Prevalence and sources of *Campylobacter* spp. contamination in free-range broiler production in Belgium

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An one year epidemiological study was carried out between February 2005 and January 2006 in Belgium to assess the *Campylobacter* prevalence in free-range broiler production. Three successive broiler flocks on 6 belgian farms were investigated for the presence of *Campylobacter* ssp. during the rearing period. Each flock was visited four times, before and after the outdoor rearing period. During each visit, samples were taken in the broiler houses (litter, cecal droppings, water-lines, feed, entrance premises) as well as from the outer rearing environment (open-air range). Conventional microbiological methods combined with biochemical tests were used for the *Campylobacter* detection, species identification and isolation. *Campylobacter* prevalence was very high in free-range broiler production during the experimental period. *C. jejuni* is the main species isolated from all contaminated samples, while mixed C. jejuni/C. coli infections sometimes occured. Contamination of the broiler flocks was increased in summer/autumn, with a 100% flocks contamination, whereas only 4 (66.7%) and 3 (50%) of the flocks became *Campylobacter* positive in spring and winter respectively, at the end of the rearing period. Moreover, about 53.8% of contaminated flocks were infected with *Campylobacter* before chicks have access to the open-air range. In 69.2% of the *Campylobacter*-positive flocks, the open-air range soil belonged partly of fully to environmental samples found to be *Campylobacter*-positive before flock infection. The other potential sources of infection were delivery tray, entrance premises floor and water-lines. The access to an open-air range seems to be an important way of contamination of broilers because *Campylobacter* prevalence in the flocks increased after going out.

Keywords: *Campylobacter*; free-range broiler production, epidemiological study

Introduction

Campylobacter is a well recognized cause of human acute enterocolitis. *Campylobacter* infections are considered a worldwide problem of economic burden and public health, causing considerable suffering and loss of productivity. During the last 30 years, reported cases of human campylobacteriosis has dramatically increased in industrialised countries. The number of confirmed cases registered in the EU Member States and the reported incidence rate per 100 000 inhabitants vary widely, i.e. from 0 in Cyprus to 249.6 in Czech Republic in 2004. The overall incidence of campylobacteriosis was 47.6 per 100 000 population in the EU-25, which is slightly higher than for *Salmonella* (European Food Safety Authority, 2004). This make *Campylobacter* the most commonly reported gastrointestinal bacterial pathogen in human in the EU. With the exception of Spain and Sweden, all EU-15 Member States reported an increase in the number of human cases of campylobacteriosis, with an overall incidence increase of 32% in 2004 compared to 2003. More than

95% of registered *Campylobacter* enteritis are caused by thermotolerant species, i.e. *C. jejuni* and *C. coli* (Butzler, 2004).

Although unpasteurized milk and contaminated drinking water has been commonly associated with epidemics and sporadic cases, campylobacteriosis is mainly recognized as a food-borne infection in developed countries. The reservoir of *Campylobacter* spp. comprises wild and domestic animals, particularly birds. Therefore, the main source of *Campylobacter* infections is the consumption of contaminated foods of animal origin, particularly raw or insufficiently cooked poultry meat. Over the last decade, the occurrence and spread of *Campylobacter* in conventional broiler flocks has been intensively studied in several countries. Flock prevalences ranging from 18% to 90% have been reported in Europe (Evans et Sayers, 2000; Refrégier-Petton *et al*., 2001; Stern *et al*., 2001; Newell et Fearnley, 2003), with the northernmost countries having substantially lower figures than southern European countries. A seasonal variation in the prevalence of *Campylobacter*-positive broiler-flocks has also been reported from Norway (Kapperud *et al*., 1993) as well as from France (Refrégier-Petton *et al*., 2001) and UK (Wallace *et al*., 1997).

Less 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 Denmark, 100% of cloacal samples from organic broiler flocks were contaminated, while only 36.7% of conventional broiler flocks were Campylobacter-positive (Heuer *et al*., 2001). These observations indicate that free-range rearing of poultry could be associated with a higher prevalence of *Campylobacter* than conventional standard production, the access to an open-air range being un additional risk factor as mentioned by Huneau-Salaün *et al.* (2005).

