International Journal of Food Microbiology 150 (2011) 95–102

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

From farm to fork follow-up of thermotolerant campylobacters throughout the broiler production chain and in human cases in a Hungarian county during a ten-months period

I. Damjanova [a], M. Jakab [a], T. Farkas [b], J. Mészáros [c], Zs. Galántai [d], I. Turcsányi [b], A. Bistyák [b], Á. Juhász [d], J. Pászti [a], I. Kiss [b], G. Kardos [b,e,*]

[a] National Center for Epidemiology, H-1097 Budapest, Gyáli út 2–6., Hungary
[b] Central Agricultural Office, Veterinary Diagnostic Directorate, Department of Microbiology and Department of Pathology, Debrecen, H-4031 Debrecen, Bornemissza u. 3–5. Hungary
[c] ÁNTSZ Laboratory Ltd., H-4024, Debrecen, Dósa nádor tér 5–6., Hungary (Medical Officer Service)
[d] Hajdú-Bihar County Agricultural Office, Foodchain Safety and Animal Health Directorate, H-4030 Debrecen, Diószegi út 30., Hungary
[e] Department of Medical Microbiology, University of Debrecen, H-4032 Debrecen, Nagyerdei krt. 98., Hungary

ARTICLE INFO

Article history:
Received 25 June 2010
Received in revised form 5 May 2011
Accepted 9 July 2011
Available online 4 August 2011

Keywords:
campylobacteriosis
Campylobacter jejuni
Campylobacter coli
pulsed-field gel electrophoresis

ABSTRACT

A study tracking thermotolerant campylobacters from the setting of the broilers throughout the whole rearing period, slaughter and sale of chicken products in five consecutive broiler rotations of the same henhouse as well as in two different other farms was conducted in a well-defined geographic area (Hajdú-Bihar county, Hungary) between March 2006 and Feb 2007. All notified cases of human campylobacteriosis in this area during the study period were also included. One hundred and one, 44, 23 and 282 *Campylobacter jejuni* and 13, 15, 20 and 60 *C. coli* were isolated from broiler houses, slaughterhouses, retail shops and human samples, respectively. Sixty-two isolates collected from broilers or their environment selected from different flocks (57 *C. jejuni*, 5 *C. coli*), 92 isolates collected from abattoirs and retail shops (72 *C. jejuni*, 20 *C. coli*), as well as 85 randomly selected human isolates (74 *C. jejuni*, 11 *C. coli*) were subjected to PFGE analysis using restriction enzymes *Kpn*I and *Sma*I.

Sixty-six of the isolates produced unique *Sma-Kpn* profiles; the majority (46) of these were of human origin. The remaining isolates formed PFGE clusters of between 2–25 isolates with 14 (12 *C. jejuni* and 2 *C. coli*) main clusters comprised of five or more isolates with identical *Kpn*I-*Sma*I patterns.

Two genetic clones of *C. jejuni* (clone A, n = 25; clone B, n = 20) included 18% of isolates from different sources. Generally, isolates from one cluster were found in 1–3 different flocks, notably, clone B was present in three rotations including those from the two independent farms. Six of the seven investigated flocks had one or two characteristic prevalent clones. Transmission of clones between consecutive flocks was frequently seen. Spread of both *C. jejuni* and *C. coli* was traced multiple times along the food chain; eight *C. jejuni*, but no *C. coli* clones were detected both in broilers and humans.

These data suggest that broilers were the major source for *C. jejuni* but not for *C. coli* in the studied area and period. For *C. jejuni* the carryover of strains between consecutive flocks may be a common event, but the strain is eventually replaced by another and consecutive carryover events seem to be infrequent. The majority of the human disease was due to nonepidemic strains; some clones were transmitted from more than one broiler flocks (including epidemiologically unrelated flocks) to humans multiple times.

