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Campylobacter jejuni contamination of broiler carcasses: Population dynamics and genetic profiles at slaughterhouse level

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ABSTRACT

Six slaughter batches deriving from six typical industrial broiler flocks were examined for the presence, quantity and genetic characteristics of contaminating *Campylobacter jejuni* (*C. jejuni*) during various stages of slaughtering and carcass processing. To assess the contamination dynamics of the carcasses, the analyses were always conducted on neck-skin samples from the same pre-selected and carefully marked carcasses in each batch. The skin samples were taken sequentially at three successive slaughter-line locations in the evisceration room, after three-day refrigeration and after three-day freezing procedure. Caecal samples from the same animals were also tested, as well as samples from the slaughterhouse environment before and after slaughtering. The samples were analysed by the ISO10272 isolation method; campylobacters from neck-skin samples were also quantified. Isolates were species-identified and genotyped by pulsed-field gel electrophoresis (PFGE). On average, the highest *C. jejuni* skin contamination was detected at the first sampling point (post-plucking), suggesting that the majority of *Campylobacter* contamination actually occurs before the entrance to the eviscerating room, probably during the preceding plucking stage. In two out of five positive batches, an additional increase in contamination was recorded after the evisceration step. An evident trend of increasing contamination level was detected when successive batches were compared at each of two initial sampling sites in the evisceration room, indicating an accumulation of contaminating *C. jejuni* at some point before the evisceration room. Three-day refrigeration and three-day freezing caused a 4.5- and 142-fold drop in mean *C. jejuni* CFU counts, respectively. All pre-slaughtering samples from the slaughterhouse environment were negative and all post-slaughtering samples, except water from the scalding tank, were positive. Pulsotypes were limited: altogether five different types were detected, typically one type per batch. The PFGE results from the slaughterhouse environment isolates indicate that cross-contamination is possible (multiple pulsotypes detected in e.g. eviscerating machine). Nevertheless, this was not confirmed in carcasses: analyses of neck-skin isolates suggest that carcasses are contaminated by their own caecal/farm/flock pulsotype.

1. Introduction

Intestinal campylobacteriosis is the leading bacterial zoonosis in Europe and worldwide (WHO, 2011). The main causative agent of this food borne acute gastroenteritis is *Campylobacter jejuni* (*C. jejuni*), a common coloniser of birds' intestines, especially in industrial poultry production (Skirrow, 1977). *C. jejuni* contaminated poultry meat and meat products are considered the most important sources of disease in humans. *Campylobacter* contamination of poultry meat occurs vastly and inevitably during slaughterhouse processing (Berrang et al., 2001); thereafter campylobacters efficiently survive throughout the food chain, constituting risk to public health. The high level of colonised broilers and contaminated broiler meat with *C. jejuni* isolates has also become of major concern in Slovenia. In 2011, Slovenia reported a prevalence of 77.0% and 92.0% in broiler faecal and skin samples, respectively (EFSA, 2013), which is among the highest in the EU. Interestingly, the number of human campylobacteriosis cases in Slovenia is not as high as expected according to the aforementioned data; the incidence is slightly under the EU average and lower than in many

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countries reporting low prevalence at broiler farms and on carcasses (EFSA, 2013). A possible explanation for this fact could be that even though the prevalence of *Campylobacter* carcass contamination was high, the detected numbers of *Campylobacter* were quite low: in 94.2% of carcasses the counts were lower than 1000 CFU/g or below the limit of detection (<10 CFU/g; EFSA, 2010) and basically in line the European Food Safety Authority (EFSA) -recommended microbiological criteria (EFSA, 2011). These numbers are expected to be further reduced by refrigeration at the production plant, at retail and consumer levels, until actual consumption (El-Shibiny et al., 2009).

Slovenia is a small country with only two poultry processing plants, which allows for individualized assessment of critical points. Consequently, the study's authors believe that further reduction of *Campylobacter* carcass contamination at slaughterhouse level is possible, despite the fact that the contaminating *Campylobacter* numbers are favourably low already. To achieve this goal it is first necessary to ascertain where, when, how and to what extent contamination occurs on a specific slaughter line. Therefore, a detailed analysis of *Campylobacter* carcass contamination was conducted at the bigger of two existing broiler slaughterhouse lines, representing approx. 80% of poultry processing in Slovenia. This paper presents and discusses the results.

