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Epidemiological study on risk factors and risk reducing measures for campylobacter infections in Dutch broiler flocks

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SUMMARY

From September 1991 until August 1993 an epidemiological study involving 20 Dutch broiler farms was conducted to identify risk factors and risk reducing measures for campylobacter infections in broiler flocks. *Campylobacter* spp. were detected in 64 (57%) of the 112 broiler flocks and in 25 (63%) of the 40 broiler cycles examined. Univariate analysis of farm management data was performed followed by logistic regression analysis of selected risk and risk reducing factors. The presence of other farm animals, including pigs, cattle, sheep and fowl, other than broilers, was found to be independently associated with an increased risk of campylobacter infections in broiler flocks (odds ratio (OR) = 11·81; $P = 0{\cdot}041$). Further, the results indicate that application of specific hygiene measures during the rearing period, such as washing hands before tending the broiler flocks, the use of separate boots for each broiler house and the use of footbath disinfection when entering a broiler house, may significantly reduce the risk of campylobacter infections in broiler flocks.

INTRODUCTION

Campylobacter jejuni/coli is recognized as a major cause of human enteritis in many developed countries. For example, in The Netherlands, the number of cases of campylobacteriosis is estimated at 300000 per year [1, 2]. Poultry meat has been identified as a major risk factor for sporadically occurring campylobacter infections [3–7]. *Campylobacter* spp. were detected in approximately 40% of chicken products, sampled in retail stores in The Netherlands between 1991 and 1993 [8], while they were isolated from 82% of Dutch broiler flocks [9]. It is generally recognized that a reduction in human campylobacteriosis should be achieved by reducing the number of campylobacter infected poultry flocks [10].

Several studies have been carried out to clarify the transmission routes involved in the infections of broiler flocks with *Campylobacter* spp. Vertical transmission from parent flocks to progeny via the eggs is considered unlikely since the organism has only been isolated occasionally from the shells of freshly laid eggs, does not easily penetrate the contents of the egg [11] and has not been isolated from fertile eggs [12], newly hatched chicks [12–14] and hatchery waste [14, 15]. Moreover, the rarity of early campylobacter infections in poultry flocks, and the results of epidemiological typing studies, indicate that flocks become infected from environmental sources [13, 16]. Feed is not likely to be a significant source of flock infection since its dryness adversely affects survival of *Campylobacter* spp. and in several studies fresh feed samples were always negative [12, 13, 16, 17]. Potable water is not considered a significant source of infection although in a British study drinking water from a bore hole was identified as the predominant source of campylobacter infection in broilers [12]. Similarly, an epidemiological study conducted in Norway identified undisinfected drinking water as a risk factor [18]. In the same study, the tending of other poultry and pigs before entering the broiler house were also identified as risk factors. Furthermore, farm workers and vermin have been suggested as potential sources or vehicles of infection [10].

In order to provide a basis for an efficient control strategy the relative importance of the different potential sources has to be assessed. The present epidemiological study was performed to identify risk factors as well as risk reducing measures for campylobacter infections in Dutch broiler flocks.

METHODS

Sampling of broiler flocks

The study, which lasted from September 1991 until August 1993, involved 20 broiler farms belonging to the same poultry company. On all farms an all-in all-out system was applied which meant that the broiler houses were depopulated, left empty for 2 weeks and restocked simultaneously. Each farm was visited twice, during two successive broiler cycles and all the broiler flocks present were sampled. The number of broiler flocks raised on each farm varied from 1–6 flocks per broiler cycle. The flocks, whose sizes ranged from 5000 to 25000 birds, were sampled a few days before depopulation. From each 10 pooled samples of faeces were collected at random by dipping a sterile cotton swab into 10 fresh caecal droppings. The swabs were transferred into tubes containing 2 ml of phosphate buffered saline and transported to the laboratory for examination for *Campylobacter* spp. The number of samples taken per flock (100) enabled the detection of flocks with 3% infected birds, at a confidence level of 95%.

Isolation of *Campylobacter* spp.

The swabs were used to inoculate campylobacter blood-free selective agar (Oxoid CM 739) with cefoperazone (32 mg/l) and cycloheximide (100 mg/l) within 2 h of collection. Plates were incubated in a microaerobic atmosphere (7% O_2, 10% CO_2 and 83% N_2) at 42 °C for 48 h. Characteristic colonies were examined under a phase-contrast microscope for typical spiral-shaped cells and rapid motility. The identity of a proportion of isolates from different flocks was confirmed by using a latex agglutination test (Meritec™-Campy (jcl), Meridian Diagnostics, Inc.).

Farm management

Information on farm management was collected using a structured questionnaire. The information collected included data on the construction of the farm premises, hygiene measures taken between successive broiler cycles (especially concerning cleaning and disinfection procedures) and hygiene measures taken during the broiler cycles, presence of pets or farm animals (other than broilers), occurrence of vermin and specific measures taken to control them, occurrence of disease and use of antibiotics, as well as general farm management data (such as the sort of litter used in the broiler houses). The time of sampling (season) was also recorded.

