A Prospective Follow-Up Study on Transmission of *Campylobacter* from Poultry to Abattoir Workers

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Abstract

Contact with poultry or poultry meat is a well-known risk factor for campylobacteriosis, but prospective studies on transmission of *Campylobacter* from chickens to humans during slaughter are scarce. In this study, we monitored transmission of *Campylobacter* from slaughtered chicken to originally culture-negative abattoir workers during the peak season of colonized chicken and human *Campylobacter* infection. Stool samples were obtained from 28 abattoir workers together with data on health status once a month between June and September 2010, with a follow-up sample collected in February 2011. *Campylobacter*-positive individuals and chicken flocks were identified by culture, and isolates were further characterized using molecular techniques. *Campylobacter* was isolated from seven asymptomatic individuals. Four of them had been newly employed and had not reported any previous *Campylobacter* infection. Four human isolates had matching genetic fingerprints with isolates from recently slaughtered chickens. Our results further support the role of chicken as the source of human *Campylobacter* infection but suggest that asymptomatic *Campylobacter* infection may occur even in individuals with only limited earlier exposure to *Campylobacter*.

Introduction

CAMPYLOBACTER INFECTION is the most commonly reported zoonosis in the European Union and the most frequent cause of bacterial gastroenteritis in the Western world, with symptoms ranging from mild watery diarrhea to bloody stools, fever, and abdominal pain (EFSA/ECDC, 2013). Possible postinfectious complications include the severe neurological disorder Guillain-Barré syndrome, which has been associated with strains that have the ability to incorporate sialic acids in the lipooligosaccharide (LOS) of the cell wall (Godschalk *et al.*, 2004). Such strains have also been proposed to be associated with increased invasivity and more severe enteritis, but the results are conflicting (Mortensen *et al.*, 2009; Louwen *et al.*, 2012; Ellström *et al.*, 2013a, b). As shown by case-control studies and epidemiological surveys using molecular typing methods, poultry meat is an important source of campylobacteriosis (Schönberg-Norio *et al.*, 2004; Jore *et al.*, 2010). Genotypes of human isolates have been shown to overlap with those of poultry origin by 30–60% in comparative studies (Lindmark *et al.*, 2004; de Haan *et al.*, 2010). However, it is not clear whether chicken may also carry *Campylobacter* isolates that are less pathogenic for humans or if all chicken *Campylobacter* isolates can

be transmitted to humans and cause disease. The prevalence of *Campylobacter* among chicken flocks in Sweden shows a considerable seasonal variation, with a peak of 25–40% positive flocks during the summer months, June to September, and only 5–10% during the rest of the year (Jore *et al.*, 2010). The incidence of domestic human infections essentially displays the same seasonal variation (Jore *et al.*, 2010). We studied the transmission of *Campylobacter* from chicken flocks to the personnel at two abattoirs during the peak season for *Campylobacter* infections in Sweden, with emphasis on the characteristics of the abattoir workers and the *Campylobacter* isolates.

Materials and Methods

Participants and stool cultures

The study was performed at two chicken abattoirs in Sweden. All abattoir workers directly involved in the chicken slaughter were asked to participate in the study. Initial stool samples were collected from 28 volunteering abattoir workers at the end of June and thereafter once a month from July to September 2010. In addition, a follow-up stool sample was collected at the end of February 2011. Participants sent stool samples in Amies transport medium with charcoal (Sarstedt,

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Nümbrecht, Germany) to the Clinical Microbiology Laboratory at Uppsala University Hospital. A total of 113 stool samples were cultured for *Campylobacter* on modified charcoal-cefoperazone-deoxycholate agar plates (Oxoid, Basingstoke, England) (Engberg *et al.*, 2000). Identification of *Campylobacter* was preliminarily based on colony appearance, oxidase test, and microscopy, and the isolates were stored at -70°C until further analysis. Data on underlying diseases, medication, use of tobacco, previously confirmed *Campylobacter* infection, length of employment at a poultry abattoir and so on were obtained by questionnaires filled out by the participants at the start of the study. Further information about intake of probiotics, proton pump inhibitors, and antimicrobials as well as gastrointestinal and other symptoms was obtained by questionnaires at each sampling occasion. These characteristics were compared between infected and noninfected participants using Fischer's two-tailed exact test (data not shown).

