs. Selective enrichment did not detect any additional positive flocks, rather campylobacters were not isolated from 8 flocks which were positive by direct plating.

Univariate analysis of risk factors

Only few of the potential risk factors examined showed a statistical significant association with campylobacter colonization (Table 1).

Drinking water. The broilers received drinking water from a variety of sources, including lakes, rivers, brooks, ponds, wells, and bore holes. Birds in campylobacter positive flocks were more likely to have drunk undisinfected water than negative flocks (OR = 3.24, *P* = 0.023). In all, 117 flocks (66%) used undisinfected

Table 1. *Univariate analysis of selected risk factors for campylobacter colonization in broiler flocks*

Risk factor	No. (%)*				Odds ratio	90% confidence interval	P value
	Positive† (n = 32)		Negative† (n = 144)				
Drinking water							
Undisinfected water	27/32	84%	90/144	63%	3.24	1.2-8.9	0.023
Undisinfected surface water	9/32	28%	10/144	7%	5.24	1.9-14.3	0.001
Coliform bacteria	13/30	43%	17/117	15%	4.50	1.7-12.1	0.0005
Faecal coliform bacteria	7/30	23%	7/117	6%	4.78	1.3-17.4	0.009
Campylobacter isolated	3/30	10%	1/117	1%	12.89	1.1-340.8	0.027
Animal contacts							
Tending other poultry‡	6/32	19%	11/114	8%	2.79	1.0-8.2	0.063
Tending pigs‡	5/32	16%	6/144	4%	4.26	1.2-15.0	0.024
Rats on the farm	4/31	13%	4/144	3%	4.12	1.0-16.3	0.044
Other factors							
Geographic area (Hedmark)	19/32	59%	53/144	37%	2.51	1.2-5.5	0.021
Season (August-November)	20/32	63%	52/144	36%	2.95	1.3-6.5	0.007

* Denominators exclude flocks with missing values on the risk factors.

† Positive, campylobacter-colonized flocks ; negative, non-colonized flocks.

‡ The flock manager tended other poultry or pigs prior to entering the broiler house.

water. Seventy-four (42%) obtained drinking water from a surface source, but this exposure was not identified as a risk factor (OR = 0.93, P = 0.86). However, using surface water which was not disinfected was strongly associated with an increased risk of colonization (OR = 5.24, P = 0.001). A considerable number of flocks received drinking water of suboptimal bacteriological quality. Coliform bacteria were detected in samples from 30 (20%) of the 147 farms examined; the total coliform count exceeded 10 cfu/100 ml in 9 cases. Faecal coliforms were detected in samples from 14 farms (10%). The total heterotrophic bacterial count was greater than 100 cfu/ml on 55 farms and exceeded 1000 cfu/ml in 17 samples. Campylobacter colonization was associated with both presence of coliforms and faecal coliforms (Table 1). The total coliform and faecal coliform counts were higher in water samples from colonized than from non-colonized flocks, whereas the total bacterial count did not differ significantly (Table 2). *C. jejuni* biotype 1 was isolated from 4 of the 147 water samples, and this factor was strongly associated with colonization (OR = 12.89, P = 0.027). On three farms where *C. jejuni* biotype 1 was recovered from the water, the chickens were infected with the same biotype as well. On one farm, the organism was isolated from the water supply but not from the flock under study.

Animal contacts. The farmers were asked to specify whether poultry or other animals were kept on the farm, or whether any mammals, birds, or insects were observed in the broiler house during the rearing period or when the house was

Table 2. Relationship between campylobacter colonization in broiler flocks and number of indicator bacteria in drinking water samples

Indicator bacteria	Colonized flocks			Non-colonized flocks			P value
	Mean	Median	Range	Mean	Median	Range	
Total bacterial count (cfu/ml)	404	97	< 1–3100	366	18	< 1–> 4000	0.81
Coliform count (cfu/100 ml)	17.2	< 1	< 1–260	2.5	< 1	< 1–155	0.0003
Faecal coliform count (cfu/100 ml)	7.1	< 1	< 1–94	< 1	< 1	< 1–13	0.0035

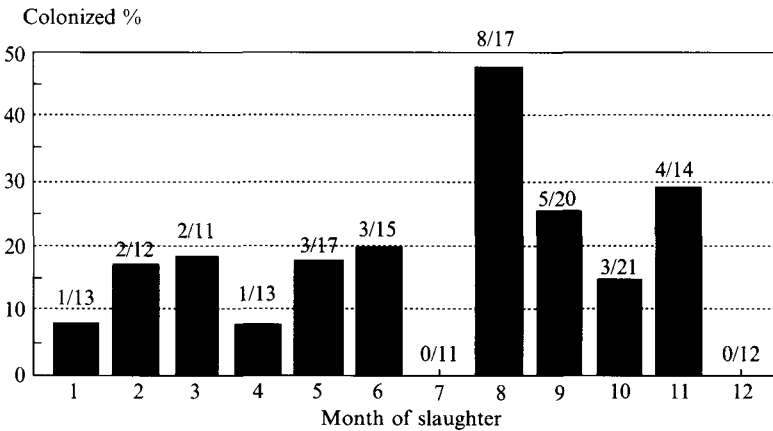


Fig. 1. Seasonal distribution of campylobacter colonization among broiler chicken flocks in southeastern Norway. Values on the Y-axis are expressed as the proportion (%) of the total number of flocks examined each month. Figures above the bars indicate no. of infected flocks/no. examined.

