

Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks

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Aims: To determine the flock prevalence and to estimate the within flock prevalence of *Campylobacter* in broiler flocks from different rearing systems, and to determine the antimicrobial susceptibility of *Campylobacter* isolates to selected antimicrobial substances.

Methods and Results: One hundred and sixty broiler flocks originating from organic, conventional and extensive indoor production farms were investigated for the presence of *Campylobacter* at the time of slaughter. *Campylobacter* isolates from a subsample of positive flocks were subjected to susceptibility testing. *Campylobacter* spp. were isolated from 100% of organic broiler flocks, from 36.7% of conventional broiler flocks and from 49.2% of extensive indoor broiler flocks. Six of 62 *Campylobacter* isolates were resistant to one or more of the antimicrobials tested.

Conclusions: These results indicate that the special characteristics of organic broiler production provide a high prevalence of *Campylobacter*-positive flocks. Antimicrobial resistance was scarce among *Campylobacter* isolates from all rearing systems.

Significance and Impact of the Study: Organic broiler flocks constitute a strong potential for introduction of *Campylobacter* to the processing line upon arrival at slaughter.

INTRODUCTION

Campylobacter is a well recognized cause of human enteritis, and food-borne campylobacteriosis is considered a main problem of public health in many developed countries. In 1999, campylobacteriosis was the most frequent food-borne zoonosis in Denmark (78 cases per 100 000 inhabitants) (Anon. 2000a). Poultry products are suspected to be an important source of infection in Denmark as well as in other countries (Kapperud *et al.* 1992; Hanninen *et al.* 2000; Studahl and Andersson 2000; Neimann 2001).

Over the last decade, the occurrence and spread of *Campylobacter* in conventional broiler flocks has been intensively studied, whereas the occurrence of *Campylobacter* in broiler flocks of organic or other non-conventional origin has received less attention. Organic and other non-conventional broiler products are now readily available for

retail in many countries, yet very little is known about the status of these broiler flocks with regard to the prevalence of *Campylobacter*.

A few reports on the prevalence of *Campylobacter* in non-conventional broiler production are available. In a French study, 85.7% of faecal samples from one flock of chickens raised in a free-range system were *Campylobacter* positive (Rivoal *et al.* 1999). In a study from Tanzania, 76.5% of samples of droppings from indigenous free-range poultry were *Campylobacter* positive (Kazwala *et al.* 1993), and in a study of *Campylobacter* in domestic free range chickens and confined commercial chickens in Peru, the isolation frequencies were 54% and 35%, respectively (Tresierra-Ayala *et al.* 1995). Although neither of these studies deal specifically with organic broilers, they do indicate that free-range rearing of poultry could be associated with a high prevalence of *Campylobacter*.

Reports from several countries on prevalence of *Campylobacter* in conventional broilers are available. Flock prevalences ranging from 18 to 82% have been reported (Humphrey *et al.* 1993; Kapperud *et al.* 1993;

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Jacobs-Reitsma *et al.* 1994a; Berndtson *et al.* 1996a; Hald *et al.* 2000). Although a direct comparison of reports on *Campylobacter* prevalence from different countries will be strongly biased by differences in sample size, isolation procedures and sample material used, the results reported suggests that in some countries, a considerable proportion of conventional broiler flocks are *Campylobacter*-negative.

In a few investigations the authors reported the proportion of *Campylobacter*-positive samples within the samples taken from one or more of the flocks included in the investigation (Berndtson *et al.* 1996b; Gregory *et al.* 1997; Evans and Sayers 2000; Hald *et al.* 2001). The results from these studies suggest that if a broiler flock is *Campylobacter* infected, a large proportion of the birds within in the flock is infected.

A seasonal variation in the prevalence of *Campylobacter*-positive broiler flocks has been reported from Denmark (Wedderkopp *et al.* 2000), as well as from Norway (Kapperud *et al.* 1993), Sweden (Berndtson 1996) and The Netherlands (Jacobs-Reitsma *et al.* 1994a).

