Finnish Food Safety Authority, Evira
Research Department
Microbiology Unit
Helsinki, Finland

and

Department of Food Hygiene and Environmental Health
Faculty of Veterinary Medicine
University of Helsinki
Helsinki, Finland

Finnish cattle as reservoir of *Campylobacter* spp.

Marjaana Hakkinen

Academic Dissertation

To be presented with the permission of the Faculty of Veterinary Medicine,
University of Helsinki, for public examination in Auditorium XII, Fabianinkatu 33, Helsinki
on 26 November 2010, at 12 o'clock noon
HELSINKI 2010

Supervising professor

Marja-Liisa Hänninen, DVM, PhD, Professor
Department of Food Hygiene and Environmental Health
Faculty of Veterinary Medicine
University of Helsinki
Helsinki, Finland

Supervised by

Marja-Liisa Hänninen, DVM, PhD, Professor
Department of Food Hygiene and Environmental Health
Faculty of Veterinary Medicine
University of Helsinki
Helsinki, Finland

Reviewed by

Thomas Alter, PhD, Professor
Department of Veterinary Medicine
Institute of Food Hygiene
Freie Universität Berlin
Berlin, Germany

Eva Møller Nielsen, PhD
Department of Microbiological Surveillance and Disease
Statens Serum Institut
Copenhagen, Denmark

Opponent

Wilma Jacobs-Reitsma, PhD
National Institute for Public Health and Environment
The Netherlands

ISSN 1796-4660, ISBN 978-952-225-076-6 (print)
ISSN 1797-2981, ISBN 978-952-225-077-3 (pdf)
Helsinki University Printing House 2010

Abstract

The reported incidence of human campylobacteriosis in Finland is higher than in most other European countries. A high annual percentage of sporadic infections is of foreign origin, although a notable proportion of summer infections is domestically acquired. While chickens appear to be a major source of campylobacters for humans in most countries, the prevalence of campylobacters is very low in chicken slaughter batches in Finland. Data on other potential animal reservoirs of human pathogenic campylobacters in Finland are scarce. Consequently, this study aimed to investigate the status of Finnish cattle as a potential source of thermophilic *Campylobacte*r spp. and antibiotic-resistant *Campylobacter jejuni* for human sporadic campylobacter infections of domestic origin.

A survey of the prevalence of thermophilic *Campylobacter* spp. in Finnish cattle studied bovine rectal faecal samples (*n*=952) and carcass surface samples (*n*=948) from twelve Finnish slaughterhouses from January to December 2003. The total prevalence of *Campylobacter* spp. in faecal samples was 31.1%, and in carcass samples 3.5%. *Campylobacter jejuni*, the most common species, was present in 19.5% of faecal samples and in 3.1% of carcasses. In addition to thermophilic *Campylobacter* spp., *C. hyointestinalis* ssp. *hyointestinalis* was present in bovine samples. The prevalence of campylobacters was higher among beef cattle than among dairy cattle. Using the enrichment method, the number of positive faecal samples was 7.5 times higher than that obtained by direct plating. The predominant serotypes of faecal *C. jejuni*, determined by serotyping with a set of 25 commercial antisera for heat-stable antigens (Penner), were Pen2 and Pen4-complex, which covered 52% of the samples. Genotyping with pulsed-field gel electrophoresis (PFGE) using SmaI restriction yielded a high diversity of *C. jejuni* subtypes in cattle. Determining the minimum inhibitory concentrations of ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid, and oxytetracycline among bovine *C. jejuni* isolates using a commercial broth microdilution method yielded 9% of isolates resistant to at least one of the antimicrobials examined. No multiresistant isolates were found among the bovine *C. jejuni* strains.

The study of the shedding patterns of *Campylobacter* spp. among three Finnish dairy cattle herds included the examination of fresh faecal samples and tank milk samples taken five times, as well as samples from drinking troughs taken once during the one-year study. The semiquantitative enrichment method detected *C. jejuni* in 169 of the 340 faecal samples, mostly at low levels. In addition, *C. jejuni* was present in one drinking trough sample. The prevalence between herds and sampling occasions varied widely. PFGE, using SmaI as restriction enzyme, identified only a few subtypes in each herd. In two

of the herds, two subtypes persisted throughout the sampling. Individual animals presented various shedding patterns during the study.

Comparison of *C. jejuni* isolates from humans, chickens and cattle included the design of primers for four new genetic markers selected from completely sequenced *C. jejuni* genomes 81-176, RM1221 and NCTC 11168, and the PCR examination of domestic human isolates from southern Finland in 1996, 2002 and 2003 (*n*=309), chicken isolates from 2003, 2006 and 2007 (*n*=205), and bovine isolates from 2003 (*n*=131). The results revealed that bovine isolates differed significantly from human and chicken isolates. In particular, the γ-glutamyl transpeptidase gene was uncommon among bovine isolates.

The PFGE genotyping of *C. jejuni* isolates, using SmaI and KpnI restriction enzymes, included a geographically representative collection of isolates from domestic sporadic human infections, chicken slaughter batches, and cattle faeces and carcasses during the seasonal peak of campylobacteriosis in the summer of 2003. The study determined that 55.4% of human isolates were indistinguishable from those of chickens and cattle. Temporal association between isolates from humans and chickens was possible in 31.4% of human infections. Approximately 19% of the human infections may have been associated with cattle. However, isolates from bovine carcasses and human cases represented different PFGE subtypes.

In conclusion, this study suggests that Finnish cattle is a notable reservoir of *C. jejuni*, the most important *Campylobacter* sp. in human enteric infections. Although the concentration of these organisms in bovine faeces appeared to be low, excretion can be persistent. The genetic diversity and presence or absence of marker genes support previous suggestions of host-adapted *C. jejuni* strains, and may indicate variations in virulence between strains from different hosts. In addition to chickens, Finnish cattle appeared to be an important reservoir and possible source of *C. jejuni* in domestic sporadic human infections. However, sources of campylobacters may differ between rural and urban areas in Finland, and in general, the transmission of *C. jejuni* of bovine origin probably occurs via other routes than food.

Acknowledgements

This study begun at the National Veterinary and Food Research Institute (EELA) and ended at the Microbiology unit of Finnish Food Safety Authority (Evira). I thank Professor Tuula Honkanen-Buzalski, previously the general director of EELA and the current head of the Research Department in Evira, and DVM PhD Vesa Myllys, the former head of the Microbiology Unit, for the opportunity to carry out this study. The financial support from the Finnish Veterinary Science Foundation, the Walter Ehrström Foundation and the University of Helsinki made this work possible. I thank these organisations for their funding, which enabled me to take study leave to write the articles and the thesis.

My warmest appreciation goes to my supervisor Professor Marja-Liisa Hänninen for her continuous encouragement and excellent scientific guidance during these years. I truly admire her vast knowledge and enthusiasm in the field of science.

Professor Thomas Alter and PhD Eva Møller Nielsen, the official reviewers of my thesis, deserve my sincere thanks for their constructive and valuable criticism, as does Stephen Stalter for his excellent revision of the English language of my thesis.

I am also grateful to my co-authors Helmi Heiska, Manuel Gonzalez, Ulla-Maija Nakari, and Professors Hilpi Rautelin and Anja Siitonen for their valuable contributions to this project and for our fruitful co-operation.

I warmly thank the veterinary inspectors for collecting the samples at the slaughterhouses, and the farmers for permitting collect samples from their barns. Without their contribution this study would have been impossible.

Completion of the project depended on the excellent work of the laboratory staff. I am especially grateful to Kirsi Eklund for her laboratory work throughout the project. Mira Kankare, Lea Nygård, Kaija Pajunen, Maaret Hyppönen, Mirva Tahvanainen, Sari Maljanen, Katriina Mälkönen, Marja Rasinperä, and all the young students who spent their summer hunting campylobacters in our laboratory also deserve my warm thanks for their skilful assistance at different stages of this project.

I offer my gratitude to all of my colleagues at the Microbiology Unit for their support and encouragement. My special thanks belong to Leila Rantala for introducing me to the secrets of Bionumerics, to Tuula Johansson and Saija Hallanvuo for handling my share while I was absent, and to Ansu Myllyniemi for her friendship and tireless optimism.

I am also grateful to all of my friends for their friendship and for reminding me of other important aspects of life during these years so full of work. My very special thanks go to my aunt Maija-Leena, whose enquiring character I wish to emulate.

My late parents always encouraged me to study and supported me in many ways, and my late sister left me an unforgettable memory of sisterhood: I miss her so much. I am deeply grateful to my parents, my sister and my brother.

My deepest gratitude goes to my dearest and nearest: my husband Jukka and our daughters Elisa and Tuuli. With you I have experienced the happiest times in my life.

Contents

List of original publications

This thesis is based on the following publications:

I Hakkinen, M., Heiska, H. & Hänninen M.-L. 2007. Prevalence of *Campylobacter* spp. in cattle in Finland and antimicrobial susceptibilities of bovine *Campylobacter jejuni* strains. Appl. Environ. Microbiol. 73, 3232-3238.

II Hakkinen, M. & Hänninen, M.-L. 2009. Shedding of *Campylobacter* spp. in Finnish cattle on dairy farms. J. Appl. Microbiol. 107, 898-905.

III Gonzalez, M., Hakkinen, M., Rautelin, H. & Hänninen, M.-L. 2009. Bovine *Campylobacter jejuni* strains differ from human and chicken strains in an analysis of certain molecular genetic markers, Appl. Environ. Microbiol. 75, 1208-10.

IV Hakkinen, M., Nakari U.-M. & Siitonen, A. 2009. Chickens and cattle as sources of sporadic, domestically acquired *Campylobacter jejuni* infections in Finland. Appl. Environ. Microbiol. 75, 5244-5249.

The publications are indicated in the text by their roman numerals. The original articles have been reprinted with the permission of their copyright holders: The American Society for Microbiology (I, III and IV) and John Wiley and Sons (II).

Abbreviations

AFLP	amplified fragment length polymorphism
ATCC	American Type Culture Collection
BIOHAZ	EFSA Panel on Biological Hazards
bp	base pair
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CLSI	Clinical and Laboratory Standards Institute
DMSO	dimethyl sulfoxide oxidoreductase
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetraacetic acid
EFSA	European Food Safety Authority
EUCAST	European Committee for Antimicrobial Susceptibility Testing
flaA	flagellin A gene
ggt	γ-glutamyl transpeptidase gene
ISO	International Organization for Standardization
mCCDA	modified charcoal cefoperazone deoxycholate agar
MIC	minimum inhibitory concentration
MLST	multilocus sequence typing
MPN	most probable number
NCFA	Nordic Committee on Food Analysis
NCTC	National Collection of Type Cultures
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
ST	sequence type
SVR	short variable region
THL	National Institute for Health and Welfare
TSI	triple-sugar iron
UV	ultra violet
WHO	World Health Organization

1 Introduction

The genus *Campylobacter* was established in 1963 (Sebald and Véron 1963). Over a hundred years ago, however, scientists had already described these *Vibrio*-like organisms, which were present primarily in bovine and ovine abortions (Smith and Taylor 1919, Skirrow 2006), and occasionally in human disease as well (Levy 1946, King 1957). The importance of thermophilic campylobacters, and of *Campylobacter jejuni* and *C. coli* in particular, as human enteric pathogens has become clear since the 1970s, after the discovery of selective isolation methods for these fastidious organisms (Dekeyser et al. 1972, Butzler et al. 1973, Skirrow 1977). Subsequent intensive research has revealed that *C. jejuni* is the most common cause of human bacterial gastroenteritis worldwide (Baker et al. 2007, EFSA 2010b).

Campylobacter infection, campylobacteriosis, is usually a self-limiting disease with clinical symptoms similar to those of other acute bacterial enteric infections (Blaser and Engberg 2008). The infective dose can be low: in experimental infections, a dose of 500 bacterial cells was sufficient to cause disease (Black et al. 1988), and outbreak data modelling has suggested that even fewer than 20 cells can induce symptoms (Teunis et al. 2005). Musculosceletal symptoms are common complications in connection with *C. jejuni* enteric infections, and reactive arthritis occurs in about 4% to 5% of cases (Doorduyn et al. 2008, Schönberg-Norio et al. 2009). The most serious, though infrequent sequela is Guillain-Barré syndrome, an acute neuromuscular paralysis (Jacobs et al. 2008).