In Belgium, the incidence of human *Campylobacter* infections is well documented, with a relatively high number of reported cases, i.e. 64.6 cases per 100 000 inhabitants, compared to others EU-member States (European Food Safety Authority, 2006). The incidence is stable since 2000 (Institut scientifique de Santé publique, 2005). On the other hand, no epidemiological informations were recorded about the prevalence of *Campylobacter* in broiler flocks, neither in conventional poultry production, nor in free-range broiler flocks.

The aim of the present study was to determine the flock prevalence of *Campylobacter* from free-range broiler production, a fast-expanding poultry rearing system in Belgium. Furthermore, the study aimed to evaluate potential contamination sources at the farm level, including among others food, litter, drinking water or free-open range. In accordance with the request related to *Campylobacter* national surveillance recorded in the EFSA scientific report (European Food Safety Authority, 2005), the collected data will help to develop an effective control program to reduce the broiler flocks prevalence of *Campylobacter*, and than the campylobacteriosis incidence, at national level.

Materials and methods

To assess the *Campylobacter* prevalence in free-range broiler production, 6 farms rearing slow-growing broiler strains were investigated. Broilers had free access to an open-air range from 6 weeks of age and were slaughtered at minimum 82 days of age. The French-speaking provinces of Belgium, i.e. the Walloon Region, comprise about 70 free-range broiler production farms, so we investigated about 9% of Walloon farms. Three successive broiler flocks were sampled on each farm, from February 2005 to January 2006, for the presence of *Campylobacter* ssp. during the rearing period. Each broiler flock was comprised of ca. 4 800 birds, with a rearing period of 12 weeks.

Each flock was visited four times, before (1 and 27 days of age) and after (54 and 81 days of age) the outdoor rearing period. The first visit was carried out just before the setting up of the chicks, and samples were taken aseptically in the disinfected broiler house (litter, water-lines, feed and swabs from exit trap doors and from the floor) as well as from the outer rearing environment (open-air range). For the 3 following visits, samples consisted of caecal droppings taken from the whole house litter, and of surface soil of the open-air range. All samples were collected on each occasion within 1 to 2h and transferred in insulated boxes containing ice packs for transport to the laboratory. They were kept at 4°C for 2 weeks maximum prior to the microbiological tests.

Campylobacter detection and isolation methods were based on the ISO 10272 procedure. Briefly, each swabs or 25g of solid material were inoculated into 100 ml selective enrichment Bolton broth (Oxoid, Belgium) supplemented with 0,5% lysed defibrinated horse blood. Water samples were filtered on a sterile 0,45 µm microporous filter (Zetapor, CUNO Benelux, Belgium) prior to add the latter to 100 ml of broth. All sample samples were then subjected to pre-enrichment step at 37°C for 4h followed by enrichissement culture at 42°C for 44h in microaerophilic atmosphere. After enrichment, the samples were streaked onto selective agar media (Karmali agar, Biokar, Belgium; mCCDA agar, Oxoid, Belgium) and the plates were incubated in jars at 42°C for 48h in microaerophilic atmosphere obtained using commercial gas generating kits (Anaerocult C, VWR International, Belgium). From each positive agar plate, several typical campylobacter colonies was subcultured onto Brucella agar (48h, 42°C) and tested for Gram-staining, motility, production of oxidase and catalase, hippurate hydrolysis and antibiotic susceptibility to nalidixic acid and cephalothin.

Results and discussion

Data for all successive flocks for the 6 farms studied are summarized in *Table 1*. The letter denotes farm codes A through F.

Table 1 Free-range production farm contamination by *Campylobacter* spp. in Belgium, according to the flock and the age of the broilers.

Farm	**Flock 1** *(Febr.-June)*		**Flock 2** *(July-Sept.)*		**Flock 3** *(Oct.-Jan.)*	
	Contamination	**Day(s) of age**	**Contamination**	**Day(s) of age**	**Contamination**	**Day(s) of age**
A	-	-	+	54	-	
B	+	27	+	54	-	
C	-	-	+	81	-	
D	+	54	+	54	+	1
E	+	27	+	1	+	1
F	+	54	+	27	+	1
	66.7 %		**100 %**		**50 %**	

1 day of age : setting up of chicks; 27 days of age: before the outdoor rearing period; 54 and 81 days of age: after the outdoor rearing period.

From February to June 2005, i.e. in spring, the *Table 1* shows that 4 farms (66.7%) were *Campylobacter*-positive. The extent of contamination increased in summer until October (flock 2), to reach a prevalence of 100 %. Furthermore, in winter, only 3 farms (50%) were contaminated by *Campylobacter*. Similar observations were reported in Denmark for organic broiler flocks (Heuer *et al.*, 2001), in the US for conventional poultry production (Stern *et al*., 2001) and in France for extensive outdoor broiler production (Huneau-Salaün et al., 2005).