Chicken isolates

Through the Swedish *Campylobacter* surveillance program (Hansson *et al.*, 2007), we had access to all *Campylobacter*

isolates from colonized chicken flocks that had been slaughtered at the two abattoirs during the study period. At the two abattoirs, a total of 135 *Campylobacter*-positive chicken flocks were slaughtered between June 2010 and February 2011. To search for the source of infection among the *Campylobacter*-positive chicken flocks, the human isolates were analyzed with pulsed-field gel electrophoresis (PFGE) together with isolates from all chicken flocks ($N=28$) slaughtered at the corresponding abattoir within 1 month before the positive human samples were obtained. For human isolates for which no chicken isolates with matching PFGE patterns were found within a month from the positive human stool culture, chicken isolates at the corresponding abattoir from within 2 months were tested (eight additional isolates).

Characterization of isolates

All 42 *Campylobacter* isolates from humans and chickens in the study were identified to the species level by polymerase chain reaction according to methods described earlier (Linton *et al.*, 1997; Denis *et al.*, 1999). PFGE was performed according to the "Campynet" standardized protocol, using the restriction enzyme *Sma*I (<http://campynet.vetinst.dk/PFGE.html>).

TABLE 1. LENGTH OF EMPLOYMENT AT ABATTOIR, PREVIOUS *CAMPYLOBACTER* INFECTION, AND TIME OF *CAMPYLOBACTER*-POSITIVE STOOL CULTURE FOR THE PARTICIPANTS OF THE STUDY

Participant	Length of employment ^a	Previously verified <i>Campylobacter</i> infection reported	Campylobacter stool culture, negative (–) or positive (+) in:				
			June	July	August	September	February
1	≤1 month	No	–	–	–		
2	≤1 month	No	–	+ ^b	–	–	–
3	≤1 month	No	–	–			–
4	≤1 month	No	–	–	–	–	
5	≤1 month	No	–	–	–		
6	≤1 month	No	–	–			
7	>1 month to 1 year	No	–	–		–	–
8	>1 month to 1 year	No	–	–	–	+	–
9	>1 month to 1 year	No	–	–	–	–	
10	>1 month to 1 year	No	–	–			
11	>1 month to 1 year	No	–			+	–
12	>1 month to 1 year	No	–	–	–	+ ^c	–
13	>1 year to 10 years	Yes	–	–	–	–	–
14	>1 year to 10 years	Yes	–	–	–		+ ^c
15	>1 year to 10 years	No	–	–	–	–	–
16	>1 year to 10 years	Yes	–			–	–
17	>1 year to 10 years	No	–	–	–	–	–
18	>1 year to 10 years	Yes	–	–	–	+ ^c	
19	>1 year to 10 years	No	–	–	–		
20	>1 year to 10 years	No	–	–	–	–	
21	>1 year to 10 years	Yes	–	–	–	–	–
22	>1 year to 10 years	No	–	–	–	–	–
23	>1 year to 10 years	No	–	–	–		–
24	>10 years	No	–	–	–	–	–
25	>10 years	No	–	–	–	–	–
26	>10 years	No	–	–	–	+ ^c	–
27	>10 years	No	–	–	–	–	–
28	>10 years	No		–	–	–	–
No. of positive chicken flocks slaughtered			10	19	34	23	5
Total no. of flocks slaughtered			91	72	78	95	80

^aLength of employment at the first stool sample.

^bThe isolate could not be resuscitated from the frozen stock.

^cChicken isolate with identical genotype verified (Fig. 1).