Antimicrobial susceptibility of *Campylobacter* isolates from broilers in Denmark has been reported since 1995 through the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) (Anon. 2000b). However, these data do not provide comparison between different broiler rearing systems. From other countries, reports on antimicrobial susceptibility of *Campylobacter* isolates from broilers of conventional origin are available (Jacobs-Reitsma *et al.* 1994b; Berndtson 1996; Saenz *et al.* 2000), whereas similar knowledge regarding broiler flocks of organic and other non-conventional production origin is absent or scarce.

The aim of the present study was to determine the flock prevalence and to estimate the within flock prevalence of *Campylobacter* in broiler flocks from organic, conventional and extensive indoor production farms in Denmark. Furthermore, the study aimed to determine the antimicrobial susceptibility of *Campylobacter* isolates from broilers from the three rearing systems to selected antimicrobial substances.

MATERIALS AND METHODS

Sampling procedure

Broiler flocks. Broiler flocks of organic, conventional and extensive indoor rearing systems were selected for the study. Organic broiler production was established in Denmark in 1998. The organic producers have adopted strict rules of production, including restricted use of antimicrobial substances, free-range rearing and use of organic feed. A slow-growing breed of broilers is used in the organic production and the minimum age at slaughter

is 81 days. Conventional broiler flocks are given high protein and high energy feed. They are reared in a confined environment at high stocking density and the age at slaughter is 36–42 days. Extensive indoor broiler flocks are produced in a less intensive rearing system in a confined environment with a low stocking density. These broilers are given low protein and low energy feed and the minimum age at slaughter is 56 days. Antimicrobial growth promoters are not in use with any of the three rearing systems.

One hundred and sixty broiler flocks from 39 farms were sampled in 1998–2000. Seventy-nine conventional broiler flocks originating from 18 houses at 18 farms, 59 extensive indoor broiler flocks originating from 16 broiler houses at nine farms and 22 organic broiler flocks originating from 12 free-range farms were sampled for the investigation.

The broiler flocks were slaughtered at four different abattoirs. In Denmark, the allocation of broiler flocks to different abattoirs is dependent on geographical location and ownership relations. One abattoir received flocks from all three rearing systems, one abattoir received conventional and extensive indoor broiler flocks, one abattoir received organic flocks only, and one abattoir received conventional flocks only.

Samples. Samples consisted of cloacal swabs taken from the broilers at slaughter. In order to enable determination of the flock prevalence as well as estimation of the within-flock prevalence of *Campylobacter*, 10 broilers from each flock were sampled individually. The sample size considerations were based on the assumption that the within-flock prevalence in *Campylobacter*-positive flocks would be 50% or higher. During transportation, the swabs were kept in sealed 10 ml tubes containing Brain Heart Infusion broth, 37 g l⁻¹ (Difco), supplemented with 5% calf blood and 0.5% agar (Oxoid). If not sent immediately to the laboratory, the swabs were refrigerated at 5°C for a maximum of 3 days after sampling, before dispatch to the laboratory.

Isolation and identification of *Campylobacter*

Upon arrival at the laboratory, each swab was immersed in a 2 ml tube containing veal infusion broth supplemented with 4% new-born calf serum. The swabs were left for 10–15 min in the broth. Subsequently, 10 µl of the broth were streaked onto modified charcoal cefoperazone deoxycholate agar (CCDA; Oxoid) and incubated for 48 h at 42°C in incubation jars filled with a gas mixture of 65% N₂, 25% H₂ and 10% CO₂. *Campylobacter*-like colonies were purified on blood agar and identified to species level on the basis of standard procedures comprising tests for hippurate and

indoxyl acetate hydrolysis, catalase production, and susceptibility to cephalotin and nalidixic acid (On and Holmes 1991, 1992).