Campylobacters have a wide range of animal hosts, including food production animals, which can be carriers of these bacteria in their intestinal tract without showing clinical symptoms (Nielsen et al. 1997, Stanley and Jones 2003, Brown et al. 2004, Devane et al. 2005, Milnes et al. 2008). A particularly favourable environment for the proliferation of *C. jejuni* is the avian intestine (Lee and Newell 2006), and accordingly, poultry appears to be a major source of *C. jejuni* in humans (EFSA Panel on Biological Hazards [BIOHAZ] 2010).

The prevalence of campylobacters in Finnish chicken slaughter batches is among the lowest in Europe (EFSA 2010c). The incidence of human campylobacteriosis in Finland, however, is among the highest in Europe, although the incidences may not be fully comparable between countries due to differences in their reporting systems (EFSA 2010b). Reporting is comparable between Nordic countries, however; the highest incidence of human campylobacter infections occurred in Finland (84/100 000 population), but was substantially lower in Norway (60.7/100 000 population), whereas the prevalence of campylobacters in chicken slaughter batches was low in

Finland (3.9%) and the lowest in Norway (3.2%) (EFSA 2010b, EFSA 2010c).

A high percentage, up to 77% in 2008 ((National Institute for Health and Welfare 2009), of human campylobacter infections reported in Finland originate from travel abroad. Nevertheless, the proportion of domestically acquired campylobacter infections peaks in the summer season, comprising approximately 40% to 70% of reported cases (Vierikko et al. 2004, National Public Health Institute 2005). Similarly, the prevalence of campylobacters in Finnish chicken slaughter batches also peaks in late summer, whereas campylobacters are rarely found in chickens in winter. The prevalence of *Campylobacter* spp. in chicken slaughter batches has remained low with no major changes after the implementation of the Finnish campylobacter monitoring programme for chickens in June 2004 (http://www.zoonoosikeskus.fi/attachments/zoonoosit/kampylobakteeri/kampylobakteeri_2.pdf). Between 2000 and 2008, only three of the ten food-related campylobacteriosis outbreaks identified in Finland were attributed to chicken, turkey or duck meat (Finnish Food Safety Authority, unpublished data). On the other hand, the sources of sporadic campylobacter infections, which constitute the majority of cases, usually remain unclear. According to a recent Finnish study (Schönberg-Norio et al. 2006), the sources of domestically acquired campylobacteriosis differ depending on the age of the patient and the geographical area. But besides chickens (Hänninen et al. 2000, Perko-Mäkelä et al. 2002, Kärenlampi et al. 2003), available data on other potential animal reservoirs of campylobacters in Finland are limited.

2 Review of the literature

2.1 *Campylobacter* spp. and human enteric diseases

2.1.1 Human campylobacteriosis

Campylobacter spp., especially *Campylobacter jejuni*, is the most frequently reported cause of human bacterial gastroenteric infections. The incidence of campylobacteriosis has been steadily rising in most countries where the disease is notifiable (Baker et al. 2007, EFSA 2010b). Reports from New Zealand have presented the highest incidences between 2000 and 2007, peaking at 422.4 cases per 100 000 people in 2006 (Baker et al. 2006, Baker et al. 2007, Mullner et al. 2010a). The EFSA report on zoonoses in 2008 (EFSA 2010b) reported incidences of campylobacteriosis from <0.1 to 193.3/100 000 population in European countries. The wide variation among countries likely reflects differences in health care and reporting systems, and in microbiological methods rather than real differences in the incidence of campylobacter infections (Olson et al. 2008, Vally et al. 2009, EFSA 2010b).

The majority of human cases are sporadic or small-scale family outbreaks, whereas large outbreaks occur infrequently (Olson et al. 2008). Identification of outbreaks, however, can be difficult due to the diffuse geographic and temporal distribution of the cases (Adak et al. 2005, Gilpin et al. 2006). The temporal association of cases can remain unclear, because the incubation period prior the onset of symptoms can vary. In addition, wide variation in the severity of the disease among individual patients complicates the detection of outbreaks. For example, patients with mild symptoms may recover without the need for medical care, and therefore remain unidentified as outbreak cases (Olson et al. 2008).

A marked seasonality is characteristic to the incidence of human campylobacteriosis, which peaks in different summer months depending on the geographical area (Nylen et al. 2002, Kovats et al. 2005, Louis et al. 2005, Baker et al. 2007, van Hees et al. 2007, Ragimbeau et al. 2008, White et al. 2009). In the Nordic countries, for example, the number of human cases consistently peaks in the end of July and in the beginning of August (Nylen et al. 2002, Jore et al. 2010), whereas in England and Wales the peak occurs in mid-June and mid-July (Louis et al. 2005). The annual increase in the incidence of sporadic infections relates to climatic factors, such as rising ambient temperature (Patrick et al. 2004, Lake et al. 2009, Stark et al. 2009, White et al. 2009, Jore et al. 2010) and relative humidity (Patrick et al. 2004, White et al. 2009), whereas the effect of rainfall appears to be negligible (Patrick et al. 2004, Kovats et al. 2005, Louis et al. 2005). A

study by Nicholson et al. (2005), however, showed a significant association between preceding rainfall and water-borne outbreaks.

Reports from different countries present the highest incidence rates among children under five years of age and in age groups between 15 and 29 years of age (Sopwith et al. 2003, Carrique-Mas et al. 2005, Baker et al. 2007, White et al. 2009, Nakari et al. 2010). Children living in rural areas seem to be at especially higher risk for contracting campylobacteriosis than those living in urban centres (Ethelberg et al. 2005, Baker et al. 2007, Garrett et al. 2007). Moreover, the incidence of campylobacteriosis in children under five years of age appears to be particularly temperature-related (Louis et al. 2005). Other factors, such as the use of acid-suppressing medication or underlying disease may also explain the higher risk of campylobacteriosis among the elderly reported in recent studies (Gillespie et al. 2009, Doorduyn et al. 2010). Besides differences among age groups, the incidence of campylobacteriosis also varies between genders. Males represent a slightly higher proportion of reported cases irrespective of age (Louis et al. 2005, Baker et al. 2007, White et al. 2009).

Evidence from various studies has suggested that the development of immunity is a consequence of repeated or long-term exposure to *Campylobacter* spp., such as the regular consumption of risky food or occupational contact with animals (Forbes et al. 2009, Tam et al. 2009). Recent experiments with human volunteers have confirmed the acquisition of immunity, which offered complete short-term protection from illness, and resistance to colonisation upon re-challenge with the same *C. jejuni* strain (Tribble et al. 2010).

2.1.2 Sources of *Campylobacter* spp. in human infection

The predominantly sporadic appearance of campylobacteriosis complicates the tracing of its sources, which in sporadic cases often remain unidentified, because the incubation period prior to the onset of symptoms can be long. Nevertheless, in sporadic foodborne cases, a major source of campylobacters appears to be the handling and consumption of fresh chicken (Studahl and Andersson 2000, Adak et al. 2005, Wingstrand et al. 2006, Stafford et al. 2007, Unicomb et al. 2008, Wilson et al. 2008, Lindmark et al. 2009). More important than eating improperly heated chicken meat, however, is probably cross-contamination from raw chicken meat during meal preparation (Kapperud et al. 2003). The importance of chicken is obvious in countries such as Belgium, Iceland, Denmark and New Zealand, where the reduced consumption of chicken meat or the implementation of measures that reduce the contamination of chicken meat have substantially reduced the incidence of human cases (Vellinga and Van Loock 2002, Stern et al. 2003, Mullner et al. 2009, Rosenquist et al. 2009). On the other hand, the numbers of reported human cases have risen in Sweden and Finland despite the steady or reduced prevalence of campylobacters in chicken flocks (Studahl and Andersson 2000, EFSA 2010b). Moreover, genotyping studies of *Campylobacter* spp.

from different sources suggest overestimation of the importance of chicken in human campylobacteriosis (Duim et al. 2000, Dingle et al. 2001, Kärenlampi et al. 2003, Levesque et al. 2008).

Besides the consumption and handling of chicken, case-control studies have identified other food-associated risk factors, including the consumption of undercooked meat, pork, pork with bones, ham and beef, offal, game and tripe, barbecued meat or undercooked seafood; eating at a restaurant; poor kitchen hygiene, and drinking unpasteurised or bird-pecked milk (Studahl and Andersson 2000, Kapperud et al. 2003, Neimann et al. 2003, Sopwith et al. 2003, Schönberg-Norio et al. 2004, Carrique-Mas et al. 2005, Gallay et al. 2006, Stafford et al. 2008, Unicomb et al. 2008, Doorduyn et al. 2010). In addition, the preparation of meat by barbecuing appears to be a risk factor for campylobacteriosis (Studahl and Andersson 2000, Kapperud et al. 2003, Neimann et al. 2003, Doorduyn et al. 2010). "Protective" food-related factors, in contrast, include for example the consumption of sausage, fish, raw vegetables, fruits or berries, chocolate and nuts and pasteurised milk (Kapperud et al. 2003, Schönberg-Norio et al. 2004, Carrique-Mas et al. 2005, Stafford et al. 2008, Doorduyn et al. 2010).

Studies focusing on defined temporal and spatial areas have elucidated the relative importance of different sources of campylobacters in human infection. Increasing evidence from recent research indicates that exposures in urban areas differ from those in rural areas (Studahl and Andersson 2000, Baker et al. 2007, Garrett et al. 2007, Strachan et al. 2009). Poultry appears to be a less likely source of campylobacters among the rural population than among urban dwellers (Ethelberg et al. 2005, Mullner et al. 2010b). Moreover, a significant correlation between agricultural activities and the seasonality of infections in rural areas suggests an association with environmental rather than food sources (Kovats et al. 2005, Louis et al. 2005, Tam et al. 2006). The contaminated environment, direct contact with farm animals and the consumption of unpasteurised milk on the farm may be the most important exposures for rural population, and especially for children (Studahl and Andersson 2000, Kapperud et al. 2003, Sopwith et al. 2003, Minihan et al. 2004, Ethelberg et al. 2005, Schildt et al. 2006, Baker et al. 2007, Garrett et al. 2007, Strachan et al. 2009, Mullner et al. 2010b).

In addition to food production animals, pet animals - especially young dogs and cats - can be carriers of thermophilic campylobacters (Hald and Madsen 1997, Hald et al. 2004, Wieland et al. 2005, Workman et al. 2005). Several studies have identified contact with dogs and cats as a risk factor for sporadic human campylobacteriosis (Kapperud et al. 2003, Neimann et al. 2003, Unicomb et al. 2008, Tam et al. 2009), particularly among infants (Carrique-Mas et al. 2005, Stafford et al. 2008, Doorduyn et al. 2010).

Comparisons of genotypes of *C. jejuni* isolates from wildlife and the environment have yielded contradictory conclusions about the

importance of wild animals as an origin of human campylobacteriosis. Common *C. jejuni* genotypes in human disease occur in wildlife, such as birds (Colles et al. 2003, French et al. 2005), whereas other studies identify predominant subtypes from wild animals and the environment as a minor source of *Campylobacter* spp. in human infections (Broman et al. 2002, Colles et al. 2003, Broman et al. 2004, French et al. 2005, Garrett et al. 2007, Wilson et al. 2008). However, a major problem in studies of environmental campylobacters is the large diversity of inputs, so the environmental sampling may only provide an indication of the diversity of isolates present (Garrett et al. 2007).

Several case-control studies have recognised the consumption of undisinfected water from a surface water source or a private well as a risk factor and, accordingly, the consumption of treated water as a "protective" factor against human sporadic campylobacteriosis (Kapperud et al. 2003, Neimann et al. 2003, Michaud et al. 2004, Nygård et al. 2004, Schönberg-Norio et al. 2004, Carrique-Mas et al. 2005, Sandberg et al. 2006). Furthermore, the largest outbreaks of campylobacteriosis have been water-borne and have often occurred as a consequence of contamination of drinking water supplies due to the washing out of faecal material of farm animals or wild birds from the environment after a heavy rain (Clark et al. 2003, Hänninen et al. 2003, Gallay et al. 2006, Pitkänen et al. 2008). Similarly, rainfall and the subsequent run-off can contaminate surface waters used for recreational purposes. Recently, recreational water exposure has appeared to be a risk factor in case-control studies (Schönberg-Norio et al. 2004, Denno et al. 2009, Doorduyn et al. 2010).