Statistical analyses

The broiler flocks present on a farm during one broiler cycle were taken as the unit of observation. Thus, a broiler cycle was scored positive if *Campylobacter* spp. were isolated from one or more flocks present and the total number of observations thus amounted to 40. The relation between the occurrence of *Campylobacter* spp. in the first and the second broiler cycle was tested using the Fisher's exact test. Univariate analyses of dichotomous variables and multivariate analyses of risk and risk reducing factors by using logistic regression were performed by using the Proc Logistic procedure of SAS version 6.10. All reported P values are two-tailed.

RESULTS

Occurrence of *Campylobacter* spp.

Campylobacter spp. were detected in 64 (57%) of the 112 broiler flocks and in 25 (63%) of the 40 broiler cycles examined. *Campylobacter* spp. were detected in 1 of the 2 broiler cycles on 7 farms, in both on 9 and in neither on 4 farms.

Univariate analysis of risk and risk reducing factors

No statistically significant relation was found between the occurrence of *Campylobacter* spp. in the first 20 broiler cycles and the results of the second 20 broiler

Table 1. *Univariate analysis of selected risk and risk reducing factors for campylobacter infections in broiler flocks*

Factor	Number* (%) campylobacter Positive	Negative	OR†	95% CI‡	P value
Presence of other farm animals§	19/25 (76·0)	5/15 (33·3)	6·33	1·54–26·00	0·010
Use of ground water for cleaning the broiler houses	10/22 (45·5)	2/14 (14·3)	5·00	0·90–27·81	0·066
Use of a detergent for cleaning the broiler houses	2/21 (9·5)	6/15 (40·0)	0·16	0·03–0·94	0·043
Cleaning and disinfection of the farm yard between successive broiler cycles	10/21 (47·6)	12/15 (80·0)	0·23	0·05–1·05	0·057
Use of separate boots for each broiler house	10/23 (43·5)	12/15 (80·0)	0·19	0·04–0·87	0·032
Washing hands before tending the broiler flocks	5/23 (21·7)	9/15 (60·0)	0·19	0·04–0·78	0·021
Use of footbath disinfection when entering a broiler house	14/25 (56·0)	12/15 (80·0)	0·32	0·07–1·41	0·132

* Number of broiler cycles with respective factor/total no. of broiler cycles. Denominators exclude broiler cycles with missing values for the respective factors.
† OR, odds ratio.
‡ CI, confidence interval.
§ Farm animals, pigs or cattle or sheep or fowl (other than broilers).

cycles (Fisher's exact $P = 0{\cdot}36$). Therefore, the 40 measurements were treated in the model as 40 independent observations. The results of the univariate analysis of risk and risk reducing factors are presented in Table 1. Two factors were associated with an increased risk ($P \leqslant 0{\cdot}10$) of campylobacter infections in broiler flocks: (i) presence of other farm animals on the farm (pigs, cattle, sheep and fowl) and (ii) use of ground water (instead of tap water) for cleaning the broiler houses between successive broiler cycles. Analysis of the individual categories of farm animals did not yield P values $\leqslant 0{\cdot}10$. Furthermore, several other factors were associated with a reduced risk ($P \leqslant 0{\cdot}10$) of campylobacter infections in broiler flocks: (i) the use of a detergent for cleaning the broiler houses, (ii) the cleaning and disinfection of the farm yard between successive broiler cycles, (iii) the use of separate boots for each broiler house, and (iv) the washing of hands before tending the broiler flocks. In addition, the use of footbath disinfection when entering a broiler house was slightly associated with a reduced risk of infection ($P = 0{\cdot}132$).

Multivariate analysis of risk and risk reducing factors

Logistic regression analysis was performed to determine which factors were independently associated

Table 2. *Multivariate analysis of risk and combined risk reducing factors for campylobacter infections in broiler flocks*

Factor	OR*	95% CI†	P value
FARM ANIMALS‡	11·81	1·10–126·26	0·041
GROUND WATER§	4·83	0·45–51·75	0·193
HYGIENE 1‖	0·20	0·02–1·68	0·138
HYGIENE 2¶	0·19	0·04–0·84	0·028

* OR, odds ratio.
† CI, confidence interval.
‡ FARM ANIMALS, presence of other farm animals on the farm (pigs or cattle or sheep or fowl (other than broilers)); variable with two levels (0, 1).
§ GROUND WATER, use of ground water for cleaning the broiler houses; variable with two levels (0, 1).
‖ HYGIENE 1, variable with three levels (0, 1, 2); HYGIENE 1 = DETERGENT + YARD, where DETERGENT = use of a detergent for cleaning the broiler houses, YARD = cleaning and disinfection of the farm yard between successive broiler cycles.
¶ HYGIENE 2, variable with four levels (0, 1, 2, 3); HYGIENE 2 = BOOTS + HANDWASH + FOOTBATH, where BOOTS = use of separate boots for each broiler house, HANDWASH = washing hands before tending the broiler flocks, FOOTBATH = use of footbath disinfection when entering a broiler house.

with an increased or reduced risk of campylobacter infections in broiler flocks. Initially, all the risk and risk reducing factors identified in the univariate analysis were placed in one model. However, the number of factors ($n = 7$) involved was too large with respect to the number of observations to enable proper analysis. Therefore, the five risk reducing factors identified in the univariate analysis were combined into two variables. The two hygiene measures applied between successive broiler cycles, i.e. the use of a detergent for cleaning the broiler houses (DETERGENT) and cleaning and disinfection of the farm yard between successive broiler cycles (YARD), were combined into one variable (HYGIENE 1) with three levels (0, 1, 2) according to the following equation:

HYGIENE 1 = DETERGENT + YARD.

