Campylobacter in the Pork Food Chain : a quantitative hazard analysis

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Abstract

Campylobacter are one of the most frequent causes of bacterial enteritis in industrialized countries and are widespread in food animals. Pigs are known to be largely contaminated in farms, but few data exist about the status of the pork food chain.
The purpose of this study was to quantify the *Campylobacter* contamination of the French pork food chain : prevalence, contamination level, bacterial species in primary production (piglets and fattening pigs when slaughtered), and in first and second transformation process (from carcasses before chilling to deboned meat cuts).
A total of 1286 rectal samples (from 1036 piglets 25 days old, and from 250 fattening pigs) and 3500 meat samples (from 550 carcasses and 300 meat cuts, 2 or 8 samples/carcass and 2 samples/cut, 25 cm^2/sample) were collected from 9 pigs from confined farrow-to-finish farms (3 batches per farm were tested over a year), randomly selected, and five slaughterhouses and six cutting plants.
Bacteriological results showed that 77% of the piglets and 100 % of the fattening pigs were infected with high levels of contamination: 40 000 cfu/g of faeces (50 to 5 10^6 cfu/g). Before chilling, 23% of the carcasses (2 sites) were contaminated with low levels (2.3 cfu/cm^2 as a mean value) with high variations between samples (0.4 to 330 cfu/cm^2), and 9,7% of the carcasses (8 sites) after chilling were contaminated. Primal cuts contamination was lower than 1%, and no *Campylobacter* detected after deboning.
On the basis of multiplex-PCR identification, 0 isolates were identified as *C. jejuni*, 91% (1028/1128) as *C. coli* and 9% (100/1128) campylobacter-like.
From these data we concluded that *Campylobacter coli* carriage is high in pork primary production, but hygiene procedures (GHP, HACCP...) are essential to maintain the low contamination of carcasses and meat cuts.
The link between *Campylobacter* porcine and human strains remains to be established, and their virulence for humans studied.

Introduction

Campylobacter are one of the most frequent causes of bacterial enteritis in industrialized countries (OMS, 2005) and are widespread in food animals. Pigs are known to be largely contaminated in farms (Weijtens, et al., 1997 ; Magras et al., 2004 ; Payot et al., 2004) and *Campylobacter* colonization seems to occur at an early age (Weijtens, et al., 1997 ; Weijtens et al., 1999 ; Magras et al., 2004). Nevertheless, few data exist on the status of the pork food chain and particularly on the contamination level of primary products (animals) and meat (Pearce et al., 2003). The purpose of this study was to quantify the *Campylobacter* contamination of the French pork food chain: prevalence, contamination level, bacterial species in primary production (piglets and fattening pigs when slaughtered), first transformation (carcasses before and after chilling) and second transformation process (deboned meat cuts).

Material and methods

In 9 confined farrow-to-finish farms situated in the western part of France, 1036 rectal samples (5g/sample) from 25 days old piglets were collected. In each farm, 3 batches were tested over a year, and within a batch 10 nursing dams and 4 piglets by litter were randomly selected.
In 5 slaughterhouses, 250 rectal samples (5g/sample) and 500 meat surface samples from corresponding carcass (25 cm^2/sample, two samples/carcass) were collected from 10 visits during 4 months.
In 6 cutting plants, 300 randomly selected refrigerated carcasses were sampled on 8 sites (25 cm^2/site) over a 2 years period. On 75 of these carcasses, 4 primal cuts were sampled before and after deboning.

Preston broth (10 ml) was added to rectal and meat samples for selective enrichment before inoculation on Karmali and Butzler media. Plates were incubated at 42°C in microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2). After 5 days, suspected colonies were confirmed by typical morphology, darting motility and Gram staining tests. Species identification of isolates was conducted by PCR-Multiplex (Van de Giessen et al. 1998).