empty. Eight farms (5%) reported rats on the premises, and this factor was found to be associated with an increased risk of campylobacter colonization (OR = 4.12, $P = 0.044$). No significant associations were demonstrated for other animal species. A total of 29 farms kept other types of poultry, and 15 raised pigs. Although the presence of other poultry or pigs *per se* was not identified as risk factors, colonized farms were more likely to report that the flock manager tended other poultry (OR = 2.79, $P = 0.063$) or pigs (OR = 4.26, $P = 0.024$) prior to entering the broiler house.

Other factors. Campylobacter colonization was significantly associated with geographic area and season; colonized flocks were more likely to be identified in the Hedmark region (OR = 2.51, $P = 0.021$) and to be slaughtered in the autumn (August–November) (OR = 2.95, $P = 0.007$) (Fig. 1).

The following factors were not found to be associated with an increased risk of colonization: flock size; floor space; average weight of day old chicks; age at slaughter; hatchery; number of days between depopulation and washing of the house, between washing and disinfection, between disinfection and restocking; the length of time before restocking; procedures for disposal of litter and manure:

routines for cleaning, washing, and disinfection of the broiler house between flocks; routines for cleaning, washing, and disinfection of equipment, implements, drinkers, water tanks, feeders, feed bins, footwear, clothes, and hands; visitors to the house; hygienic measures when the caretaker or visitors entered the broiler room; hours spent in the house; number of times entering the house; presence of other poultry or animals at the farm; contact with poultry on other farms; birds, mammals, or insects seen in the room during rearing or when empty; procedures for control of rodents or insects; asphalt or concrete in front of doors and gates; doors or gates kept open before restocking; distance between the broiler house and disposed litter, manure, farmland, other livestock, and garbage dumps; types of waterers, feeders, feed bins, and silos; feed producer; cracks or holes in feed bins or top covers; types of litter, litter wetness, and litter refill procedures.

Multivariate analysis of risk factors

The following variables were found to be independently associated with campylobacter colonization when placed simultaneously in a linear logistic regression model (Table 3): (i) the use of undisinfected water (OR = 3.42, $P = 0.045$), (ii) tending other poultry prior to entering the broiler house (OR = 6.43, $P = 0.007$), (iii) tending pigs before entering the house (OR = 4.86, $P = 0.037$), (iv) geographic region (Hedmark versus Østfold county) (OR = 2.91, $P = 0.023$), (v) season (autumn versus other seasons) (OR = 3.43, $P = 0.008$). Presence of rats at the farm was associated with an increased risk, but this factor did not reach statistical significance (OR = 3.96, $P = 0.083$). This variable was kept in the model on the basis of analysis of deviance. No significant interactions were detected among the variables included in the analysis. The population attributable fraction for consumption of undisinfected water was 0.53.

Productivity and health

No significant associations were detected between campylobacter infection and the following factors: mortality during the rearing period, carcass rejection or downgrading at processing, mean carcass weight, or total feed consumption per broiler. Likewise, there were no associations with disease signs such as diarrhoea (soiling around the cloaca), or abnormal variation in flock behaviour, feed and water consumption, or colour or consistency of faecal droppings.

DISCUSSION

The epidemiological approach employed in the present study enabled identification of several risk factors for campylobacter colonization in broiler flocks and allowed an assessment of their relative importance, thereby providing a basis for a specific control strategy. A number of potential risk factors were not found to be associated with colonization. It is important to emphasize that for most of these variables the number of exposed flocks was too low to enable detection of statistically significant associations except those with the highest risk.