2.2 Subtyping of *Campylobacter jejuni*

The control of human campylobacteriosis requires a thorough understanding of the epidemiology of campylobacters. The special characteristics of these organisms, such as high diversity, weak clonality, frequent recombination within the genus, wide host distribution, and the sporadic nature of the disease, complicate the tracing the sources of these pathogens (Wassenaar and Newell 2000, Dingle et al. 2001, Strachan et al. 2009). Subtyping beyond the species level is therefore fundamental in gathering information on the relative importance of different sources in human campylobacteriosis from outbreak investigations, source attribution studies, and studies on the population genetics of pathogenic bacteria.

2.2.1 Serotyping

Serotyping is a traditional phenotypic subtyping method for epidemiological studies of *C. jejuni* and *C. coli*. Two serotyping schemes based on different antigens are available. The Penner serotyping scheme exploits the passive hemagglutination of heat-stable antigens of campylobacters (Penner and Hennessy 1980, Penner et al.

1983), later identified as capsular polysaccharides (Karlyshev et al. 2000), whereas the Lior scheme uses bacterial heat-labile antigens and slide agglutination (Lior et al. 1982). These previously widely used methods offer relatively low discriminatory power (Garrett et al. 2007, Gilpin et al. 2008b), and a high proportion of strains remains untypeable (Rautelin and Hänninen 1999, Desai et al. 2001, Devane et al. 2005). Therefore, either serotyping technique alone is ineffective as subtyping method. Additional disadvantages of serotyping include the limited commercial availability, high cost and poor quality of the antisera (Rautelin and Hänninen 1999, Desai et al. 2001).

2.2.2 Pulsed-field gel electrophoresis (PFGE)

PFGE is based on restriction site polymorphism throughout the entire genome using rare-cutting endonucleases. Immobilisation of the bacterial suspension in agarose plugs prior to cell lysis prevents the mechanical breakage of the genomic DNA (Wassenaar and Newell 2000). The genomic fragments (20 to 200 bp) are separated on agarose gel under particular conditions of electrophoresis in which the orientation of the electric field changes in a pulsed manner (Lukinmaa et al. 2004).

The most commonly used restriction enzyme in PFGE for *Campylobacter* spp. is SmaI, which produces profiles that are sufficient to demonstrate the dissimilarity of isolates. However, demonstrating the similarity of isolates requires the use of two enzymes in digestion (Lindmark et al. 2004, Gilpin et al. 2006). Some studies have shown that digestion with KpnI alone is almost as discriminatory as the combination of SmaI and KpnI (Michaud et al. 2001, Gilpin et al. 2006). However, the reproducibility of results obtained with KpnI digestion appears to be poorer than those obtained with SmaI (Gilpin et al. 2006), which offers high reproducibility under standardised conditions (Ribot et al. 2001).

The discriminatory power of PFGE is high (Hänninen et al. 2001, Sails et al. 2003). Variation among PFGE patterns arises from chromosomal insertions, deletions and recombination, which increases the discriminatory power of the method and its ability to detect rapidly occurring chromosomal changes (Levesque et al. 2008). Consequently, PFGE is a useful tool in focused short-term epidemiological studies, such as outbreak investigations, whereas it is less suitable for long-term longitudinal studies of epidemiology of campylobacters due to the wide genetic variability of these organisms (Engberg et al. 1998, Sails et al. 2003).

The interpretation of PFGE patterns is, despite computer-aided analysis methods, based largely on the subjective visual comparison of profiles. The lack of standardisation limits comparisons of typing results among different laboratories. The protocols of Pulsenet (Ribot et al. 2001) and Campynet (http://campynet.vetinst.dk) are attempts towards harmonisation of this genotyping method.

2.2.3 Amplified fragment length polymorphism (AFLP)

AFLP method is based on the selective amplification of chromosomal DNA fragments obtained by the use of two restriction endonucleases. After digestion of DNA and the subsequent ligation of restriction site-specific adapters and preselective PCR, the final selective amplification of DNA fragments with radioactively or fluorescently labelled primers results in products from 50 to 500 bp. The final PCR products are separated on denaturing polyacrylamide gels and analysed using an automated sequencer (Duim et al. 1999).

AFLP is a highly discriminatory subtyping method (de Boer et al. 2000, Hänninen et al. 2001), which appears to be less sensitive than PFGE to the genetic instability (Wassenaar and Newell 2000) However, the cost of the equipment and the difficulty of making interlaboratory comparisons are major disadvantages of this method (Wassenaar and Newell 2000, Schouls et al. 2003).

2.2.4 *Fla*-SVR typing

Fla-SVR typing is a technique which uses PCR amplification of the short variable region (SVR) of the *flaA* flagellin gene for sequencing. This region, although short (321 bp), is hypervariable and can discriminate even closely related campylobacter strains (Meinersmann et al. 1997, Dingle et al. 2001, Meinersmann et al. 2005); the technique is therefore valuable in outbreak investigations (Sails et al. 2003). However, the *flaA* locus may be unsuitable for longitudinal epidemiological studies due to intra- and intergenomic recombination (Harrington et al. 1997, Sails et al. 2003).

The major advantage of this method, like other sequence-based methods, is the objective interpretation and standardised nomenclature of the subtypes which permit interlaboratory comparisons and electronic distribution (Sails et al. 2003)

2.2.5 Multilocus sequence typing (MLST)

Multilocus sequence typing utilises the genetic variation of the nucleotide sequences of ca. 500-bp fragments from seven housekeeping genes, which are slowly evolving as they are essential to metabolic function (Dingle et al. 2001, Wareing et al. 2003). Using the nucleotide sequence data, isolates can be assigned a sequence type (ST), which represents a combination of seven numbers obtained by assigning a number to each unique allele at a specific locus. This typing method allows the examination of the population structure of campylobacters in terms of clonal complexes. Each clonal complex, representing a lineage believed to originate from a common ancestor, consists of a central genotype, a founder ST, after which the complex is named, together with closely related genotypes. Generally, the

founder represents a frequently occurring genotype, whereas the other members of the clonal complex are less common (Dingle et al. 2001, Wareing et al. 2003).

MLST was developed to be a tool in studies of population genetics and evolutionary studies (Dingle et al. 2001, Wareing et al. 2003), and is especially suitable for identification of clonal complexes among genetically diverse bacterial species such as *C. jejuni* (Wareing et al. 2003). Due to the wide geographical distribution of sequence types or clonal complexes, MLST is an especially an invaluable tool for long-range epidemiological studies (Dingle et al. 2008).

The discriminatory power of MLST is comparable to that of *flaA* SVR typing (Levesque et al. 2008). However, MLST is less discriminatory than PFGE, and is therefore less suitable for outbreak investigations (Sails et al. 2003, Levesque et al. 2008). The applicability of MLST to short-term epidemiological studies increases when additional loci, such as the *flaA* SVR or nucleotide sequences of genes encoding antigens, are included in the analysis (Sails et al. 2003, Dingle et al. 2008).

The advantages of the method are its objectivity, reproducibility and simplicity of interpretation of the results (Dingle et al. 2001). As a sequence-based typing method, MLST is portable, and the sequence data are comparable between laboratories due to its unified nomenclature (McCarthy et al. 2007, Levesque et al. 2008). A freely accessible international database of *Campylobacter* MLST data is available (http://mlst.zoo.ox.ac.uk).

2.2.6 DNA microarray

Microarray technology enables comparisons of DNA from whole bacterial genome sequences, and, in combination with sophisticated mathematical algorithms, permits the determination of phylogenetic relationships between bacterial populations. Comparative phylogenetics provides an approach to investigate differences in the genomes of isolates from different sources and to identify specific genes associated with particular animal hosts or with the virulence of pathogenic bacteria (Dorrell et al. 2002, Taboada et al. 2004, Champion et al. 2005).

In studies on the comparative phylogenetics of *C. jejuni*, the genomic sequence of pathogenic isolate NCTC 11168 (Parkhill et al. 2000) is the reference strain most commonly used as the basis of whole-genome DNA microarrays (Champion et al. 2005). The exploitation of the complete genome data is a definite advantage of this approach in comparison to other subtyping methods (Taboada et al. 2004). However, a disadvantage is its use of only a single reference strain, which may exclude a fraction of the gene pool of *C. jejuni* (Champion et al. 2005).

2.3 *Campylobacter* spp. in cattle

2.3.1 Prevalence of *Campylobacter* spp. in cattle

Thermophilic campylobacters are typically the most frequently isolated human bacterial pathogens from healthy cattle at slaughter (Beach et al. 2002, Gharst et al. 2006, Madden et al. 2007, Milnes et al. 2008). In slaughterhouse surveys, the prevalence of bovine intestinal campylobacter colonisation has varied between 12.5% and 89.4% (Table 1). Furthermore, studies on campylobacters on cattle farms or in cattle herds have reported percentages from 12% to 100% (Busato et al. 1999, Wesley et al. 2000, Nielsen 2002, Englen et al. 2007, Oporto et al. 2007, Parisi et al. 2007, Gilpin et al. 2008b, Kwan et al. 2008b, Ragimbeau et al. 2008, Ellis-Iversen et al. 2009a), and within-herd prevalences from 0% to 100% in dairy cattle (Humphrey and Beckett 1987, Oporto et al. 2007, Gilpin et al. 2008a, Gilpin et al. 2008b, Pradhan et al. 2009), and from 5.4% to 83% in beef cattle (Inglis et al. 2003, Berry et al. 2006, Oporto et al. 2007). However, the results of different studies are not fully comparable due to variations in study designs and laboratory methods. The intestinal sampling site in slaughterhouse surveys (Garcia et al. 1985, Grau 1988, Stanley et al. 1998, Inglis et al. 2005), the sampling methods on farms (Hoar et al. 1999), the age of animals (Nielsen 2002) and the detection methods in the laboratory (Stanley et al. 1998, Inglis et al. 2003, Gharst et al. 2006) all influence the results.

Table 1. Prevalences of thermophilic *Campylobacter* spp. in cattle in slaughterhouse surveys.

Sample type	No. of animals examined	Proportion of positive samples, %	Reference
Rumen, calves	23	74	Grau 1988
Faeces, calves	24	54	
Rumen, adult cattle	89	3.4	
Faeces, adult cattle	96	12.5	
Gallbladder	100	33	Garcia et al. 1985
Large intestine	100	35	
Small intestine	100	31	
Liver	100	12	
Lymph node	70	1.4	
Intestinal contents	360	89.4	Stanley et al. 1998
Rectal swab, feedlot cattle	100	68	Beach et al. 2002
Rectal swab, adult cattle	96	7	
Gallbladder, intestinal contents, liver or faeces	1154	26.1	Acik and Cetinkaya 2005
Faeces, beef cattle	252	19	Gharst et al. 2006
Faeces, dairy cattle	358	95	
Intestinal contents, calves	74	46	Johnsen et al. 2006
Intestinal contents, adult cattle	715	28.5	
Faeces, beef cattle	220	24.8	Madden et al. 2007
Rectal contents (1999/2000)	667	54.6	Milnes et al. 2008
Rectal contents (2003)	891	24.5	
Liver	108	45	Enokimoto et al. 2007
Bile	108	5	
Liver	60	31.7	Ghafir et al. 2007
Bile	290	23	Matsumoto et al. 2008
Liver	148	1.4	
Faeces, calves	747	39.1	Chatre et al. 2010
Faeces, beef cattle	754	6.0	
Faeces, culled cows	754	4.6	

2.3.2 *Campylobacter* species in cattle

C. jejuni has been predominant, whereas *C. coli* has become a minor species in cattle in most of the slaughterhouse and farm studies (Garcia et al. 1985, Giacoboni et al. 1993, Stanley et al. 1998, Wesley et al. 2000, Minihan et al. 2004, Acik and Cetinkaya 2005, Bae et al. 2005, Berry et al. 2006, Madden et al. 2007, Oporto et al. 2007, Parisi et al. 2007, Gilpin et al. 2008a, Gilpin et al. 2008b, Milnes et al. 2008, Ragimbeau et al. 2008, Ellis-Iversen et al. 2009a, Chatre et al. 2010). Beside these well-known human pathogens, other *Campylobacter* spp. of unclear importance to human health appear to be common in bovine intestines. Some surveys have identified *C. hyointestinalis* (Grau 1988, Atabay and Corry 1998, Pezzotti et al. 2003), and a new species, *C. lanienae,* as the most prevalent *Campylobacter* species in the intestines of cattle (Inglis et al. 2003, Inglis and Kalischuk 2004, Inglis et al. 2004). A minor bovine intestinal species is *C. fetus* (Giacoboni et al. 1993, Atabay and Corry 1998, Busato et al. 1999, Inglis et al. 2003, Inglis et al. 2004), the two subspecies of which cause genital infections and abortions in cattle and can infect immunodeficient humans (Debruyne et al. 2008). Co-colonisation of at least two *Campylobacter* spp. can occur in cattle faeces (Inglis et al. 2003, Inglis et al. 2004) or in the gallbladder (Enokimoto et al. 2007). An animal's age appears to influence to the proportions of different *Campylobacter* spp. present in the faeces of cattle (Giacoboni et al. 1993, Busato et al. 1999, Bae et al. 2005).