1. Introduction

Gastrointestinal infection with thermotolerant campylobacters cause significant morbidity both in developing and developed countries. The two most frequent species, *Campylobacter jejuni* and *C. coli*, are among the most frequent causes of bacterial enteritis in the European Union and in the United States (Humphrey et al., 2007; Moore et al., 2005). The caused disease burden is comparable to that of salmonellosis, and markedly exceeds those of other bacteria causing enteritis (European food safety authority, 2010b). In Hungary, the estimated number of cases of campylobacteriosis is 6000–10000 cases annually, corresponding to an incidence of 57.7 per 100000 inhabitants vs. a median morbidity of 47.1 per 100000 persons in the

* Corresponding author at: Central Agricultural Office, Veterinary Diagnostic Directorate, Department of Microbiology, Debrecen, H-4031 Debrecen, Bornemissza u. 3–5., Hungary, Tel.: +36 52 418 133; fax: +36 52 310 823.
E-mail address: kardosg@gmail.com (G. Kardos).

0168-1605/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2011.07.011

EU in 2007 (European food safety authority, 2010b). Besides gastroenteritis, severe sequelae may also occur (Humphrey et al., 2007). Most notified *Campylobacter* infections in Hungary are considered as sporadic and the sources of infection as well as possible related cases remain undiscovered in most cases.

Poultry has been recognized as the primary reservoir of *C. jejuni* and a significant reservoir of *C. coli* (Friedman et al., 2004; Humphrey et al., 2007; Sheppard et al., 2009). Epidemiological evidence points to the major role of zoonotic transmission of these pathogens from poultry to humans (Hakkinen et al., 2009; Moore et al., 2005; Sheppard et al., 2009). Recent studies suggest that some genotypes or clonal lineages adapted to animal host may never or only rarely cause disease in humans, whereas others are common human pathogens with one or several potential food-borne sources (Duim et al., 2003; Lindmark et al., 2004; Ragimbeau et al., 2008). A number of studies followed these bacteria between different points of the food chain (e.g. Hakkinen et al., 2009; Lienau et al., 2007; Lindmark et al., 2004; Parisi et al., 2007; Thakur et al., 2010; Zorman et al., 2006). Most of these studies investigate animal to food or food to human transmission, but reports encompassing the whole food chain from farm to fork are scant.

The aim of our study was to follow thermotolerant *Campylobacter spp*. throughout the whole food chain from farm to fork, i.e. from the broiler premises through the abattoir and the retail shops to human cases occurring in the region where the majority of the broiler meat from the studied stocks was sold.

2. Material and methods

2.1. Sample collection: broiler farms

During the study period (April 2006 – January 2007) five consecutively set and reared flocks (K1-K5, 15000 birds/flock) of a commercial broiler chicken farm (K) located in an eastern county of Hungary were visited and sampled regularly. At the end of the study period one broiler flock each from two other farms (farms N and D, 21000 and 15000 birds/flock, respectively) located in other municipalities of the same county were also included. The farms K and N belonged to the same owner (Owner 1), while the farm D belonged to another one (Owner 2). All three farms were uniform in setting, they reared conventionally, with mechanically ventilated closed henhouses with bell drinkers and pan feeders; in summer supplementary evaporative cooling pads were used. A combination of whole-house and spot brooding was used for the brought-in chicks; the lighting programme was 23 hours of lighting plus one hour of darkness; flocks finished at the same time without thinning (all-in-all-out). The *Salmonella* status of the flocks was published earlier (Nógrády et al., 2008).

