Campylobacter contamination during poultry slaughter in Belgium

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Summary

The relation between internal carriage and surface contamination with thermophilic Campylobacter species in broilers was examined by molecular typing methods. Samples from 39 flocks were collected from three Belgian poultry slaughterhouses. From each flock, crop swabs before slaughter, and intestines and neck skins during slaughter were collected. A total of 308 isolates were identified at species level and further characterized by flagellinA PCR/Restriction Fragment Length Polymorphism and Pulsed Field Gel Electrophoresis. Isolates were identified as Campylobacter jejuni (88.9%), Campylobacter coli (8.8%) and Campylobacter lari (2.3%) and 27 genotypes could be distinguished. In only six flocks, genotypes isolated from the neck skins were also found in the alimentary tract from previously slaughtered flocks. Four of these flocks were initially Campylobacter free.

Introduction

The thermophilic Campylobacter species are leading causative agents of bacterial enteritis in the developed countries. Consumption or inadequate handling of raw or undercooked poultry products have been identified as major risks for infection. The initial source of contamination of broilers is still unidentified. In living birds, infection can be introduced by horizontal transmission during rearing. The lack of a hygiene barrier, dirty footwear, rodents and insects, thinning of the flock, inappropriate cleaning of the broiler house or a too short down period have been identified as risk factors for transmission (Berndtson, 1996, Hald, 2001, Petersen, 2001). Once introduced, campylobacters spread very quickly throughout the broiler house, probably via the drinking nipples (Berndtson, 1996). At the age of five to six weeks, the birds are loaded into crates and transported to the slaughterhouse. These crates are washed and disinfected after use. However, inadequately washed and disinfected crates have been identified as an additional source of contamination for chickens subsequently transported in such crates. Newell et al. (2001) observed that carcasses from a Campylobacter free flock were contaminated with the same genotype that was previously isolated from the crates used to transport the birds.

During slaughter, carcass contamination may occur due to the plucking and the evisceration process. The rubber fingers applied in the defeathering process exert pressure on the carcasses, forcing potential contaminated fecal material out and spreading it on the carcasses and the slaughter equipment (Berrang, 2001, Oosterom, 1983). Logistic slaughtering is not established in Belgium. Therefore, it is possible that a Campylobacter free flock becomes contaminated during slaughter by previously slaughtered flocks.

The aims of the study were to determine the presence of campylobacters on poultry carcasses and to study the genetic diversity in the alimentary tract and on the carcasses in order to identify the contamination source for successive flocks.

Materials and methods

The study was conducted in three Belgian poultry slaughterhouses (A, B, C) from January to July 2002. Each slaughterhouse was visited three times with a minimum interval of two weeks between visits. The slaughterhouses were visited on Mondays. In total, 39 broiler flocks were examined. All farms applied an all-in, all-out system. Birds were between 35 and 42 days old when slaughtered. In slaughterhouse A, B and C there were respectively 13 (A1 to A13), 16 (B1 to B16), and 10 (C1 to C10) flocks sampled. From each flock, 30 living birds were randomly selected from 15 crates or different
transport containers. Of each bird, one swab sample of the crop was taken and stored and transported in Buffered Peptone Water (BPW, Oxoid, Basingstoke, UK). Additionally, from each flock 30 gastrointestinal tracts and 20 neck skin samples were collected at random immediately after evisceration and before chilling respectively. All samples were packed in sterile plastic bags and transported to the laboratory under cooled conditions and processed the same day.

From each of the 30 gastrointestinal tracts, 1 g content of the duodenum and 1 g content of one cecum were aseptically collected. These samples were pooled to create three subsamples of 10 g cecae content and three subsamples of 10 g duodenum content. The pooled samples were homogenized with 90 ml BPW in a stomacher blender at normal speed. Thirty crop swabs were pooled to three subsamples and homogenized with 25 ml BPW. From each neck skin sample, 10 g was homogenized with 90 ml BPW. Of each of the homogenates, 1 ml was added to 9 ml selective Preston broth (Nutrient Broth n°2 CM 67, Oxoid, Basingstoke UK, enriched with 5% (v/v) lysed defibrinated horse blood and 1% Preston Supplement (5000 IU polymixin B, 0,010 g rifampicin, trimethoprim and 0,010 g amphotericin dissolved in 10 ml ethanol)). The homogenates were incubated for 24 to 48 h at 42°C under microaerobic conditions. After 24 h, 10 µl was plated onto modified Cefoperazone Charcoal Deoxychololate Agar (mCCDA, CM 739 plus SR155, Oxoid, Basingstoke, UK) and incubated for 24 h to 48h at 42°C under microaerobic conditions. If there was no Campylobacter growth on mCCDA after 24 h of incubation, 10 µl of the 48 h incubated homogenates was plated on a new mCCDA plate. Morphologically typically colonies were picked, examined by Gram-staining and subcultured on a blood agar plate (CM 965 and L13, Oxoid, Basingstoke, UK and 5% (v/v) defibrinated horse blood). One colony of each of the pooled samples of the crop, the small intestines and the ceca and a maximum of nine isolates of the neck skin samples were stored in whole horse blood at −80°C for further examination.