FIG. 1. *SmaI* restriction patterns after pulsed-field gel electrophoresis (PFGE) for the six human *Campylobacter* isolates and the corresponding chicken isolates with identical patterns. The column "Isolation date" denotes in which month each positive human sample was obtained as well as how many days earlier the corresponding chicken isolates with matching PFGE patterns were obtained. *Isolates from different flocks of the same breeder. LOS class, lipooligosaccharide locus class (range from A to S); MLST, multilocus sequence typing; ST, sequence type (specific combination of sequence data from seven housekeeping genes in the *C. jejuni* genome); CC, clonal complex (related STs with at least 4 identical alleles); 18df, the isolate harbored the open reading frame orf18 and thus had any of the LOS locus classes D, F, I, J, K, N, S, or Q which all are unable to incorporate sialic acids; ND, not determined.

Gels were analyzed using the Bionumerics software for analysis of PFGE fingerprints (Applied Maths, Kortrijk, Belgium). Dendrograms were constructed using the Dice coefficient and the unweighted pair-group method with arithmetic means. Multilocus sequence typing (MLST) of the five human *C. jejuni* isolates was performed according to the methods described earlier (Dingle *et al.*, 2001). MLST is a sequence-based genotyping method utilizing internal fragments of seven housekeeping genes of *C. jejuni*. The combined profile of the seven alleles (sequences that differ by at least one base pair are considered distinct alleles) corresponds to a sequence type (ST), and related STs with at least four identical alleles belong to the same clonal complex (CC). The Bionumerix software was used for sequence assembly. Allele numbers, STs, and CCs were assigned using the PubMLST database (<http://pubmlst.org>). All five human *C. jejuni* isolates and the chicken isolates with matching PFGE patterns were typed for LOS locus class according to the method described by Parker *et al.* (Parker *et al.*, 2005).

Ethical considerations

The study was approved by the regional board of the ethical committee at Uppsala University. All participants gave their written informed consent.

Results

In total, 28 workers at 2 poultry abattoirs in Sweden were included; 17 women (mean age 33.9 years) and 11 men (mean age, 32.2 years). Twelve participants had been employed ≤ 1 year (median, 1.3 months) at the beginning of the study (Table 1). All human stool cultures were *Campylobacter*

negative at the beginning of the study in June, but follow-up samples revealed positive *Campylobacter* stool cultures from a total of seven abattoir workers: five of them in September (Table 1). Importantly, no symptoms were reported by any of the *Campylobacter*-positive participants at the time of positive stool culture. Four of the *Campylobacter*-positive participants had been employed within a year from the beginning of the study. These individuals had not reported any previous employment at a poultry abattoir or any previously documented *Campylobacter* infection. Two of the five participants with earlier confirmed *Campylobacter* infection also became culture positive during the study.

Five of the seven human *Campylobacter* isolates were identified as *C. jejuni* and one as *C. lari*. One isolate was lost as it could not be resuscitated from the frozen stock. The five human *C. jejuni* isolates were further identified by MLST to belong to four different clonal complexes: ST-48CC, ST-658CC, ST-677CC, and ST-45CC (two isolates) (Fig. 1). Four of the human isolates—three *C. jejuni* and one *C. lari*—had a *SmaI* pattern identical to that of chicken isolates from flocks slaughtered within 1 month (Fig. 1). For two positive abattoir workers, no chicken isolates with matching PFGE patterns were found at the corresponding abattoir even within 2 months of infection.

To determine whether any specific LOS locus class was over-represented among the isolates only causing asymptomatic infections, the LOS locus class was determined for the five human *C. jejuni* isolates and the corresponding chicken isolates with matching PFGE patterns. The isolates belonged to four different LOS locus classes and hence there was no over-representation of a specific LOS locus class (Fig. 1). In this study, none of the host characteristics collected in

the questionnaires was significantly associated with positive stool cultures.