Prior to setting of the birds, hygienic samples of drag swabs were collected aseptically (by wearing plastic gloves) from 1 m^2 of the floor and walls as well as from drinkers and feeders. Meconium samples from the 1-day-old chicks were collected on sterile papers (altogether 25 g/broiler house) and investigated. After setting of the birds, floor faeces samples were collected at weeks 3 and 6 from all flocks. Later sampling was extended to the age of 1 week (flocks K3 and K4), and further extended to the age 1–2 days, 1 week and 2 weeks (flocks K5, N and D). At each occasion 60 faecal samples, pooled as 12 by 5 samples were collected into Preston broth (nutrient broth supplemented with Preston Campylobacter Selective Supplement, Oxoid, Basingstoke, UK). Together with faecal samples, samples from the chicken feed (1 kg), drinking water (1 l) and litter (500 g) were also taken. Air samples were taken by sedimentation for two hours onto selective agar medium (cefoperazone-amphotericin B-teicoplanin agar, CAT, Oxoid). If available, caecal samples collected from one to seven day old chickens found dead were also included. Carcasses of potential vector animals like rodents (mice, rats) and lesser mealworm (*Alphitobius diaperinus*) were also examined when found. All samples were processed on the day of the sample collection. At the three farms altogether 129 hygienic, seven meconium and 300 chicken floor faeces sample pools (1500 floor faeces samples) and 100 samples of other origin (litter, feed, drinking water and suspected vector animals) were collected and tested for the presence of thermotolerant campylobacters.

2.2. Sample collection: processing plants and retail shops

From the farms K and N the flocks were transported to the same commercial slaughterhouse (S1), while the investigated flock of the farm D was processed in another one (S2). Both processing plants were non-automatic, but somewhat dissimilar in design. In S1, the processing started with electric stunning, followed by manual neck cutting. After bleeding out, carcasses passed through a scalding tank operated at 53–55 °C, which was followed by machine-plucking, vent opening, eviscerating, neck cropping prior to chopping off of the breast fillet and the wings. After evisceration, chilling was performed by evaporation; carcasses were kept cool using dry-air. The line speed was 1000 birds per hour. The processing line of the S2 was similar, but the scalding tank operated at 61 ± 1 °C, chilling was achieved by dry-air only and the line speed was only 188 birds per hour. Hygienic samples of peptone-watered drag swab samples were taken before and during processing; the area sampled was 20 cm^2, the following surfaces were sampled: table for breast fillet taking, balance tray, blade of the chopping knife, chopping board, chilling line, eviscerating line, table for gizzard taking, scalding line as well as hands and aprons of workers. During processing, 15 neck skin (approx. 10 g/bird, pooled as 3 by 5 samples) and 5 post-chilled breast fillet (collected individually) samples were taken randomly from all flocks according to the Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. Five guts were also sampled individually for all flocks. Altogether, 149 hygienic as well as 70 carcass and meat samples were collected from the two slaughterhouses.

Raw chicken meat from the processed flocks of the farms K and N were distributed in the same retail shops, one of which (RS1) was sampled for all flocks of farms K and N. The meat from the flock D1 was sold and sampled at a different retail shop (RS2). As practically all Hungarian meat shops, both shops sold various poultry meat (chicken, turkey, occasionally duck and goose) from the same counter, where different species and different parts were stored and sold together, without individual packaging. Possibility of cross-contamination from other poultry meat was considerable. Both shops, again according to the common Hungarian practice, sold pork and beef at the same time, but these were stored and sold separately from poultry meat. Both shops were in relatively large municipalities with numerous meat shops and supermarkets, therefore the shops samples were definitely not the only source for meat in the area.

Raw chicken parts (breast fillet, leg, liver, wing and parson's nose), 5 or 10 samples per flock, have been bought at the day when the meat arrived to the retail shop and processed according to the Commission Regulation (EC) No. 2073/2005. Altogether 55 retail raw meat samples were collected (retail meat was not investigated for flock K1 and only five samples were bought for flock D).

The schedule of the sampling visits, processing of the flocks and buying of retail meat is shown in Table 1.

2.3. Sample collection: human stool samples

Stool samples (one from each worker) were collected from the workers of the farms and the processing plants and investigated for the presence of *Campylobacter spp*. in the first month of the study. *Campylobacter* strains isolated from reported cases of campylobacteriosis by the Medical Officer Service during the study period in Hajdú-Bihar County (an area of 6211 km^2, with approximately 550000 inhabitants in the year of sampling) were also collected. These were inoculated onto