All isolates were identified at species level using the PCR assay of Vandamme et al. (1997) and Linton et al. (1996). All isolates were further characterized by FlagellinA PCR/Restriction Fragment Length Polymorphism (FlaA typing). The method of Nachamkin et al. (1993) using Ddel (Promega, Madison, Wisconsin, USA) as restriction enzyme was applied.

For each FlaA type within one flock, at least one from the crop, one from the small intestines, one from the ceca and one isolate from the neck skins was randomly chosen for further characterization by Pulsed Field Gel Electrophoresis (PFGE) using Smal as restriction enzyme (Invitrogen, Paisley, UK).

Gels were stained in ethidiumbromide and digitally captured under UV light. Gel images were analyzed with GelCompar version 3.0 (Applied Maths, Sint-Martens-Latem, Belgium).

**Results and discussion**

Twenty eight flocks arriving at the abattoir were infected with campylobacters. This prevalence of 72% is similar to another Belgian study with 67% of the flocks slaughtered carrying campylobacters in the ceca (Herman et al., 2003) and a Dutch study with a prevalence of 82% (n=187) (Jacobs-Reitsma et al., 1994). Other studies reported prevalence of flocks infected with campylobacters at farm level. This prevalence ranges between 27% (n=287) in Sweden (Berndtson, 1996) and 95% (n=100) in the UK (Evans, 1997). This difference may be due to different isolation methods, the health herd management on the farms and probably the season in which the chickens were raised. In the present study, 11 (28%) flocks were considered as Campylobacter free, since no campylobacters could be isolated from the crop, the duodenum nor the ceca. After slaughter, no campylobacters were found on the neck skins of seven flocks (18%). These seven belonged to the 11 flocks which were considered as Campylobacter free. The remaining four flocks had 1 to 18 contaminated neck skins.

In total, 308 isolates were identified at species level. Two hundred seventy four (88.9%) isolates were identified as C. jejuni, 27 (8.8%) as C. coli and 7 (2.3%) as C. lari. In abattoir A, three successively slaughtered flocks harbored C. lari in their small intestines and/or ceca. C. coli were isolated from the small intestines and/or ceca of one flock slaughtered in abattoir A and from three flocks slaughtered in abattoir B. All isolates of flocks slaughtered in abattoir C were identified as C. jejuni.

Characterizing the 308 isolates with FlaA genotyping resulted in 22 different FlaA genotypes. A total of 133 isolates were further characterized with PFGE, using the Smal restriction enzyme. Twenty five genotypes were distinguished. Combining the two methods resulted in 27 genotypes.

The contamination on the carcasses correlated with the carriage of campylobacters in the crop, the duodenum or the ceca of the chickens just before slaughter. The carcass contamination by the intestinal content of the own flock seems to be more important than cross-contamination by previously slaughtered flocks, since there were only six flocks surface contaminated with genotypes isolated from the crop or intestines from one or two previously slaughtered flocks. Four of these six flocks were
considered as initially *Campylobacter* free. These four flocks may have had no contaminated carcasses after logistic slaughtering.

On six occasions, flocks slaughtered on the same day in the same abattoir, harbored the same genotype in crop or intestines. Since the flocks are reared on different farms, the possibility that these flocks carried the same genotype in their intestines is small unless the different farms were in contact during raising time. One possible explanation is that farms were in contact during raising time since they belonged to the same integrated system. For example, three flocks which carried the same genotype in their intestines at arrival at the abattoir, were all supplied by the same feed supplier and were all visited by the same veterinarian within 2 weeks before slaughter. Another possible explanation is that the flocks became contaminated during transport and resting time. Newell *et al.* (2001) and Slader *et al.* (2002) showed that during transport birds can become contaminated on the surface with campylobacters, excreted by the flock transported previously but still present in the washed and disinfected crates. These campylobacters were found on the processed carcasses. However, the hypothesis if birds can become internally contaminated requires further investigation.

References