Results and discussion

On all the 9 farms, pigs were heavily contaminated by *Campylobacter*. *Campylobacter* was recovered from 77 % (95% CI :74-79) of the faecal samples collected from the 1036 piglets. Nevertheless some differences between the 9 farms in the number of pigs tested positive for *Campylobacter* were statistically significant ($p < 0.005$ SNK test). On the basis of identification with multiplex-PCR, *C.coli* was the only species recovered from the faecal samples.
The high prevalence rates reported in this study agree with other results indicating prevalence of *Campylobacter* of 85 % amongst piglets (Weijtens, et al., 1997 ; Young et al., 2000). This study confirms that piglets are already intestinal carriers of *C. coli* at the age of 25 days on the piggeries. Young et al. (2000) have described a predominant infection of pigs by *C.jejuni* in the USA. In our study and in agreement with Nesbakken et al. (2003), *C.jejuni* had never been isolated from the faecal samples. These findings suggest that the prevalence of the respective species might differ considerably between countries. An other explanation may be the use of different identification procedures.

At slaughter, bacteriological results showed that 100 % of the pigs were infected with high levels of contamination : 40 000 cfu/g of faeces on average (50 to 5 10^6 cfu/g). Before chilling, 23% (95% CI : 18-29) of the carcasses (2 sites) were contaminated with low levels (2.3 cfu/cm^2 as a mean value) with high variations between samples (0.4 to 330 cfu/cm^2).
Our prevalence is in agreement with the 29% observed by Nesbakken et al. (2003), but in other studies hot carcasses contamination varies from 6.7% to 66% (Pearce et al., 2003 ; Sorensen et Christensen, 1997) ; differences in sampling and analytical methods may explain these results.
At cutting plants, 9,7% (95% CI : 6.6-13.4) of the refrigerated carcasses (8 sites) were contaminated. When calculated with the same sites than on the hot carcasses (2 sites), prevalence was 4,4 % (95% CI : 2.2-7.4). After further processing, primal cuts contamination was lower than 1%, *Campylobacter* being recovered from only 2 samples, and no positive sample was found on deboned cuts.
In a study conducted by the USDA on market hogs in 1995 and 1996 (USDA, 1996), *Campylobacter* could be enumerated from 20,5% of chilled carcasses, with an average contamination of 0,1 cfu/cm^2 (0.03 to 46 MNP/cm^2) by destructive method. Zerby et al. (1998) reported a prevalence of 7.9% using a 3 sites swabbing method. Sampling method (destructive or non destructive), carcass sites sampling (pooled or individual samples from, number and and size of sampled sites) and analytical methods (detection level and media) could explain the difference in the observed prevalence.
Some authors have previously reported a diminution of the prevalence during refrigeration process (Pearce et al., 2003 ; Gürtler et al., 2004), and the effect of different chilling systems have been studied (Oosterom et al., 1983 ; Chang et al., 2003 ; Laroche et al., 2004). Complex factors as

humidity, ventilation, thermal stress, and oxidation are involved in Campylobacter survival during chilling and could explain these results.

A total of 1128 isolates were obtained from piglets to meat cuts. On the basis of identification with multiplex-PCR, no *C. jejuni* was recovered from the samples. *C. coli* was the only species identified with 91% (1028/1128) of the isolates, 9% (100/1128) being identified as campylobacter-like.

Conclusions

According to these results, *Campylobacter coli* appears to be specific to the French pork chain. Despite an early intestinal carriage at farms and a high faecal contamination levels of pigs at slaughter, carcass contamination is lower than it could be expected at the end of the slaughter line. If 23% of the carcasses are contaminated before chilling, the average contamination level is only about 2 cfu/cm^2, thanks to good hygiene procedures (GHP) during slaughtering, preventing evisceration accident and cross contamination.
After chilling, carcass prevalence droped to 4,4% (2 sites), *Campylobacter* survival being affected by industrial refrigeration conditions. During further processing, from primal cuts to deboned meat, contamination is reduced as reported for other pathogenic bacteria (Salmonella or STEC) due to skin removal and GHP limiting cross contamination.

From these data we concluded that *Campylobacter coli* carriage is high in French pork primary production, but efficient hygiene procedures (GHP, HACCP...) in slaughter and cutting plants, together with *Campylobacter* decrease during refrigeration, contribute to a very low contamination of meat cuts.
The link between *Campylobacter* porcine and human strains remains to be established, and their virulence for humans studied.

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Topic n.2