The three hygiene measures applied during the rearing period, i.e. the use of separate boots for each broiler house (BOOTS), washing hands before tending the broiler flocks (HANDWASH) and the use of footbath disinfection when entering a broiler house (FOOTBATH), were combined into one variable (HYGIENE 2) with four levels (0, 1, 2, 3) according to the following equation:

HYGIENE 2 = BOOTS + HANDWASH + FOOTBATH.

These two variables were then placed in a model with the other two risk factors identified in the univariate analysis. The results are presented in Table 2. The presence of other farm animals was found to be independently associated with an increased risk of campylobacter infections in broiler flocks (odds ratio (OR) = 11·81; $P = 0{\cdot}041$). The use of ground water for cleaning the broiler houses was associated with a non-significant increase in risk (OR = 4·83; $P = 0{\cdot}193$). Furthermore, variable HYGIENE 2 was significantly associated with a reduced risk of campylobacter infections in broiler flocks (OR = 0·19; $P = 0{\cdot}028$). Since this variable was categorized into four levels, the presence of two and three factors (i.e. the use of separate boots for each broiler house and washing hands before tending the broiler flocks and the use of footbath disinfection when entering a broiler house) resulted in a reduced risk of 0·04 and 0·01 respectively. The variable HYGIENE 1 was also associated with a reduced risk of infection, but it was not of statistical significance (OR = 0·20; $P = 0{\cdot}138$). There appeared, however, to be a strong correlation between the variable HYGIENE 1 and the variable HYGIENE 2 (Spearman correlation coefficient = 0·49; $P = 0{\cdot}002$). No significant interactions among the variables were found.

DISCUSSION

A large number of environmental factors including the poultry house environment, untreated drinking water, old litter, other farm animals, domestic pets, rodents, insects, wild birds, farm workers, equipment and transport vehicles have been suggested as sources of campylobacter infections in broiler flocks [10], but the relative importance of these potential sources is not clearly understood.

In this study, no relation was found between the first and the second broiler cycles possibly because thorough cleaning of the broiler houses between the first and the second broiler cycle was carried out on all farms. In addition, disinfection was carried out in all but three cases and in only one of these were *Campylobacter* spp. detected in both broiler cycles. Moreover, on all farms the broiler houses were empty for at least one week after cleaning and disinfection was completed. Considering these facts, and taking into account the rarity of early campylobacter infections, direct transmission of *Campylobacter* spp. from the first flock to the second via the broiler houses itself would appear unlikely, and thus the results of the first and second broiler cycles are likely to be independent. Furthermore, for several potential risk and risk reducing factors the number of broiler cycles with the respective factor was either very low or very high and no statistically significant association was demonstrable.

The presence of other farm animals on the farm, including pigs, cattle, sheep and fowl, other than broilers, was strongly associated with an increased risk of campylobacter infections in broilers. This finding is not surprising, however, since these animals frequently excrete *Campylobacter* spp. in their faeces [10, 16, 18]. The failure to demonstrate statistically significant associations between campylobacter infections in broilers and the individual categories of farm animals indicates that this association found is not restricted exclusively to one of the categories of farm animals.

The results of this study further indicate that hygiene measures applied during the rearing period, i.e. the use of separate boots, footbath disinfection and hand washing may significantly reduce the risk of campylobacter infections in broilers flocks. This suggests that farm workers are important in transmitting *Campylobacter* spp. to broiler flocks. These findings are in agreement with results of a previous study in which campylobacter infections in broiler flocks were prevented by the strict application of hygiene measures including the three risk reducing measures identified in this study [16]. Similarly, a British study [14] indicated that campylobacter infections in broilers could be prevented by using footbath disinfection.

The use of ground water for cleaning the broiler houses did not reach statistical significance in the multivariate analysis, which may indicate that this factor was confounded by the presence of other farm animals in the univariate analysis. On the other hand, considering the wide confidence intervals for the OR the number of observations may have been too small for a statistically significant association to be demonstrated. This may also be true for the hygiene measures applied between successive broiler cycles (variable HYGIENE 1). However, the association of these measures with a reduced risk of infection may be explained in part by the strong correlation found between this variable and the variable HYGIENE 2.

In summary, the results of this study indicate that the presence of other farm animals on the farm, including pigs, cattle, sheep and fowl, may significantly increase the risk of campylobacter infections in broiler flocks. This risk may significantly be reduced by application of specific hygiene measures during the rearing period preventing horizontal transmission of the organism via the farm workers.

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