2.3.3 *Campylobacter jejuni* in cattle at farm

Cattle are usually symptomless carriers of campylobacters (Stanley et al. 1998). However, *C. jejuni* can cause diarrhoea - sometimes with severe symptoms - in young cattle (Dilworth et al. 1988, Gilpin et al. 2008b). Although free of campylobacters at birth, calves acquire these organisms in an early phase of life due to exposure to a contaminated environment (Stanley et al. 1998, Gilpin et al. 2008b), and are more frequent carriers of campylobacters than adult cattle (Giacoboni et al. 1993, Nielsen 2002, Johnsen et al. 2006, Gilpin et al. 2008b, Chatre et al. 2010). In addition, calves excrete higher numbers of campylobacters in their faeces than do older animals (Stanley et al. 1998, Nielsen 2002), although the diversity of *C. jejuni* subtypes in adult cattle may be greater (Nielsen 2002, Kwan et al. 2008b).

Studies of the shedding patterns of *C. jejuni* in cattle herds have reported that individual animals can be persistent carriers and shedders of high numbers of *C. jejuni* or even of a single subtype of *C. jejuni*, whereas others excrete *Campylobacter* spp. intermittently (Humphrey and Beckett 1987, Hänninen et al. 1998, Stanley et al. 1998, Inglis et al. 2004, Minihan et al. 2004, Gilpin et al. 2008b, Kwan et al. 2008b).

Nevertheless, some individuals appear to be resistant to colonisation in an environment where the exposure rate is high (Minihan et al. 2004). The variety of environmental sources of *C. jejuni* is great, when the cattle are grazing outdoors (Oporto et al. 2007, Grove-White et al. 2010). For example, one farmland study detected an association between the presence of *C. jejuni* in bird faeces and a higher probability of isolating the organism from cattle (Brown et al. 2004). On the other hand, indoor housing can allow re-infection from a faecally contaminated environment or due to closer contacts with carriers of *Campylobacter* spp. (Stanley et al. 1998, Busato et al. 1999, Minihan et al. 2004, Ellis-Iversen et al. 2009a, Ellis-Iversen et al. 2009b). Large herd size, which can relate to higher stocking density of cattle, is likely to increase contact between animals and appears to be a risk factor for faecal shedding of *Campylobacter* spp. (Ellis-Iversen et al. 2009b, Grove-White et al. 2010).

An important factor in the transmission of campylobacters among cattle is drinking water hygiene. Water from private supplies appears to be a risk factor for colonisation of *Campylobacter* spp. in young cattle (Ellis-Iversen et al. 2009b). In addition, campylobacter contamination of water trough surfaces appears to increase (Minihan et al. 2004), and, unsurprisingly, the frequent emptying and cleaning of water troughs reduces the risk for campylobacter infection (Ellis-Iversen et al. 2009a). Without cleaning, the chlorination of drinking water alone seems insufficient to prevent transmission of the organism among cattle reared indoors (Wesley et al. 2000, Besser et al. 2005). During the grazing period, campylobacter colonisation may persist due to the cattle's access to natural waters (Humphrey and Beckett 1987, Hänninen et al. 1998).

A strong seasonal fluctuation in the occurrence of *Campylobacter* spp. is evident in dairy cattle with highest prevalences occurring in late spring or summer when the cattle are grazing (Hänninen et al. 1998, Stanley et al. 1998, Kwan et al. 2008b, Grove-White et al. 2010). Besides the water source, changes in diet can affect the colonisation and shedding of campylobacters in cattle at pasture (Stanley et al. 1998, Ellis-Iversen et al. 2009b, Grove-White et al. 2010). In addition, the presence of wildlife may increase the exposure of cattle to campylobacters, whereas direct transmission between individuals in a herd may occur less frequently than when animals are housed indoors (Grove-White et al. 2010).

The transmission of *Campylobacter* spp. from other production animals, such as pigs, on the same farm can occur at low levels (Boes et al. 2005): one study has identified the presence of horses as a risk factor for the campylobacter colonisation of young cattle (Ellis-Iversen et al. 2009b). Other factors that may increase the risk for campylobacter colonisation of cattle include the type of feed, manure disposal on the farm, the accessibility of feed to wild birds (Wesley et al. 2000), the effects of reproductive hormones (Stanley et al. 1998), or metabolic stress due to the demands on production animals (Grove-White et al. 2010). Among intensively raised feedlot cattle, for

example, the faecal shedding of *Campylobacter* spp. can substantially increase during the relatively short feeding period (Minihan et al. 2004, Besser et al. 2005).

2.3.4 Genetic diversity and host adaptation of bovine *Campylobacter jejuni* strains

C. jejuni strains isolated from cattle represent a wide variety of genotypes. Farm studies have identified as many as nine different genotypes simultaneously present in a herd (Nielsen 2002, Oporto et al. 2007, Parisi et al. 2007, Gilpin et al. 2008a, Ragimbeau et al. 2008), and co-colonisation of two or more non-related *C. jejuni* genotypes in one animal has also occurred (Gilpin et al. 2008a, Gilpin et al. 2008b). The diversity of campylobacter genotypes in cattle may reflect the number of various sources of these organisms due to different farming practices (Nielsen 2002, Parisi et al. 2007), although it may also indicate that the bovine intestinal tract is a favourable environment for the exchange of genetic material among campylobacter strains (French et al. 2005, Meinersmann et al. 2005, McCarthy et al. 2007). Through intragenetic or intergenetic recombination, *C. jejuni* can adapt to persistent colonisation in the intestines of a specific host and acquire a host signature in the genome, which can predict the source of the organism in human infections (Dingle et al. 2001, Champion et al. 2005, McCarthy et al. 2007).

An example of cattle- or ruminant-associated genotypes is the *C. jejuni* ST-61 clonal complex, which, according to reports from a few countries in Europe and from New Zealand, occurs predominantly in cattle (Colles et al. 2003, Manning et al. 2003, French et al. 2005, Kärenlampi et al. 2007, Kwan et al. 2008b, Ragimbeau et al. 2008, Mullner et al. 2010a). Evidence from MLST studies suggests that this clonal complex of *C. jejuni* has evolved in the intestines of cattle and other ruminants, and that the particular allele (*uncA17*) which defines the ST-61 likely originates from *C. coli* (Dingle et al. 2002, French et al. 2005, Meinersmann et al. 2005).

2.4 *Campylobacter* spp. in foods of bovine origin

2.4.1 Beef and edible offal

Although cattle frequently carry campylobacters when arriving at the slaughterhouse, (Besser et al. 2005, Garrett et al. 2007), red meat appears to be a minor source of these organisms (Table 2). The faecal campylobacter contamination of carcasses is possible during processing, but a high-level slaughter hygiene reduces overall contamination (Minihan et al. 2004, Garrett et al. 2007), and drying, along with exposure to oxygen during chilling further decreases the survival of *Campylobacter* spp. on carcasses and in red meat (Grau 1988). Minced meat, rather, can provide favourable conditions for the

survival of campylobacters at refrigerator temperature (Svedhem et al. 1981). However, studies on ground beef at retail have typically failed to detect *Campylobacter* spp. (Ghafir et al. 2007, Medeiros et al. 2008, Phillips et al. 2008).

Table 2. Occurrence of thermophilic *Campylobacter* spp. in retail beef

Total No. of samples	No. of positive samples	Proportion of positive samples, %	Reference
182	1	0.5	(Zhao et al. 2001)
151	2	1.3	(Pezzotti et al. 2003)
221	7	3.2	(Whyte et al. 2004)
230	8	3.5	(Wong et al. 2007)
250	3	1.2	(Hong et al. 2007)
451	49	10.9	(Hussain et al. 2007)
50	1	2.0	(Vindigni et al. 2007)
1514	71	4.7	(Little et al. 2008)
198	22	11.1	(Bostan et al. 2009)
210	5	2.4	(Rahimi et al. 2010)
142	20	14.1	(Sammarco et al. 2010)

Apparently healthy cattle may carry *Campylobacter* spp. in the gallbladder (Garcia et al. 1985, Enokimoto et al. 2007). Bile can therefore transmit campylobacter contamination to the liver during the slaughter process (Acik and Cetinkaya 2005, Enokimoto et al. 2007, Little et al. 2008, Matsumoto et al. 2008). Surveys at slaughter have reported campylobacter prevalences between 1.4% and 45% (Table 1), and retail studies have presented prevalences of 12% to 54% in the liver (Kramer et al. 2000, Little et al. 2008, Medeiros et al. 2008).

2.4.2 Milk and milk products

The common presence of *Campylobacter* spp. in the intestines of dairy cattle warrants the possibility of faecal contamination of raw milk. The contamination of milk can occur due to lapses in hygiene or failures in the milking process, but can be avoided or at least reduced by applying proper hygiene at milking, and pasteurising milk, which destroys campylobacters (Humphrey et al. 2007). The prevalences of *Campylobacter* spp. in raw milk have varied from 0% to 27% (Table 3), and concentrations from lower than 10 cfu/ml up to 100MPN/100 ml (Humphrey and Beckett 1987, Heuvelink et al. 2009).

Few studies have explored the presence and survival of *Campylobacter* spp. in milk products. The preparation processes of Brie and Camembert cheeses or hard and semi-hard cheeses seem unfavourable to campylobacters (Bachmann and Spahr 1995, Medeiros et al. 2008), and the survival of *C. jejuni* in yoghurt is poor (Birk and Knochel 2009), probably due to low pH, and the presence of organic acids and other metabolites produced by lactic acid bacteria. *C. jejuni*, however, was able to survive up to 18 days in garlic butter at refrigerator temperature when the initial inoculum was large (Zhao et al. 2000).

Table 3. Prevalence of *Campylobacter* spp. in raw milk in different studies

Total No. of samples	No. of positive samples	Proportion of positive samples, %	Reference
108	1	0.9	(Doyle and Roman 1982)
210	3	1.4	(Lovett et al. 1983)
111	9	8.1	(Humphrey and Beckett 1987)
111	1	0.9	(Hudson et al. 1999)
131	12	9.2	(Jayarao and Henning 2001)
300	82	27.3	(Yang et al. 2003)
62	1	1.6	(Whyte et al. 2004)
248	5	2.2	(Jayarao et al. 2006)
127	13	10.2	(Hussain et al. 2007)
59	0	0	(Medeiros et al. 2008)

2.5 Cattle as a source of *Campylobacter* spp. in human infections

2.5.1 Outbreak investigations and case-control studies

Investigations have attributed numerous outbreaks of campylobacteriosis to the consumption of unpasteurised or improperly pasteurised milk, or of products prepared from unpasteurised milk (Robinson et al. 1979, Morgan et al. 1994, Fahey et al. 1995, Lehner et al. 2000, Peterson 2003, Centers for Disease Control and Prevention (CDC) 2009, Heuvelink et al. 2009, Unicomb et al. 2009). The consumption of raw milk during farm visits (Evans et al. 1996, Kalman et al. 2000), in camps (McNaughton et al. 1982, Lehner et al. 2000), festivals (Morgan et al. 1994) schools or day-care centres (Jones et al. 1981, Robinson and Jones 1981) has resulted in wide outbreaks in many countries. In Finland, the faecal contamination of milk due to a failure in the milking process caused a long-lasting outbreak of campylobacteriosis among members of a farming family who consumed raw milk (Schildt et al. 2006).