Discussion

Poultry is an important source of human *Campylobacter* infections. During this prospective follow-up study on 28 poultry abattoir workers, 7 participants became stool culture-positive for *Campylobacter* by routine diagnostic methods. Four of the human isolates had PFGE *Sma*I restriction patterns identical to isolates from chickens slaughtered at the corresponding abattoir within a month of human infection, providing evidence for transmission of *Campylobacter* from chicken to the personnel at the abattoirs. Immunity developed after years of employment at chicken abattoir might explain the asymptomatic infections of the long-term abattoir workers. However, it is noteworthy that four of the *Campylobacter*-positive asymptomatic individuals had most probably only had very limited earlier exposure to *Campylobacter*: short duration of employment at any poultry abattoir, employment before the study mostly during the season with only few contaminated chicken flocks, and no previously verified *Campylobacter* infection.

Campylobacter infection has been reported previously among poultry abattoir workers (Christenson *et al.*, 1983; Cawthraw *et al.*, 2000; de Perio *et al.*, 2013). However, in all these studies asymptomatic *Campylobacter* infection has been exclusively reported among experienced workers, whereas all newly employed *Campylobacter*-positive staff members have reported symptoms of the infection. In a study of a *Campylobacter* outbreak at an abattoir in Sweden, 17 of 24 newly employed workers were infected together with 20 of 70 experienced workers. Only five asymptomatic *Campylobacter*-positive individuals were detected; all of them were among the experienced staff members (Christenson *et al.*, 1983). In another study, eight *Campylobacter* culture-positive abattoir workers were identified and one of them, the only short-term worker, developed symptoms (Cawthraw *et al.*, 2000). Furthermore, in a register-based study of *Campylobacter*-positive symptomatic poultry-processing workers at an abattoir in the United States between 2008 and 2011, 83% of the infected individuals had worked at the plant for <1 month before the infection (de Perio *et al.*, 2013). Together, these studies indicate that continuous bacterial exposure may protect to some extent the experienced workers from symptomatic *Campylobacter* infection. However, our findings in the present prospective study, although with a low number of participants, show that asymptomatic infection may also occur among employees with only limited earlier exposure to *Campylobacter* and thus could further suggest that all *Campylobacter* isolates of chicken origin might not be equally virulent and cause symptomatic infection in humans. The *C. jejuni* isolates from the newly employed participants in this study belonged to clonal complexes previously found among symptomatic humans, and it is possible that host factors may explain the lack of symptoms even among these individuals. On the other hand, virulence determinants and phenotypes have been shown to vary even within clonal complexes (Revez *et al.*, 2011).

One suggested virulence trait in the pathogenesis of Guillain-Barré syndrome is the ability of some *C. jejuni* strains to incorporate sialic acids in the LOS, resulting in structures mimicking human gangliosides (Godschalk *et al.*,

2004). Such strains also displayed increased invasion of epithelial cells *in vitro*, but results are conflicting regarding their ability to cause more severe symptoms in patients (Louwen *et al.*, 2012; Ellström *et al.*, 2013a). Screening for LOS locus class of the *C. jejuni* isolates in the present study did not reveal any obvious association between a certain LOS locus class and the asymptomatic infections among the abattoir workers. However, the numbers are too small to draw any further conclusions.

Conclusions

This prospective follow-up study further supports the role of chicken as the source of human *Campylobacter* infection but suggests that asymptomatic *Campylobacter* infection might occur even in individuals with only limited earlier exposure to *Campylobacter*.

Acknowledgments

The skilled technical assistance of Anna Nilsson, Boel Harbom, Mattias Myrenäs, and Ninni Pudas is gratefully acknowledged. This work was financially supported by grants from the Swedish Research Council (grant number: 521-2011-3527) and the Swedish Research Council FORMAS (grant number: 221-2012-1442). The funding sources had no involvement in any part of the study.

Disclosure Statement

No competing financial interests exist.

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