2. Material and methods

2.1. Study design

Six typical industrial broiler flocks, three in spring 2012 and three in autumn 2012, from different farms and diverse geographic locations were selected for this longitudinal study. One week before slaughtering the flocks were examined for their *C. jejuni* status. The birds of the same flocks were then slaughtered at the same processing plant at 41 days of age and analysed as corresponding slaughter batches (spring: B1–B3, autumn: B4–B6) at the slaughterhouse level: ten evenly distributed carcasses per batch were selected to be followed, carefully marked and analysed for *C. jejuni* presence, quantity and genetic characterisation during successive stages of slaughtering and carcass processing. To assess the possibility of cross-contamination, the slaughterhouse environment and processing equipment were checked for *Campylobacter* contamination before and after slaughtering.

2.2. Samples

2.2.1. Farms – broiler faeces

On the farms, ten samples of fresh faecal droppings were collected from each flock one week before slaughtering and analysed by the culture method.

2.2.2. Slaughterhouse – carcasses

At the slaughterhouse, the monitored flocks were processed as the first three batches on the scheduled day, with *C. jejuni* negative flock(s) having priority over the others. Ten carcasses per batch were selected at the entrance to the evisceration room, with an approximate 5-min time interval between them, each distinctively marked and then sampled sequentially down the slaughter line in the evisceration room. The same carcasses were also sampled after the cooling and freezing steps. Approximately 2 g (2–4 cm^2) of neck-skin from each individual carcass was taken to analyse for presence and genotype of *C. jejuni* and to assess the *C. jejuni* contamination level of each carcass at three successive sampling positions in the evisceration room: at carcass entry immediately after scalding and plucking (post-plucking, pPL), immediately after the evisceration step (post-evisceration, pEV) and after the final rinse before leaving the evisceration room and the start of the dry air-cooling step (post-final rinse, pFR). To analyse the effect of refrigeration and freezing, five of the ten monitored carcasses were neck-skin sampled and *C. jejuni* -analysed after three days of refrigeration and the other five after three days of freezing.

2.2.3. Slaughterhouse – caecal contents

To estimate the genetic heterogeneity of the *C. jejuni* population in each flock/batch at the time of slaughtering, *caeca* of the same birds were collected immediately after the evisceration step and caecal contents were cultured for the presence and PFGE analysis of *C. jejuni.*

2.2.4. Slaughterhouse – environment

To evaluate the possibility of cross-contamination, the slaughterhouse environment and processing equipment were sampled at selected critical locations before and after slaughtering of the three batches investigated: water from the stunning tank (0.5 l), water from the scalding tank (0.5 l), carcass debris (5 g) from the cloaca/vent-cutter-machine and carcass debris (5 g) from the eviscerating machine.

2.3. Isolation and identification methods

Campylobacter isolation from faecal samples was performed by the direct plating method using mCCDA and Skirrow selective agars (Oxoid, UK), incubated for 48 h at 41.5 °C in a microaerophilic atmosphere created by GENbox generators (BioMerieux, France). Neck-skin samples were analysed by the standard ISO-10272-2:2006 enumeration method (ISO, 2006b). Briefly, 1 ml of each initial sample suspension was applied onto three mCCDA plates and 0.1 ml of further 5 decimal dilutions on single CCDA plates. After 40–48 h incubation at 41.5 °C in microaerophilic atmosphere, two plates from two successive dilutions with less than 150 *Campylobacter* – suspected colonies per plate were selected to count the Colony Forming Units (CFU-s) and calculate the CFU content in the initial suspension. Campylobacters from the slaughterhouse environment and processing equipment samples were detected according to ISO-10272-1:2006, using selective enrichment in Bolton broth (Oxoid, UK) followed by plating on mCCDA and Skirrow agars (ISO, 2006a). Liquid samples (water) were processed as sediment after 20.000 g/15 min of centrifugation. The isolates were genus and species-identified using the same standard, according to results of hippurate, indoxyl acetate, catalase and sensitivity to cephalotine and nalidixic acid tests.

2.4. Pulsed-field gel electrophoresis genotyping

For genotyping with PFGE, a PulseNet standardised protocol was used (Ribot et al., 2001). All isolates were subjected to PFGE analysis using the restriction endonuclease *Sma*I. The fragments generated were separated by electrophoresis for 18 h at 6 V/cm and 14 °C with pulse times from 6.7 s to 35.4 s in a CHEF-DR II system (BioRad, Hercules, CA, USA). PFGE profiles were subjected to computer-assisted analysis with BioNumerics software (version 6.0, Applied Maths, Sint-Martens-Latem, Belgium). *Salmonella* Braenderup H9812 was used for band normalisation. Dendrograms with 1% optimisation and 1% position tolerance were created using an UPGMA (Dice coefficient) algorithm.

3. Results

On farms, *C. jejuni* was detected in broiler faeces in five out of six flocks; one of the autumn flocks (B4) remained negative. At the positive farms, *C. jejuni* was isolated from every faecal sample. At