Reports from case-control studies have also identified the consumption of unpasteurised milk as an important risk factor for campylobacteriosis among humans (Studahl and Andersson 2000, Kapperud et al. 2003, Neimann et al. 2003, Michaud et al. 2004), especially among children (Carrique-Mas et al. 2005). Other risk factors related to food of bovine origin include the consumption of steak tartare (a raw beef product) (Doorduyn et al. 2010) or barbecued red meat (Neimann et al. 2003).

Several recent case-control studies have examined the different risks of campylobacteriosis available in rural and urban areas. In a Danish study, the risk for infection appeared higher among people - particucarly children - living in areas of low population density or in farm houses than in urban–type housing (Ethelberg et al. 2005), and another study reported rising campylobacteriosis incidence associated with increasing ruminant density in Sweden (Nygård et al. 2004). In Walkerton, Canada, campylobacters originating from neighbouring cattle farms contaminated the municipal water supply after a heavy rain and caused a large-scale water outbreak (Clark et al. 2003). Furthermore, an increased risk for campylobacteriosis has been associated with contact with cattle (Kapperud et al. 2003, Neimann et al. 2003), or with farm animals more generally, including cattle (Michaud et al. 2004, Doorduyn et al. 2010). Indeed, direct contact with diarrhoeic calves or with bovine faecal material appeared to be the cause of a campylobacter infection of farm workers or children living on farm (Dilworth et al. 1988, Gilpin et al. 2008a).

2.5.2 Genotyping and source attribution studies

Subtyping campylobacters enables the attribution of human infections to specific sources. Source attribution studies that have compared campylobacter isolates from human infections and from potential sources of infection, and examined risk factors related to specific *C. jejuni* genotypes have provided additional information about the role of cattle in human campylobacteriosis. PFGE of human and cattle isolates in temporally and spatially defined studies has shown genotypic similarities indicating cattle as potential source of *Campylobacter* spp. in humans (Fitzgerald et al. 2001, Devane et al. 2005, Johnsen et al. 2006, Garrett et al. 2007, Gilpin et al. 2008b). Comparisons of human and animal isolates from various collections representing several time periods have indicated that some ST complexes, especially ST-61 complex, commonly isolated in human infections (Dingle et al. 2001) are unexpectedly common in cattle (Dingle et al. 2002, Manning et al. 2003, Schouls et al. 2003, Kärenlampi et al. 2007). Recent spatio-temporally focused MLST studies in the farm environment have confirmed the association of ST-61 complex with cattle (French et al. 2005, Kwan et al. 2008a), and have identified additional bovine-adapted STs occurring in humans as well (Rotariu et al. 2009, Sheppard et al. 2009). The importance of bovine sources was evident in a study reporting that 42% of campylobacter isolates from infections in young children in a rural area represented STs similar to those from cattle (Strachan et al. 2009).

Cattle have become a potential origin of human campylobacter infections in genotype-specific risk factor studies. Human infections by *C. jejuni* STs associated with ruminants, especially among children in rural areas have been more frequent in rural areas than in urban areas (Mullner et al. 2010b). Furthermore, ST-48 (a sequence type occurring especially in cattle) in patients was associated with eating and tasting raw minced meat (Kärenlampi et al. 2007), and a certain *flaA* subtype (the third most common type in human infections in Australia) was associated with the consumption of undercooked beef (Unicomb et al. 2008).

2.6 Antimicrobial resistance of *Campylobacter* spp.

As a self-limiting disease, campylobacter enteritis rarely requires antimicrobial therapy, which may, however, be necessary for patients with severe symptoms, prolonged duration of the infection, or an underlying disease (Blaser and Engberg 2008). The first choice of treatment of the disease is the macrolides: erythromycin, clatrithromycin or azithromycin (Bywater et al. 2004, Gupta et al. 2004, Blaser and Engberg 2008). The previous practice of using fluoroquinolones, especially in travel-related enteric infections, may be ineffective in the treatment of campylobacteriosis due to the rapidly rising antimicrobial resistance of *Campylobacter* spp. (Gupta et al. 2004), which is common among the *C. jejuni* and *C. coli* isolates from

animals and food in several European countries (EFSA 2010a). The development of resistance to fluoroquinolones among campylobacters has occurred concurrently with the extensive use of these antimicrobials in food production animals (Endtz et al. 1991, Levesque et al. 2008), and the veterinary use of fluoroquinolones appears to be a plausible explanation for the increased resistance among *Campylobacter* spp. rather than their use in human medicine (Engberg et al. 2004). Fluoroquinolone treatment can, in rare occasions, induce the emergence of resistant strains in human patients (Wistrom and Norrby 1995). However, patients are insignificant as sources of resistant campylobacter strains due to the minor role of person-to-person transmission in the epidemiology of campylobacteriosis (Engberg et al. 2004). Nevertheless, with regard to human campylobacter infections, decreased susceptibility to antimicrobial agents among *Campylobacter* spp. is a major concern, because the range of antimicrobial agents available for the treatment of severe infections may be considerably compromised, and failures in treatment are possible (Anderson et al. 2001). Furthermore, evidence from some studies indicates that human infections caused by resistant campylobacter strains may be prolonged or become more serious then those caused by susceptible strains (Engberg et al. 2004, Gupta et al. 2004, Helms et al. 2005, Feodoroff et al. 2009). Recently, the WHO has defined fluoroquinolones and macrolides as critically important antimicrobials in human medicine (WHO 2007) and recommended urgent development of risk management strategies for maintaining the effectiveness of these agents.

The determination of minimum inhibitory concentrations (MICs) is the recommended method for examination of the antimicrobial susceptibility of pathogenic bacteria (EUCAST 2003, CLSI 2008). To monitor the development of antimicrobial resistance, the European Food Safety Authority (EFSA 2007) recommends interpreting of the data according to epidemiological cut-off values (Table 4), which separate the wild-type bacterial population and isolates with reduced susceptibility to antimicrobial agents (Kahlmeter et al. 2003), instead of the clinical breakpoint values, which are the criteria in the therapeutic approach (Schwarz et al. 2010).

Table 4. Epidemiological cut-off values and clinical breakpoints of antimicrobial susceptibility for *Campylobacter jejuni* and *C. coli*

Antimicrobial agent	Epidemiological cut-off value, mg/l[a]		Clinical breakpoint, mg/l[b]
	C. jejuni	*C. coli*	*C. jejuni/coli*
Cloramphenicol	>16	>16	ND[c]
Ciprofloxacin	>1	>1	≥4
Erythromycin	>4	>16	≥32
Gentamicin	>1	>2	ND
Nalidixic acid	>16	>32	ND
Streptomycin	>2	>4	ND
Tetracycline	>2	>2	≥16

[a] EUCAST http://www.eucast.org/mic_distributions/
[b] CLSI 2006
[c] Not determined for *Campylobacter* spp.

3 Aims of the study

The aim of this study was to investigate the role of Finnish cattle as a potential reservoir of thermophilic *Campylobacter* spp., and antibiotic-resistant *Campylobacter jejuni*, and as a source (besides chicken) of domestically acquired sporadic human campylobacteriosis in Finland.

The specific objectives were:

I. to determine the prevalence of thermophilic *Campylobacter* spp. in Finnish cattle at slaughter as well as the diversity and antimicrobial susceptibility of bovine *C. jejuni* isolates.

II. to investigate the colonisation dynamics of *C. jejuni* in three Finnish dairy cattle herds.

III. to develop genetic markers for investigation of the host association of *C. jejuni* strains isolated from cattle, chickens and humans.

IV. to evaluate the contributions of chickens and cattle as sources of domestically acquired sporadic human *C. jejuni* infections in Finland in the summer of 2003.

4 Materials and methods

4.1 Sampling

In study I, 952 rectal faecal samples and 948 carcass surface samples were collected from 12 Finnish slaughterhouses from January to December 2003. The number of samples and the frequency of sampling were determined on the basis of the slaughter volumes of each slaughterhouse during the previous year. The faecal material from randomly chosen animals was collected into plastic sampling jars, leaving only a small air space in order to prevent the adverse effects of oxygen on the survival of the campylobacters. Carcass surface samples including the brisket, inner and outer thigh, and the pelvic cavity of the same animals, were taken using premoistened sterile gauze pads placed in sterile plastic bags for transportation.

In study II, three campylobacter-positive dairy cattle herds (15, 20 and 90 animals) located 60 km apart from each other in Southern Finland, were sampled over a one-year period on five occasions: 1) after the grazing period in November 2006, 2) in the middle of winter housing period in January-February 2007, 3) before the new grazing period in April 2007, 4) during the grazing in August 2007, and 5) after the grazing period in November 2007. On each sampling occasion, between 17 and 33 samples of newly-avoided faeces from individual animals were collected from the floor. Animals recently treated with antimicrobials were excluded from the sampling. In addition, tank milk samples were taken on each occasion. During the last sampling, drinking troughs of the animals were sampled using sponge swabs (Medical Wire & Equipment, Corsham, Wiltshire, UK).

4.2 Isolation of *Campylobacter* spp. (I, II)

All faecal samples of the slaughterhouse survey (I) and the farm study (II) were examined using enrichment. Ten grams of faecal material were weighed into 90 ml of Bolton broth (Campylobacter Enrichment Broth, Lab 135 plus selective supplement X131 [LAB M, Bury, England] plus lysed horse blood). In study II, a 10-fold dilution series up to 10^{-6} in Bolton broth was cultured for the semiquantitative detection of *Campylobacter* spp. (NCFA [Nordic Committee on Food Analysis] 2007). Broth cultures were incubated at 41.5°C for 24 h in a microaerobic incubator (ThermoForma [Thermo Electron Corporation, Marietta, OH]) (O2, 5%; CO2, 10%; N2, 85%). One loopful (10 µl) of enrichment culture was spread onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) plates (Campylobacter Blood Free Selective Medium Lab 112 plus supplement X112 [LAB M, Bury,

England]), which were incubated under the same conditions. The gauze samples from carcasses (I) and the sponge swab samples from drinking troughs (II) were similarly enriched in 225 ml of Bolton broth. In study I, an additional 10-µl loopful of 730 faecal samples was directly cultured on mCCDA for comparison of the two detection methods.

The most probable number (MPN) technique was applied to quantify *Campylobacter* spp. in the tank milk samples (II). Either 10×100 ml or 10×20 ml of raw milk was enriched in Bolton broth (100 ml of milk + 500 ml of Bolton broth or 20 ml of milk + 80 ml of Bolton broth). The enrichment cultures were incubated microaerobically at 37˚C for 48 h and plated on mCCDA plates which were incubated microaerobically at 37˚C for 48 h.

A minimum of two typical colonies from each mCCDA plate were subcultured onto Brucella agar (BBL, Becton Dickinson, MD) supplemented with 5% whole bovine blood treated with sodium citrate. A minimum of two isolates per campylobacter-positive sample were biochemically identified to the species level according to the standard method ISO 10272-1:2006 (ISO 2006). H_2S production in triple-sugar iron agar (TSI, pH 8) (LAB M, Bury, England) and the urease production of hippurate-negative, indoxyl acetate-hydrolysing isolates were examined to identify *C. hyointestinalis* strains. The isolates were stored in Brucella broth (BBL, Becton Dickinson, MD) supplemented with 15% glycerol at -70°C.

4.3 *Campylobacter jejuni* isolates (III, IV)

4.3.1 Human isolates (III, IV)

In study III, domestically acquired human *C. jejuni* isolates (*n*=309) were isolated in six local laboratories from July to September 1999 (Kärenlampi et al. 2003) and at the Helsinki University Central Hospital Laboratory throughout the year in 1996, 2002 and 2003 (Kärenlampi et al. 2007).

Altogether 175 domestic human *C. jejuni* isolates, collected in nine clinical microbiology laboratories (Figure 1) across the country from June to August 2003 were included in study IV. The strains were isolated from faecal samples of diarrhoeic patients by direct culture on mCCDA. These laboratories submitted all domestic isolates to the National Public Health Institute (KTL; currently the National Institute for Health and Welfare [THL]) for further examination. An isolate was considered domestic if the patient had not travelled abroad within ten days prior to the onset of symptoms or within 17 days before the specimen was taken. Isolates from identified outbreaks were excluded.

4.3.2 Chicken isolates (III, IV)

The chicken *C. jejuni* isolates in study III represented all chicken slaughter batches from the three Finnish slaughterhouses in the summer of 1999 (Perko-Mäkelä et al. 2002) and retail chicken meat samples from the Helsinki area from July to September 2003 (Kärenlampi et al. 2007).