Table 3. *Multivariate analysis of risk factors for campylobacter colonization in broiler flocks: Logistic regression model*

Risk factors	Odds ratio	95 % Confidence interval	P value
Feeding the broilers undisinfected water	3.42	1.0–11.4	0.045
Tending other poultry before entering the house	6.43	1.7–24.7	0.007
Tending pigs before entering the house	4.86	1.1–21.4	0.037
Geographic region (Hedmark <i>v.</i> Østfold county)	2.91	1.2–7.3	0.023
Season (autumn <i>v.</i> other seasons)	3.43	1.4–8.6	0.008
Presence of rats on the farm	3.96	0.8–18.8	0.083

Although some broiler chicken flocks have been shown to escape campylobacter colonization, many surveys have demonstrated that campylobacter is commonly encountered in the intestinal contents of healthy poultry [1]. In the present investigation, the bacteria were recovered from only 18% of the flocks. Among the factors which may contribute to this low prevalence rate are the reasonably good hygienic standard and the low stocking rates. However, the number of birds sampled was small relative to the size of the flocks monitored, and the possibility that some colonized flocks may have been missed cannot be excluded. On the other hand, with a sample size of 26–28 randomly selected chickens per flock, there is a 95% probability of detecting at least one positive individual if the percentage of colonized chickens is 10% or higher. The low prevalence of flock colonization is in agreement with the results of a recent case-control study designed to identify risk factors for sporadic human cases of campylobacter infection in southeastern Norway [7]. Although poultry consumption during the 2 weeks prior to illness was found to be independently associated with campylobacteriosis, the risk of eating poultry was largely accounted for by consumption of poultry produced in Denmark or Sweden, while eating poultry in Norway was not significantly associated with illness.

Chicks challenged experimentally with campylobacter-contaminated water become rapidly colonized [11]. The water supply was identified as the predominant source of *C. jejuni* on a broiler farm in southern England [25]. In the present study, feeding the chickens undisinfected water was associated independently with an increased risk of campylobacter colonization. It should be emphasized, however, that Norway is different from many other European countries in that undisinfected surface water is not infrequently used on agricultural premises. The possibility that water may act as a vehicle of transmission is supported by a recent investigation which showed that surface water sources in southeastern Norway are frequently contaminated by *Campylobacter* spp. [32]. The prevalence of campylobacters in surface water was greater in the autumn [32], and since a parallel seasonality was observed in the prevalence of colonized chicken flocks (Fig. 1), it is tempting to suggest that the peak prevalence among chicks corresponded with an increase in the occurrence of the organism in their drinking water. However, water disinfection and seasonality were independently associated with campylobacter colonization in the multivariate model. This implies that there are likely to be factors in addition to water quality which account for the seasonal trend. A seasonal variation in the isolation of *Campylobacter* spp. from

poultry and poultry products, with a peak during the warm seasons, have been noted in several countries [17, 22, 33].

Although campylobacter colonization was found to be associated with consumption of undisinfected water, *Campylobacter* spp. were only occasionally recovered from the water supply on colonized farms. This apparent discrepancy may be explained by the presence of viable but non-culturable campylobacter cells in water [24, 25, 33], or by the fact that the water samples were collected several weeks after slaughter. Moreover, the number of campylobacters present, if any, may have been too low to be detected by the culture technique used.

Campylobacter is known to be a common intestinal commensal in pigs [1], including those slaughtered in Norway [35]. Likewise, the presence of campylobacters in Norwegian poultry, including laying hens, turkeys, and broiler chickens has been documented previously [36]. Thus, the finding that contact with other poultry and pigs was associated with an increased risk of campylobacter colonization in broiler flocks was not unexpected. This emphasizes the need for strict hygienic routines when the farm workers enter the rearing room and supports the suggestion that stringent hygiene barriers are required to prevent colonization during the rearing period [10–12, 17]. Since campylobacters tend to die out rather than multiply in the environment under normal ambient conditions, transmission of the organism to subsequent flocks in the same house is probably interrupted by efficient cleaning and disinfection before restocking [11, 17, 18]. Insufficient routines for cleaning and disinfection were not associated with an increased risk of campylobacter colonization in our study. However, the number of flocks exposed to these factors was small. Moreover, a majority of the farms used long empty periods between flocks, a practice which promotes a decline in campylobacter numbers regardless of disinfection procedures.

The failure to demonstrate a relationship between campylobacter colonization and reduced productivity or disease, is in accordance with experimental findings which indicate that campylobacter is usually a non-pathogenic commensal in the gastrointestinal tract of chickens [11, 37].

Based on the results, the following preventive measures are likely to have the greatest impact on the occurrence of campylobacter in broiler chicken flocks in the study area: (i) disinfection of drinking water supplies, and (ii) emphasis on hygienic routines when entering the chicken room, especially after contact with other poultry or pigs. Two of the risk factors found to be associated with the presence of campylobacter were uncommon; contact with other poultry or pigs was found in only 10% and 6% of the flocks, respectively. The fact that these factors remained independently associated with campylobacter colonization in the multivariate model reinforces their importance, but implies that they would explain only a small part of the campylobacter problem. In contrast, undisinfected water was used more commonly (67%). Therefore, proper disinfection of drinking water is the single most important measure. About 53% of the cases of campylobacter colonization could potentially be prevented by proper disinfection of drinking water (population attributable fraction = 0.53).

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