Susceptibility testing of *Campylobacter* isolates

The MIC of one *Campylobacter* isolate from each of 62 flocks to tetracycline, ampicillin, erythromycin, streptomycin and enrofloxacin was determined by the agar dilution method. The test was performed on Mueller-Hinton II agar (Becton Dickinson) supplemented with 5% bovine blood. The test strains were inoculated on the agar plates with a multipoint inoculator (Denley Instruments A400, West Sussex, UK). The dilution ranges used were as follows: tetracycline 0.5–32 $\mu\text{g ml}^{-1}$, ampicillin 1–64 $\mu\text{g ml}^{-1}$, erythromycin 0.25–32 $\mu\text{g ml}^{-1}$, enrofloxacin 0.03125–4 $\mu\text{g ml}^{-1}$ and streptomycin 1–64 $\mu\text{g ml}^{-1}$. The following break-points were used: tetracycline, > 4 $\mu\text{g ml}^{-1}$; ampicillin, > 16 $\mu\text{g ml}^{-1}$; erythromycin, > 16 $\mu\text{g ml}^{-1}$; enrofloxacin, > 2 $\mu\text{g ml}^{-1}$ and streptomycin, > 32 $\mu\text{g ml}^{-1}$. Four control strains, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 were used with every test plate.

Statistical analysis

Two levels of analysis were performed on the prevalence data: (i) flock level analysis (the proportion of *Campylobacter*-positive flocks) and (ii) animal level analysis (the proportion of *Campylobacter*-positive swabs). A broiler flock was considered positive if *Campylobacter* was isolated from at least one swab sample from the flock. The data structure was hierarchical (animal/flock/house/farm/rearing system) and longitudinal (repeated measurements over time within each broiler house). In order to model the within-group correlation imposed by the hierarchical structure, the data were analysed in a Generalized Linear Mixed Model (GLMM)

$$(a) \text{Flock}_{status} = X\beta + u_{farm} + v_{house} + \varepsilon,$$

$$(b) N_{pos}/N_{Swabs} = X\beta + u_{farm} + v_{house} + \varepsilon,$$

with $X\beta$ equal to the fixed part of the model (season and rearing system), u_{farm} and v_{house} as random farm and house effects, respectively, and ε as the within-house error term. The error term ε was assumed binomial. The model parameters were estimated with Restricted Maximum Likelihood (REML) applying the SAS-macro glmm800.sas (SASv8-00), using the method of Wolfinger and O'Connell (1993). Furthermore, the results were checked in S-plus (Anon. 2000c) applying the *reglm* procedure, using the method of Schall (1991). Data on differences in numbers of

resistant *Campylobacter* isolates between rearing systems were analysed by the Fisher exact test.

RESULTS

Campylobacter spp. were isolated from all of the 22 (100%) organic broiler flocks, from 29 of 79 (36.7%) conventional broiler flocks and from 29 of 59 (49.2%) extensive indoor broiler flocks. Thus, the proportion of *Campylobacter*-positive flocks was significantly higher for organic flocks compared with conventional flocks ($P < 0.001$) and extensive indoor flocks ($P < 0.001$). The difference between conventional flocks and extensive indoor broiler flocks was non-significant ($P > 0.05$).

The overall proportion of *Campylobacter*-positive flocks was significantly higher in the period from May to October (61 of 87) compared with the period from November to April (19 of 73), resulting in an odds ratio (OR) of 6.7. In both seasons, the proportion of *Campylobacter*-positive flocks was significantly higher for organic flocks compared with conventional flocks ($P < 0.001$) and extensive indoor flocks ($P < 0.001$), while the difference between conventional flocks and extensive indoor flocks was non-significant ($P > 0.05$).

When the total number of positive and negative swab samples within each flock in each rearing system was included in the calculation, the proportion of *Campylobacter*-positive swab samples was higher for organic flocks compared with conventional flocks (OR: 4.15) and extensive indoor flocks (OR: 3.31). The overall proportion of positive swab samples was significantly higher in the period from May to October compared with the period from November to April (OR: 4.44). The random farm and house effects were small compared with the within-house error, indicating a large variation in the proportion of positive samples in flocks from the same house. The parameter estimates and 95% C.I. of the odds ratios are given in Table 1.

