International Journal of Food Microbiology 124 (2008) 188–194

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination

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ARTICLE INFO

Article history:
Received 8 June 2007
Received in revised form 6 February 2008
Accepted 24 March 2008

Keywords:
Campylobacter
Poultry
Slaughterhouse
PCR-RFLP
Disinfection
Survival

ABSTRACT

Campylobacters are a primary cause of human bacterial enteritis worldwide. They are usually considered susceptible to the disinfectant molecules used in the food industry. The purpose of this study was to see if campylobacters could survive cleaning and disinfection in poultry slaughterhouses and whether the strains recovered could contaminate carcasses during processing. Samples obtained from the environment before and after cleaning and disinfection (transport crates, processing equipment surfaces, scald tank water) and from birds (fresh droppings, neck skins) were collected during 7 investigations in 4 different slaughterhouses. Out of 41 samples collected, 30 *Campylobacter jejuni* strains were recovered from the surfaces of processing equipment before cleaning and disinfection procedures in three slaughterhouses and 9 *C. jejuni* out of 51 samples collected were found after cleaning. The study was then focused on one slaughterhouse to trace passage of the pathogen on poultry carcasses. The antimicrobial resistance phenotypes (P) (minimum inhibitory concentration, MIC) of the *C. jejuni* isolates collected in this slaughterhouse were determined. Nine phenotypes could be distinguished. Three of these were of interest as they were found in isolates recovered after cleaning and disinfection procedures. The genotypes (G) were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) of isolates with one of the three phenotypes of interest. Clusters constructed by combining the phenotype and genotyping observations (P*G type) were compared between isolates obtained after cleaning and disinfection, and isolates from droppings, neck skin and transport crate samples of slaughtered poultry flocks. Only one P*G type of strain was recovered from surfaces after cleaning and disinfection and from neck skin samples but was also recovered from transport crates. Our findings indicate that *C. jejuni* is able to survive overnight on food processing equipment surfaces, after cleaning and disinfection procedures, and that these strains may contaminate carcasses during the slaughter process. These results add to our understanding of poultry carcass contamination and highlight the need to develop ways of reducing the risk of human infection with *Campylobacter* through the consumption of poultry products.

1. Introduction

Campylobacter is one of the most common causes of human bacterial enteritis worldwide. *Campylobacter* infections are predominantly caused by thermophilic campylobacters, in particular *Campylobacter jejuni* and its close relative *Campylobacter coli* (Anonymous, 2003, 2004). The major sources of human infection are the consumption and handling of poultry and poultry products (Corry and Atabay, 2001). Data from EFSA indicates that in 2004, 81.7% of French poultry meat at slaughter were contaminated by *Campylobacter* sp (EFSA 2004). However, the sources of carcass contamination and the mechanisms by which the organisms spread between birds during processing are not fully understood. This knowledge is needed to develop efficient strategies to reduce the risk of this foodborne zoonosis for consumers. Carcasses from *Campylobacter*-free chickens can be contaminated with strains present in the crates used to transport the birds to slaughterhouses (Newell and Fearnley, 2003). *Campylobacter* present in the intestinal tract of poultry can contaminate the environment and other carcasses during evisceration and at various steps in the slaughter process, via equipment and water (Alter et al., 2005; Berndtson et al., 1996; Miwa et al., 2003; Rasschaert et al., 2006; Rivoal et al., 1999). However, because of the high speed and technical nature of poultry slaughter and processing, combined with the extensive use of potable water during slaughter, it is difficult to prevent cross-contamination between birds and between flocks within the slaughterhouse (Borck and Pedersen, 2005). This may be a significant source of contamination when *Campylobacter*-free flocks follow *Cam-*

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doi:10.1016/j.ijfoodmicro.2008.03.030

pylobacter-positive flocks through the process. This has led to the recommendation that *Campylobacter*-free flocks should be slaughtered first to reduce the risk to the consumer.

Little information is available about *Campylobacter* susceptibility to disinfectants. *Campylobacter* species are generally considered susceptible to the disinfectants commonly used in the food processing industry (Avrain et al., 2003; Blaser et al., 1986). However the survival of *Campylobacter* on slaughterhouse surfaces after cleaning and disinfection has been poorly documented. No studies have been reported on the isolation of *Campylobacter* species from swabs of surfaces in contact with food after cleaning and disinfection procedures (Borck et al., 2002; Cools et al., 2005; Malakauskas et al., 2006; Miwa et al., 2003). In one study, *Campylobacter* contamination of kitchen polypropylene surfaces was significantly decreased after chlorine disinfection, but was still detectable (Cogan et al., 1999), suggesting that campylobacters are likely present on slaughterhouse surfaces after routine cleaning and disinfection procedures, and this could be another source of carcass contamination. The first aim of the present study was to determine if *Campylobacter* could be isolated from surfaces in four French slaughterhouses after routine cleaning and disinfection procedures.