Chicken *C. jejuni* isolates (*n*=43) represented all chicken batches (*n*=955) slaughtered between May and August 2003 in two of the three Finnish broiler slaughterhouses (IV) (Figure 1). The strains were isolated in slaughterhouse laboratories by direct culture on mCCDA of the caecal contents from three to five chickens per slaughter batch. One isolate from each campylobacter-positive slaughter batch was submitted to the Finnish Food Safety Authority (Evira) for further investigation.

4.3.3 Bovine isolates (III, IV)

The bovine *C. jejuni* isolates (*n*=131) in study III were selected from the isolates from bovine faeces in study I.

In study IV, we compared all faecal *C. jejuni* isolates (*n*=186) collected in the cattle slaughterhouse survey (I) throughout the entire year to human domestic isolates collected during the seasonal peak, because we assumed that the herds from which the campylobacter - positive animals came continuously carried the same PFGE types (as occurred in the three herds in study II). Consequently, these types could infect humans during the summer. In addition, all carcass isolates (*n*=15) from sampling between May and August 2003 were included to represent possible transmission via beef.

4.4 Serotyping of *Campylobacter jejuni* isolates (I)

C. jejuni isolates from bovine faecal and carcass samples were serotyped using a set of 25 commercial antisera for the serotyping of heat-stable antigens (Penner) of *C. jejuni* using the passive hemagglutination method (Denka Seiken Co., Ltd., Tokyo, Japan). Tests were performed, and the results were interpreted according to the manufacturer's instructions.

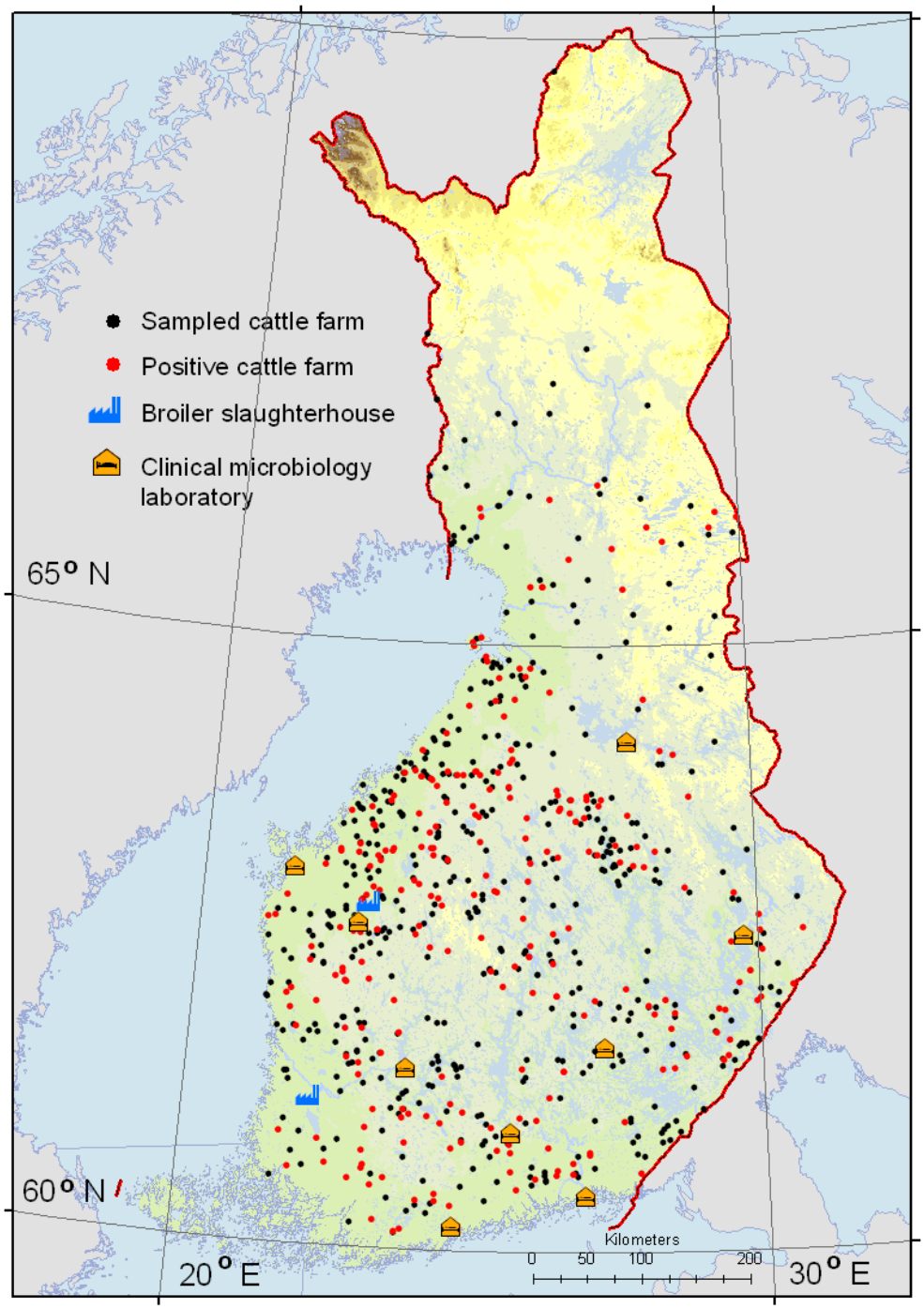

Figure 1. Location of clinical microbiology laboratories and chicken slaughterhouses included in study IV, and cattle farms in the slaughterhouse survey (I).

4.5 Pulsed-field gel electrophoresis (I, II, IV)

The agarose plugs for PFGE analysis were prepared according to the PulseNet protocol (www.cdc.gov/pulsenet/protocols, (Ribot et al. 2001) and stored in Tris-EDTA buffer at 4°C. The DNA was digested overnight at 25°C with 20 U of SmaI, or for a minimum of 4 h at 37°C with 20 U of KpnI restriction endonuclease (New England Biolabs Inc., Ipswich, MA) in a final volume of 200 µl with 2 µl of bovine serum albumin (New England Biolabs Inc., Ipswich, MA). An agarose gel (1%) was prepared in 0.5 × Tris-buffered EDTA (Sigma-Aldrich Co, Baltimore, MD). Fragments were separated by electrophoresis for 18 h at 6 V and 14°C with ramped pulse times from 6.8 to 35.4 s with a CHEF-DRIII pulsed-field electrophoresis system (Bio-Rad, CA). The gels were stained for 45 min with ethidium bromide (0.5 µg/ml) and photographed under UV light.

The PFGE data were analysed with Bionumerics V5.10 (Applied Maths, Kortrijk, Belgium) at 0.5% optimisation and 1.0% tolerance. The PFGE pattern of *Salmonella* Braenderup H9812 (ATCC BAA-664) served as the fragment size marker. Profiles differing by one or more bands were considered different subtypes. The criteria presented by (Tenover et al. 1995) were applied to assess the relationship of the subtypes (I).

4.6 PCR of genetic markers of *Campylobacter jejuni* (III)

Four genetic markers were selected from the completely sequenced genomes of *C. jejuni* strains 81-176 (Hofreuter et al. 2006), RM1221, and NCTC 11168 using comparative genomics (Chaudhuri et al. 2008), and primers were designed for the detection of these markers, which were *ggt*, the γ-glutamyl transpeptidase gene; *dmsA* (Cju34), a subunit of the putative tripartite anaerobic dimethyl sulfoxide (DMSO) oxidoreductase (DMSO/trimethylamine *N*-oxide reductase) gene; Cj1585c, coding for a putative oxidoreductase; and CJJ81176-1371, a putative serine protease gene.

The presence of these four genes in *C. jejuni* isolates from bovine faecal samples (n=131), chicken caecal or meat samples (n=205), and human patients (n=309) was examined using PCR to assess their applicability for host association studies. PCR primers designed for the amplification of the fragments appear in Table 5. Twelve PCR products for each gene fragment were sequenced to find the similarity of the sequences within a gene.

Table 5. Primers used in amplification of the fragments of the four genetic markers

Gene marker	Primer sequence		Size of the product (bp)
	Gene marker	Primer sequence	
ggt	TTTTAGCCATATCCGCTGCT	AGCTGCTGGAGTACCAA	339
dmsA	GATAGGGCATTGCGATGAGT	CTTGCTAGCCCAATCAGGAG	238
Cj1585c	TGTTGTGGGTTTGCTGGATA	TTGCTTCACTGCATTCATCC	202
CJJ81176-1367/1371	TGCAAAGCAGGGCTAAGAAT	TTATGGAGCTGGGGTGTTTC	318

4.7 Determination of antimicrobial susceptibility of *Campylobacter jejuni* isolates (I)

The minimum inhibitory concentrations (MICs) of ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid, and oxytetracycline for *C. jejuni* isolates from rectal faecal samples (I) were determined using a commercial broth microdilution method, VetMIC Camp (National Veterinary Institute, Uppsala, Sweden; www.sva.se/en/Target-navigation/Services--Products/VetMIC/). Epidemiological cut-off values for resistance, based on MIC distributions, were used in the interpretation of the results. A *C. jejuni* isolate was considered resistant to a specific antimicrobial when its MIC was distinctly higher than those of inherently susceptible *C. jejuni* isolates.

4.8 Statistical methods

Statistical analysis was performed using Excel or SPSS software. The χ^2 test was used to investigate the association between the month of sampling and the prevalence of *Campylobacter* spp., *C. jejuni* and *C. hyointestinalis* ssp. *hyointestinalis* in study I, to test the similarity in the frequencies of marker genes among the isolates from different hosts in study III, and to investigate the association between human *C. jejuni* genotypes and different animal reservoirs, as well as the similarity of human *C. jejuni* genotypes and those isolated from beef and dairy cattle herds in study IV. In addition, the host association of the combined set of the four genetic markers in study III was examined using the paired two-tailed Student's *t* test.

5 Results

5.1 Prevalence of *Campylobacter* spp. in cattle at slaughter and on three dairy farms (I, II)

Campylobacter spp. were isolated from 296 of 952 (31.1%) bovine rectal faecal samples and from 33 of 948 (3.5%) bovine carcass surface samples at slaughter (Table 6). The sampled animals originated from 747 farms. The prevalence of *Campylobacter* spp. was higher in beef cattle than in dairy cattle in terms of the individual animals and the proportions of their farms of origin (Table 7). Among the three dairy cattle herds in study II, *Campylobacter* spp. were isolated from 65% (221/340) of all the faecal samples, and from one of the sponge swab samples from the drinking troughs. No campylobacters were detected in the milk samples, whereas *Arcobacter butzleri* was detected in three milk samples from herd 3 and in one milk sample from herd 1.

Table 6. Prevalence of *Campylobacter* species in bovine faecal and carcass samples at slaughter.

Species	Faecal samples (*n*= 952)		Carcass samples (*n*=948)	
	Number	Prevalence	Number	Prevalence
Campylobacter jejuni	186	19.5	29	3.1
Campylobacter coli	21	2.2	2	0.2
Campylobacter hyointestinalis	103	10.8	2	0.2
Campylobacter spp., total	296	31.1	33	3.5

C. jejuni was the most commonly isolated thermophilic *Campylobacter* species in both studies (I and II). The prevalence of *C. jejuni* at slaughter was 19.5% (186/952). This species was more common in cattle under three years of age than in those from three to seven years of age (Table 8). In the farm study (II), *C. jejuni* was detected in 49.7% (169/340) of the faecal samples, and was also present in one of the drinking-trough samples. *C. coli* was detected in 3.2% (11/340) of the faecal samples taken on farms, and was also a minor species in samples taken at slaughter (Table 6). In herd 1, where the same ten animals were sampled on every sampling occasion, *C. jejuni* was isolated from all the samples of one animal, whereas two other animals tested campylobacter-negative on all occasions throughout the sampling period.

Table 7. Distribution of campylobacter-positive animals among beef and dairy cattle

Herd type	No. of animals	No. of positive animals	Proportion of positive animals, %	No. of farms	No. of positive farms	Proportion of positive. farms, %
Beef	337	154	45.7	283	121	42.7
Dairy	615	142	23.1	463	133	28.7
Total	952	296	31.1	746	254	34.0

Beside thermophilic *Campylobacter* spp., *C. hyointestinalis* subsp. *hyointestinalis* was detected in bovine faeces and carcasses at slaughter (Table 6), and on average in 15.3% (52/340) of the faecal samples of the three dairy herds in study II. In addition, catalase- and urease-negative, H_2S-producing *Campylobacter* sp. was detected in the faecal samples of herd 1 throughout the sampling period.