Within-flock prevalence of *Campylobacter*

The estimation of within-flock prevalence was based on the proportion of positive samples within each flock. Only *Campylobacter*-positive flocks were included in the calculation. The estimated within-flock prevalence was 65% for organic flocks, 68% for conventional flocks and 60% for extensive indoor flocks. These differences between rearing systems were non-significant. The mean prevalence of *Campylobacter*-positive samples in positive flocks was 64.2%. Among positive flocks only, the difference in the proportion of positive swab samples between the period from May to October and the period from November to

Table 1 Parameter estimates, odds ratios and 95% C.I. based on all flocks sampled

	May–October versus November–April	Extensive versus conventional	Organic versus extensive	Organic versus conventional	u_{farm}^*	v_{house}^\dagger	ϵ_{\ddagger}
REML Est.	1.49	0.23	1.20	1.42	0.66	0.02	4.50
Odds ratio (OR)	4.44	1.25	3.31	4.15			
OR 95% CI	[2.49; 7.89]	[0.51; 3.13]	[1.13; 9.73]	[1.57; 11.0]			

*Random farm effects.

†Random house effects.

‡Within-house error term.

Table 2 Parameter estimates, odds ratios and 95% C.I. based on *Campylobacter*-positive flocks only

	May–October versus November–April	Extensive versus conventional	Organic versus extensive	Organic versus conventional	u_{farm}^*	v_{house}^\dagger	ϵ_{\ddagger}
REML Est.	0.24	0.37	0.66	0.67	0.10	< 0.01	2.99
Odds ratio (OR)	1.27	1.45	1.94	1.96			
OR 95% CI	[0.69; 2.34]	[0.68; 2.73]	[0.56; 2.45]	[0.42; 1.47]			

*Random farm effects.

†Random house effects.

‡Within-house error term.

April was not significant. The parameter estimates and 95% C.I. of the odds ratios are given in Table 2.

Species distribution

The species distribution among the positive flocks was as follows: 70 flocks *C. jejuni* (87.5%), eight flocks *C. coli* (10.0%), while two flocks (2.5%) yielded a mixture of *C. jejuni* and *C. coli*. The species distribution of *Campylobacter* in the three rearing systems was as follows: organic flocks *C. jejuni* 91%, *C. coli* 4.5% and mixed infections (*C. jejuni/C. coli*) 4.5%; conventional flocks *C. jejuni* 86.2%, *C. coli* 10.3% and mixed infections (*C. jejuni/C. coli*) 3.5%; extensive indoor flocks *C. jejuni* 86.2%, *C. coli* 13.8% and mixed infections (*C. jejuni/C. coli*) 0%.

Antimicrobial susceptibility of *Campylobacter* isolates

Three of 53 *C. jejuni* isolates and three of nine *C. coli* isolates were resistant to one or more of the antimicrobials tested. Among 19 *C. jejuni* isolates from organic flocks, one isolate was resistant to ampicillin and tetracycline. Two *C. coli* isolates from organic flocks were susceptible to five antimicrobials. Among 24 *C. jejuni* isolates from extensive indoor flocks, one isolate was resistant to enrofloxacin and one isolate was resistant to streptomycin. Five *C. coli* isolates from extensive indoor flocks were tested; one isolate was resistant to erythromycin and one was resistant to strepto-

mycin. Ten *C. jejuni* isolates from conventional flocks were susceptible to five antimicrobials. Two *C. coli* isolates from conventional flocks were tested; one isolate was resistant to enrofloxacin and streptomycin. Isolates resistant to enrofloxacin were also resistant to nalidixic acid.

DISCUSSION

The results indicate that the special characteristics of organic broiler production provide a high prevalence of *Campylobacter*. Thus, organic broiler flocks constitute a strong potential for introduction of *Campylobacter* to the processing line upon arrival at slaughter. In other studies, a high prevalence of *Campylobacter* in poultry reared under free-range conditions has been reported (Kazwala *et al.* 1993; Tresierra-Ayala *et al.* 1995; Rivoal *et al.* 1999). Unlike conventional and extensive indoor broiler flocks, which are reared in a confined environment, organic broiler flocks have unimpeded access to soil and water in the open. Horizontal transmission from the environment has been put forward as a likely route of *Campylobacter* infection in broilers (Kazwala *et al.* 1990; Jacobs-Reitsma *et al.* 1995), and the use of hygiene measures has been shown to reduce the risk of infection in conventional rearing systems (van de Giessen *et al.* 1998; Evans and Sayers 2000; Hald *et al.* 2000; Gibbens *et al.* 2001). Hence, the presence of *Campylobacter* in water in the open (Hanninen *et al.* 1998; Obiri-Danso and Jones 1999) is a possible explanation for the high *Campylobacter* prevalence observed in organic broilers.