Highly sensitive and reproducible methods of strain differentiation are required to trace *Campylobacter* through the food chain (Hazeleger et al., 1998). As *C. jejuni* and other thermophilic campylobacters grow optimally at a relatively high temperature (42 °C), and do not grow at 30 °C (Hazeleger et al., 1998), they are not able to grow in slaughterhouse environment or in samples collected. It can therefore be hypothesized that antimicrobial phenotype will remain stable even if the strains are stressed by the physical conditions, such as low temperature, found in slaughterhouses. The antibiotic and disinfectant phenotypes can be used as first line markers. Phenotypic methods are less discriminating than genotyping methods (Wassenaar and Newell, 2000), in order to increase the discriminatory power in our study, restriction fragment length polymorphism (RFLP) method was performed on selected isolates. PCR-RFLP is a recommended method for typing poultry *Campylobacter* strains during the slaughtering process because of its low levels of strain non-typeability, acceptable levels of discriminatory power, and cost-effectiveness (Newell et al., 2001).

The second aim of our study was to detect with antimicrobials phenotyping and PCR-RFLP genotyping possible routes of carcass contamination by investigating the contamination of carcasses by strains isolated after cleaning and disinfection in a single slaughterhouse.

2. Materials and methods

2.1. Collection of samples in four poultry processing plants

Four unrelated French poultry slaughterhouses (designated A, B, C and D, located in Brittany and Pays de la Loire) were visited from August 2005 to March 2006. Each plant processed poultry to finished products as carcasses and portions with capacities of 6000 to 9000 birds per hour for broilers, 5000 guinea fowls per hour and 2000 turkeys or ducks per hour. Plant A was examined three times, plant B twice, and plants C and D once only. Plants A and C processed chicken, turkeys and guinea fowl on the same chain. Plant B processed poultry and guinea fowl on one chain and turkeys and ducks on a second chain. Plant D only processed turkeys. The slaughter chains for broilers and guinea fowl were entirely automated. For turkeys and ducks, most of the evisceration chain after plucking was carried out manually.

For each investigation, the planned sampling on two consecutive days was organised as shown in Fig. 1. At the end of day 1, birds of the last flock slaughtered were sampled as well as their transport crates. A flock was defined as all birds reared in the same poultry house and slaughtered at the same time. The surfaces of several evisceration machines and scald tank water were also sampled before the cleaning and disinfection procedures. At the beginning of the second day, the same surfaces were sampled after cleaning and disinfection and before processing of the first flock slaughtered. The birds in subsequent flocks (one to five flocks, depending on slaughterhouse activity and flock size) were then sampled along with their transport crates.

2.2. Sampling of birds (dropping and neck skins) in slaughterhouse A

For the second part of the study, the presence of *Campylobacter* was traced during a single campaign of sampling in slaughterhouse A. Six flocks of birds were sampled: the last flock slaughtered before cleaning and disinfection procedures in the slaughterhouse and the first five flocks slaughtered on the next day. A pooled sample of 10 fresh droppings was taken from the transport crates for each flock slaughtered. Neck skin samples were collected during processing before the carcasses entered the chilling room. Five neck skin samples were taken from the last flock slaughtered before cleaning and disinfection, 5 neck skin samples were taken from the first flock slaughtered after cleaning and disinfection, and 2 neck skin samples were taken from the 4 flocks slaughtered subsequently. A total of 6 pooled samples of droppings and 18 neck skin samples were collected. All samples were kept at 4 °C until further processing (within 48 h).

2.3. Sampling of processing equipment and scald tank water

In the 4 slaughterhouses visited, pre-cleaning of the equipment was done with high-pressure water. Cleaning and disinfection procedures were known for 3 of the 4 slaughterhouses. Cleaning was made using a device with foam containing alkaline-chlorinated molecules in slaughterhouses B and D and with a neutral detergent in slaughterhouse A. Quaternary ammonium compounds combined with glutaraldehyde were used as disinfectant for equipment in the slaughterhouses A and D. In slaughter B, disinfection of equipment was realised with poly (hexamethylene biguanide) chlorohydrate.

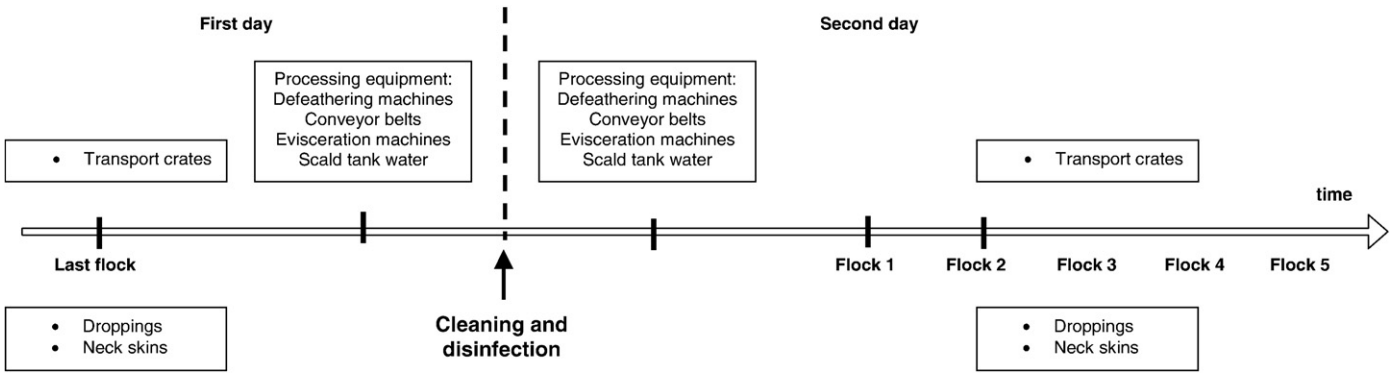

Fig. 1. Chronology of sampling.