Table 8. The prevalence of *Campylobacter* spp. in Finnish cattle representing different age groups

Age at slaughter	Total No. of samples	*C. jejuni* Positive	%	*C. coli* Positive	%	*C. hyointestinalis* Positive	%
1 to 3 years	667	171	25.6	13	1.9	67	10.0
3 to 7 years	238	10	4.2	7	2.9	29	12.2

The prevalence of *C. jejuni* in faecal samples at slaughter showed a slightly rising trend towards the end of summer to 29.2% at its peak in August 2003 (I). The association between the sampling month and the prevalence of *C. jejuni* was not statistically significant. Among the three dairy herds (II), the average monthly prevalence of *C. jejuni* was highest (64%) in November 2006, and lowest (37%) in November 2007. The prevalences between the three herds varied widely (Figure 2). In herd 3, the prevalence was consistently higher than in the other two herds (II).

Enrichment was able to detect *Campylobacter* spp. from 273 (37.4%), and direct culture from 32 (4.4%) of the 730 faecal samples (I). In the semiquantitative detection of study II, the levels of *C. jejuni* in the faecal samples were generally low (Figure 3). Of the faecal samples that tested positive, 42% were detected from the enrichment of dilution 10^{-2} at its peak. In herd 3, *C. jejuni* occurred at high levels on all sampling occasions except in August 2007.

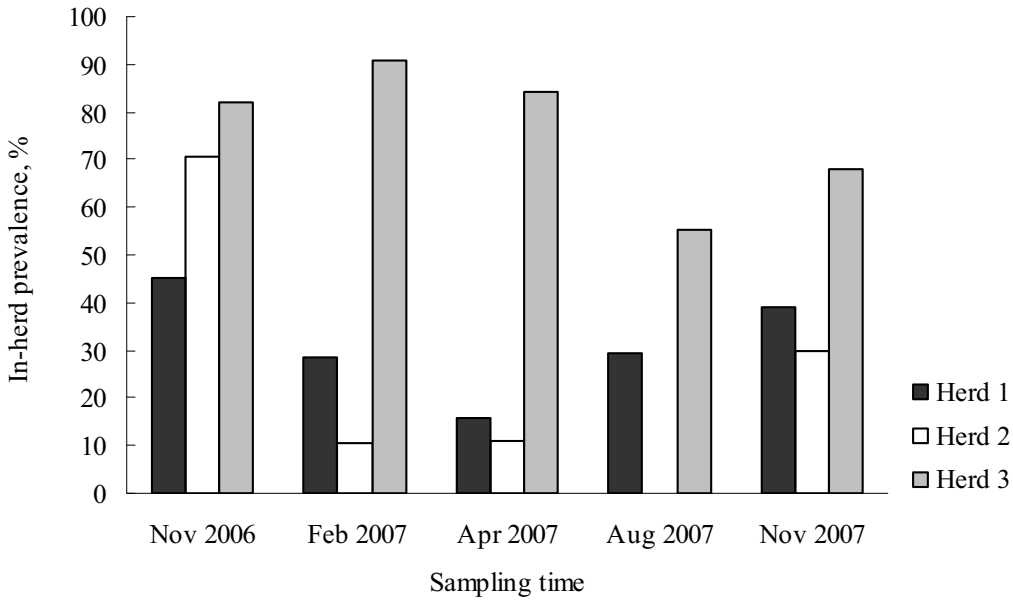

Figure 2. Prevalence of *Campylobacter jejuni* in three Finnish dairy cattle herds on different sampling occasions between November 2006 and November 2007.

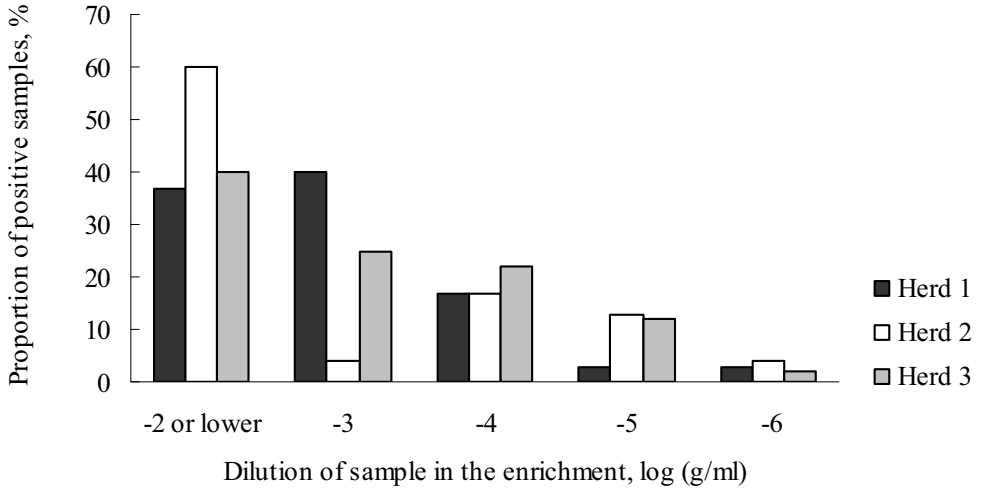

Figure 3. The distribution of campylobacter levels among positive faecal samples of three dairy cattle herds determined by semiquantitative detection.

5.1.2 Subtypes of *Campylobacter jejuni* (I, II, IV)

The faecal *C. jejuni* isolates from study I were classified into 17 serotypes according to the 25 commercially available antisera used for typing. Isolates from 22 samples (12.5%) were untypeable. The predominant serotypes isolated from the faecal samples were Pen2 and Pen4-complex, present in 52% (96/186) of the campylobacter-positive faecal samples and in 76% (16/21) of the carcass samples. The *C. jejuni* isolates representing the serotype Pen2 were further divided into 23 PFGE types with SmaI, and 13 SmaI subtypes were identified among isolates representing Pen4-complex. Pen2/S1 was the most common combined sero-PFGE type among the isolates from the faecal and carcass samples (Table 9).

Table 9. The predominant combined sero/PFGE types of *Campylobacter jejuni* isolates from bovine faecal samples at slaughter

Penner serotype	SmaI subtype	No. of positive samples	% of positive samples
2	S1	19	10.8
2	S5	10	5.7
2	S11	7	4.0
2	S18	5	2.8
4-complex	S2	10	5.7
4-complex	S3	7	4.0
12	S7	8	4.5
1,44	S6	5	2.8
Total		71	40.3

In total, PFGE with SmaI restriction enzyme identified 56 different subtypes of *C. jejuni* among the 330 isolates from the faecal samples of slaughter cattle and 20 subtypes among the 33 isolates from the carcass samples taken before chilling (I). Isolates from 30 *C. jejuni*-positive animals (16.1%) and from 11 (33.3%) carcasses represented unique subtypes. The DNA from five faecal isolates was not digestible with SmaI.

In study II, a total of thirteen SmaI genotypes were identified among the *C. jejuni* isolates (n=403) from the three dairy herds. One to four SmaI subtypes were detected from each of the herds on each sampling occasion, except in August 2007, when no campylobacters were isolated from herd 2. In herds 1 and 3, however, two subtypes

persisted throughout the entire sampling period from November 2006 to November 2007. A few additional types emerged in August 2007, whereas in herd 2, only two *C. jejuni* subtypes occurred during the entire sampling period, and no *Campylobacter* spp. were detected in August 2007. In study I, *C. jejuni* isolates from animals originating from the same farm during the same sampling (16 occasions) represented indistinguishable or related SmaI types on nine occasions and unrelated types on five occasions. *C. jejuni* isolates from the same farms on two sampling occasions (six farms) represented unrelated subtypes.

Two different SmaI subtypes were detected in 3 of the 169 positive faecal samples in study II, and in study I, the PFGE of multiple isolates from 106 faecal samples identified different SmaI types in eight samples. Two different types were detected from two carcass samples. On 12 sampling occasions, the faecal and carcass samples from the same animal yielded indistinguishable *C. jejuni* SmaI types (I). In addition, identical subtypes were isolated from one animal's faecal sample and from another animal's carcass sample during six samplings. In study II, isolates from each of the animals in herd 1 that yielded multiple campylobacter-positive samples were consistently indistinguishable, with the exception of a previous carrier of subtype S7, from which subtype S64 was isolated after two negative samples. Furthermore, the *C. jejuni* isolates from the drinking trough at farm 3 represented the most frequently detected two SmaI subtypes among animals in that herd.

In study IV, PFGE with SmaI restriction identified 43 subtypes among the 175 *C. jejuni* isolates from human domestic infections between June and August 2003, and 15 subtypes among the 43 isolates from chicken slaughter batches between May and August 2003. SmaI was unable to type 18 isolates from humans and one from chickens. Bovine faecal isolates from the entire year (n=186) and carcass isolates from May to August 2003 (n=15) represented a total of 61 subtypes.

Fourteen SmaI subtypes of *C. jejuni* (32.6% of all 43 human subtypes) representing 114 (65.1%) of 175 human isolates overlapped with those of chicken or bovine isolates. In total, 83.7% (36/43) of chicken isolates and 30.8% (62/201) of bovine isolates represented SmaI subtypes shared with humans. Further subtyping of 212 *C. jejuni* isolates (114 human, 36 chicken, and 62 cattle isolates), representing the 14 overlapping SmaI subtypes with KpnI restriction enzyme yielded 44 subtypes, 17 of which were shared between human and animal isolates (Table 10). The combined type S6/K12 predominated among the isolates from human patients (12%), and occurred in chickens and cattle as well. In total, the SmaI/KpnI profiles of 97 (55.4%) human isolates were indistinguishable from those of chicken or cattle isolates. The overlapping combined SmaI/KpnI subtypes accounted for 69.8% (30/43) of the chicken isolates and 15.9% (32/201) of the cattle isolates. The occurrence of identical SmaI/KpnI subtypes with human *C. jejuni* isolates was significantly associated with animal host species ($P < 0.001$).

All ten bovine subtypes overlapping with those of humans represented isolates from dairy cattle (*n*=31), with the exception of S22/K16, isolated from only one beef cattle. The occurrence of identical SmaI/KpnI subtypes with human *C. jejuni* isolates in cattle was not significantly related to herd type (*P* =0.056).

A temporal association of the SmaI/KpnI subtypes among isolates from chickens and patients was possible in 55 (31.4%) of 175 human infections (Table 11). Isolates from 27 (15.4%) of human cases with no temporal relation to chickens were identical to bovine isolates.