However, the high age at slaughter (> 81 days) of organic broilers may influence the flock prevalence of *Campylobacter*. Other investigators have reported that the risk of flock infection increased with the age of the broilers (Berndtson *et al.* 1996b; Evans and Sayers 2000). The use of a slow-growing breed of broilers is a special characteristic of organic broiler production. Different breeds of chicken may not be equally susceptible to *Campylobacter* colonization. Stern *et al.* (1990) found that resistance to caecal colonization by *C. jejuni* was significantly influenced through chicken host lineage.

Thus, due to co-linearity among factors such as age, breed, housing, feed, abattoir and rearing system, no single factor related to organic broiler production can be pointed out as the sole determinant of high *Campylobacter* prevalence. Rather, the prevalence results reported in this study reflect the combined effect exerted by factors that are inextricably related to each broiler rearing system.

A relatively high prevalence of *Campylobacter*-positive flocks was observed among extensive indoor flocks (49.2%) compared with conventional flocks (36.7%). Although not significant in the present investigation ($P > 0.05$), the observed difference between the two groups can probably be attributed to the effect of age at the time of testing, 56 days for extensive indoor flocks *vs.* 36–42 days for conventional flocks. Higher age would make extensive indoor flocks more likely to be *Campylobacter* infected (Lindblom *et al.* 1986; Berndtson *et al.* 1996b; Evans and Sayers 2000). The flock prevalence of 36.7% observed in conventional broiler flocks differs from the prevalence of 52% reported in a previous Danish study performed in 1995 (Hald *et al.* 2000). Meanwhile, the widespread implementation of hygiene barriers in recent years, and pricing of *Campylobacter*-free flocks in Denmark, are potential explanations for this difference.

Although large and significant differences in flock prevalence of *Campylobacter* were observed between organic and conventional flocks, and between organic and extensive flocks, the differences in within-flock prevalence between the three rearing systems were non-significant. Thus, the origin of a *Campylobacter*-positive flock did not seem to affect the within-flock prevalence. Results of other studies indicated that a large proportion of the individual broilers in *Campylobacter*-positive flocks were colonized (Berndtson *et al.* 1996b; Gregory *et al.* 1997; Evans and Sayers 2000; Hald *et al.* 2001) while in this study, most positive flocks yielded some negative swab samples. This may reflect actual differences in within-flock prevalence of *Campylobacter* at the study locations at the time of sampling, or differences in sample handling procedures and sample material used (caecal samples, swab sampling of faecal droppings or cloacal swabs).

For conventional and extensive indoor flocks the observed species distribution (*C. jejuni*/*C. coli*) was in agreement with

results of previous studies of *Campylobacter* in Danish broiler flocks (Hald *et al.* 2000; Wedderkopp *et al.* 2000). For organic flocks, the proportion of *C. jejuni*-positive flocks and flocks that yielded a mixture of *C. jejuni* and *C. coli*, was slightly elevated compared with the other production categories.

A low level of antimicrobial resistance was found among *Campylobacter* isolates from the three broiler rearing systems. This observation correlated with results reported through the DANMAP (2000b). A low level of antimicrobial resistance in *Campylobacter* isolates from broilers was also reported from Sweden (Berndtson 1996). Other investigators found high levels of resistance to ampicillin and tetracycline in *Campylobacter* isolates from broilers (Jacobs-Reitsma *et al.* 1994b; Saenz *et al.* 2000). These variations might reflect differences in consumption of antimicrobial agents in the broiler production in different countries. In the present investigation, low numbers of resistant isolates (six of 62 isolates) hampered comparison of resistance patterns of *C. jejuni* as well as of *C. coli* isolates between the three rearing systems. Thus, no relation between resistance pattern and origin of the *Campylobacter* isolates could be established.

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