Except for the third flock, farm contamination was mainly detected after the exit of the chickens on the free-open range. In 60 % of the positive first and second flocks, actually, the appearance of infection was a relatively late event, being detected at 8 weeks of age or later. So, unlike Stern *et al*. (2001) who found 92.8 % of the *Campylobacter*-positive flocks being detected at 4 to 6 weeks of age, a not negligible number of contamination cases were observed before 4 weeks of age. This may have been due partly to environmental contamination of the house surroundings from which the infection could have arisen. Samples from the entrance premises floor were actually frequently contaminated by *Campylobacter*.

Table 2 gives details of flocks in which environmental samples were found to be *Campylobacter*-positive before flock infection was detected. In 69.2% of the flocks did positive environmental samples come partly of fully from the open-air range.

Table 2 Environmental samples found to be *Campylobacter*-positive prior to the appearance of flock infection.

Flock	Farm code	Day(s) before infection detected	Positive samples in period preceding infection
1	A	0	-
	B	27	Open-air range
	C	0	-
	D	54	Open-air range, litter
	E	27	Open-air range, entrance premises
	F	54	Open-air range
2	A	54	Open-air range
	B	54	Entrance premises
	C	81	nd
	D	54	Entrance premises
	E	1	Open-air range, exit trap door
	F	27	nd
3	A	0	-
	B	0	-
	C	0	-
	D	1	Open-air range, delivery tray
	E	1	Open-air range, water-line
	F	1	Open-air range, water-line, entrance premises, delivery tray

nd: not determined

Moreover, results for the third flock, from October to January, are distinctive. Contamination for the 3 *Campylobacter*-positive farms was revealed just before the setting up of the chicks, only from the free-air range soil, the water-line, the delivery tray and the indoor premises swabs. Broilers fecal droppings were infected later, at 4 to 8 weeks of age. It can be concluded that unsuitable hygiene practices at the farm level, especially poor cleaning and disinfection of the house and not dedicated protective clothing, could be a major reason of *Campylobacter* contamination persistence in poultry flocks, as summarized by Allen and Newell (2005). The free-air open range seems, however, to be a major source of *Campylobacter* contamination, and a more difficult parameter to control.

The species distribution among the *Campylobacter*-positive flocks shows that *C. jejuni* is predominant in free-range broiler production in Belgium, with a prevalence from 50 to 75% according to the flock considered. Particularly, mixed infections *C. jejuni*/*C. coli* represented 42.8%, while *C. jejuni* and *C. coli* only represented 42.8% and 14.4% respectively. These results are in agreement with Van looveren *et al*. (2001) who found 79% *C. jejuni* in broiler carcasses and meat from Belgian slaughterhouses. In organic flocks, Heuer et al. (2001) determined a prevalence of 91%, 4.5% and 4.5% for *C. jejuni, C. coli* and *C.jejuni*/*C. coli* respectively. On the other hand, Frediani-Wolf and Stephan (2003) found that all isolated *Campylobacter* strains from poultry carcasses in a Swiss slaughterhouse were identified as *C. jejuni*. It should be mentioned that poultry carcasses could be cross-contaminated by different *Campylobacter* species during the slaughter processing, leading to different results compared to *Campylobacter* contamination of the broiler flocks.

In summary, the results of this study provided for the first time informations about the *Campylobacter* prevalence in Belgian free-range broiler production and pointed out potential sources of *Campylobacter* for this kind of rearing system. The data, recorded from February 2005 to January 2006, showed a high prevalence of enteric *Campylobacter* in free-range broiler production in Belgium, similar to results from other European states. The *Campylobacter* prevalence increased in summer-fall, in relation with increased reported cases of human campylobacteriosis during this period.

As approaches like vaccination are not yet feasible, suitable biosecurity measures to exclude campylobacters from free-range broiler flocks are currently the only intervention available. Nevertheless, this study showed that the access to an open-air range seems to be an important way of contamination of broilers because *Campylobacter* prevalence in the flocks increased after going out. The open-air range, which represents an obligatory step for free-range broiler production, appears consequently as a burdensome parameter to take into account in further development of Campylobacter control programs.

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