Table 10. Occurrence of overlapping SmaI/KpnI subtypes of *Campylobacter jejuni* in domestically acquired human sporadic infections, chickens and cattle in Finland in summer 2003

PFGE subtype		Origin of isolates: Human		Chicken		Cattle	
SmaI	KpnI	No. of isolates	% of isolates	No. of isolates	% of isolates	No. of isolates	% of isolates
S4	K29	1	0.6	1	2.3	1	0.5
S5	K27	1	0.6	0	0.0	10	4.9
S6	K12	21	12.0	2	4.7	7	3.4
S7	K1	12	6.9	2	4.7	7	3.4
S7	K2	4	2.3	2	4.7	2	1.0
S7	K3	17	9.7	2	4.7	1	0.5
S22	K16	1	0.6	0	0.0	1	0.5
S54	K10	6	3.4	2	4.7	0	0.0
S54	K11	3	1.7	1	2.3	0	0.0
S64	K19	7	4.0	1	2.3	1	0.5
S66	K18	4	2.3	0	0.0	1	0.5
S74	K4	5	2.9	8	18.6	0	0.0
S74	K5	8	4.6	4	9.3	1	0.5
S74	K7	2	1.1	2	4.7	0	0.0
S76	K20	3	1.7	1	2.3	0	0.0
S77	K30	1	0.6	1	2.3	0	0.0
S78	K6	1	0.6	1	0.0	0	0.0
Isolates of shared subtypes		97	55.4	30	69.8	32	15.6
Total No. of isolates		175		43		201	

Table 11. Temporal association between *Campylobacter jejuni* isolates from humans and chicken slaughter batches in summer 2003 in Finland

SmaI/KpnI Subtype	Number of human isolates							
	June		July		August		Total	
	associated	not associated	associated	not associated	associated	not associated	associated	not associated
S4/K29	0	0	0	1	0	0	0	1
S6/K12	0	0	7	0	14	0	21	0
S7/K1	0	1	8	0	3	0	11	1
S7/K2	0	0	0	4	0	0	0	4
S7/K3	0	2	1	5	9	0	10	7
S54/K10	0	0	0	6	0	0	0	6
S54/K11	0	0	0	1	1	1	1	2
S64/K19	0	0	0	5	1	1	1	6
S74/K4	0	0	5	0	0	0	5	5
S74/K5	0	0	0	8	0	0	0	8
S74/K7	0	0	0	0	2	0	2	2
S76/K20	0	0	0	0	3	0	3	3
S77/K30	0	0	0	0	1	0	1	1
S78/K6	0	0	0	1	0	0	0	1
Total	0	3	21	31	34	2	55	47
Total No. of human isolates		11		106		58		175

5.1.3 Occurrence of genetic markers among *Campylobacter jejuni* isolates from humans, chickens and cattle (III)

The γ-glutamyl transpeptidase and *dmsA* genes were more frequently detected among human and chicken *C. jejuni* isolates than among bovine isolates. In addition, *dmsA*-positive chicken isolates occurred with a similar high annual frequency in 2003, 2006, and 2007. In contrast, the Cj1585 oxidoreductase and the CJJ81176-1371 serine protease genes were more common among the bovine isolates than among the human and chicken isolates (Table 12). The bovine isolates differed significantly (P <0.05) from human and chicken isolates in the *t* test.

Table 12. Occurrence of four marker genes (*ggt*, *dmsA*, Cj1585c and CJJ81176-1371) in *Campylobacter jejuni* isolates from humans, chickens and cattle

Marker gene	Number of isolates harbouring the gene (%)					
	Human (*n*=309)		Chicken (*n*=205)		Cattle (*n*=131)	
ggt	169	(54.7)	75	(36.6)	11	(8.4)
dmsA	256	(82.8)	151	(73.3)	18	(13.7)
Cj1585c	99	(32.0)	49	(23.9)	83	(62.6)
CJJ81176-1367/1371	117	(37.8)	74	(36.1)	96	(73.3)

5.1.4 Antimicrobial susceptibility of bovine *Campylobacter jejuni* isolates (I)

Of the 187 *C. jejuni* isolates examined for antimicrobial susceptibility, 16 (9%) proved resistant to at least one of the antimicrobials tested (Table 13). Resistance to nalidixic acid was most common. Six of the 11 nalidixic acid-resistant isolates were also resistant to enrofloxacin. None of the isolates presented multiresistance.

Table 13. Distribution of minimum inhibitory concentrations (MICs) among bovine *Campylobacter jejuni* isolates (*n*=187)

Substance	% resistant isolates (95% CI[a])	Distribution of MICs (mg/l)[b]															
		≤ 0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Ampicillin	1.6 (0.3-4.6)					5.9	7.0	41.2	40.1	3.2	1.1	0.5	1.1				
Enrofloxacin	3.2 (1.2-6.9)	1.1	8.0	49.2	33.7	4.8	1.1	0.0	1.6	0.5[c]							
Erythromycin	0 (0.0-2.0)			1.1	1.6	22.5	51.9	20.9	2.1								
Gentamicin	0 (0.0-2.0)				3.2	54.0	42.2	0.5	1.1								
Nalidixic acid	5.9 (3.0-10.3)							1.6	15.5	61.0	16.0	3.7	0.0	0.5	1.6		
Tetracycline	1.1 (0.1-3.8)				92.0	5.9	0.5	0.5	0.5	0.5							

Bold vertical lines indicate cut-off values for resistance. Hatched fields denote range of dilutions tested for each substance.

[a] CI, confidence interval.

[b] MICs equal to or lower than the lowest concentration tested are given as the lowest concentration.

[c] MIC greater than the highest concentration in the range of dilutions tested.

6 Discussion

6.1 *Campylobacter* spp. in Finnish cattle

The sampling in our survey on *Campylobacter* spp. in cattle at slaughter (I) covered all major Finnish slaughterhouses representing 98% of the cattle slaughtered in Finland in 2003. The prevalence of *Campylobacter* spp. among Finnish cattle was lower than that in several other studies (Garcia et al. 1985, Stanley et al. 1998, Beach et al. 2002, Johnsen et al. 2006, Milnes et al. 2008). However, the results from different studies are not fully comparable due to different study designs and laboratory methods. The enrichment in our survey detected 7.5 times more campylobacter-positive faecal samples than did direct plating. The high number of false negative results obtained from direct plating probably reflected the low levels of *Campylobacter* spp. in the faeces of slaughter cattle, which is actually consistent with study II. In this study, which focused on three dairy herds on farms, and in accordance with previous studies (Stanley et al. 1998, Nielsen 2002, Heuvelink et al. 2009), most of the animals excreted lower levels of *Campylobacter* spp. than levels of *C. jejuni* in the caeca of chickens, which can reach up to 10^8 cfu/g (Reich et al. 2008). Only a few animals in study II excreted high numbers (>10^6 cfu/g) as determined by the semiquantitative detection method, which was based on the enrichment of 10-fold dilutions of the faecal samples (NCFA [Nordic Committee on Food Analysis] 2007).

The higher prevalence of *Campylobacter* spp. observed among beef cattle (I) may reflect the age distribution of the animals, because most of the beef cattle at slaughter were under three years of age, and the overall prevalence in that age group was higher than among older animals. Similar observations of the age-related prevalence of campylobacters in cattle are common in previous studies as well (Giacoboni et al. 1993, Nielsen 2002, Johnsen et al. 2006, Gilpin et al. 2008b). However, some studies have suggested that the higher prevalence of *Campylobacter* spp. in beef cattle could derive from different farming practices, such as different feed and higher animal density than among dairy cattle (Wesley et al. 2000, Minihan et al. 2004).

Similar to our results from studies I and II, most other studies on bovine campylobacters have reported *C. jejuni* as the most common *Campylobacter* sp. in cattle (Wesley et al. 2000, Berry et al. 2006, Madden et al. 2007, Milnes et al. 2008, Ragimbeau et al. 2008), whereas others, applying specific methods, have detected the predominance of other species such as *C. hyointestinalis* ssp. *hyointestinali*s or *C. lanienae* (Grau 1988, Atabay and Corry 1998, Inglis et al. 2003, Pezzotti et al. 2003). We detected *C. hyointestinalis*

in 10.8% of the faecal samples at slaughter, but no *C. lanienae*, which is anaerobic and requires specific cultivation conditions. However, on the basis of current knowledge these two species appear to be minor human pathogens (Lastovica and Allos 2008), whereas *C. jejuni* is the most commonly reported species in human infections (Baker et al. 2007, EFSA 2010b).

The results from study II indicate that dairy cattle can be long-term carriers of *C. jejuni* with varying shedding patterns among herds. Unlike in some other studies (Stanley et al. 1998, Kwan et al. 2008b, Grove-White et al. 2010), we cannot draw general conclusions in regard to seasonal variation of shedding due to the small number of herds, the few sampling occasions and the study period of only one year. While in study I the prevalence of *C. jejuni* peaked in August, study II detected no peak in the prevalence of *C. jejuni* among the herds in August, although the herds had been grazing since May, and were therefore probably exposed to a variety of potential environmental sources of campylobacters (Oporto et al. 2007, Grove-White et al. 2010). None of the herds had access to natural water sources, however, which may indirectly illustrate the importance of natural waters as a reservoir of campylobacters for cattle during grazing (Humphrey and Beckett 1987, Hänninen et al. 1998). The last sampling that occurred after grazing, however, yielded high prevalences in all of the herds, possibly due to changes in the diet (Stanley et al. 1998). In addition, the prevalence of *C. jejuni* rose in herd 3 during indoor housing in winter. The water trough samples taken on the last sampling occasion provide a plausible explanation: the predominating *C. jejuni* subtype in herd 3 was present at a detectable level in one of those samples, and probably contributed to the persistent colonisation of the herd when housed indoors (Minihan et al. 2004, Ellis-Iversen et al. 2009a). Unfortunately, we took no samples from the dug well, which was the drinking water supply for herd 3. The persons living on the farm, however, consumed water obtained from the same supply without any symptoms of the disease.

6.2 The diversity of *Campylobacter jejuni* in Finnish cattle

The *C. jejuni* sero/PFGE types in bovine faecal samples revealed high diversity in the slaughterhouse survey (I). Nevertheless, in studies I and II only one subtype was usually detected in the samples of individual animals, from which up to six isolates were genotyped. In addition, *C. jejuni* isolates from different animals originating from the same farm in study I consistently represented identical subtypes on the same sampling occasion. Moreover, in the three cattle herds in study II, only one or two persistent PFGE subtypes of *C. jejuni* were detected among each herd throughout the study, although earlier studies have reported a wider range of subtypes in adult cattle (Nielsen 2002, Kwan et al. 2008b). The presence of a small number of subtypes suggests only a few sources of *C. jejuni* or re-infection with the same

strains during the study period (Nielsen 2002, Minihan et al. 2004). In two of the herds, additional subtypes of *C. jejuni* occurred mainly during the grazing period, thus indicating new sources from the environment (Brown et al. 2004, Oporto et al. 2007, Grove-White et al. 2010). Beside the few sources on the farms, the presence of only a few subtypes of *C. jejuni* in herds may suggest ecological competition between strains in bovine intestines (Kwan et al. 2008b). Subtypes available at an early stage of an animal's life, probably have fewer competitors in the immature gut, whereas later exposure to other subtypes may result in only intermittent shedding due to the competitive advantage of the earlier colonisers. In addition, re-infection with the same few subtypes present in a herd is probably an important contributor to the colonisation of animals, as was apparent in herd 3 in study II (Ellis-Iversen et al. 2009b)

Subtyping of the *C. jejuni* isolates from the same animals on different sampling occasions in herd 1 revealed that some of the animals were intermittent carriers of campylobacters, whereas others appeared to be persistent shedders of a single subtype (Hänninen et al. 1998, Gilpin et al. 2008b, Kwan et al. 2008b). In addition, one of the animals was campylobacter-negative in all samplings, which may indicate acquired immunity, different intestinal microbiota or other individual characteristics that prevent colonisation (Minihan et al. 2004).

6.3 Chickens and cattle as sources of *Campylobacter jejuni* in sporadic human infections in Finland

6.3.1 Comparison of subtypes of *Campylobacter jejuni* from human infections, chickens and cattle

Serotyping, while comparable between laboratories, offers low discriminatory power in the typing of *C. jejuni*. Consequently, serotyping results are merely suggestive, and inconclusive for source attribution. The predominant serotypes of *C. jejuni* identified among cattle (I) - Pen2, Pen4-complex, Pen1,44 and Pen12 - occur in domestic human infections in Finland as well (Rautelin and Hänninen 1999, Vierikko et al. 2004, Nakari et al. 2005, Schönberg-Norio et al. 2006). Studies from other countries have also reported the common presence of Pen2 and Pen4-complex in the faeces of dairy cattle (Nielsen et al. 1997, Nielsen 2002, Devane et al. 2005, Ishihara et al. 2006), which may indicate the adaptation of these serotypes of *C. jejuni* to the bovine intestinal tract. In addition, the serotype Pen2 was present only in human isolates representing rural areas of Finland in a previous study that compared different geographical areas (Schönberg-Norio et al. 2006), and is uncommon in Finnish chickens (Perko-Mäkelä et al. 2002), which may indicate, in accordance with studies from other countries (Studahl and Andersson 2000, Baker et al. 2007, Garrett et al. 2007, Strachan et al. 2009) the contribution of cattle as source of *C. jejuni* in human infections in rural areas of Finland.

The comparison of domestic human, chicken and bovine isolates of *C. jejuni* focused on the isolates present during the summer months from June to August 2003, because the incidence of human campylobacteriosis in Finland consistently peaks in July-August. Furthermore, most of the human infections are domestically acquired in summer, whereas those in winter are mainly travel-related (National Institute for Health and Welfare 2009). Our study included domestic human isolates from nine clinical microbiology laboratories across the country, chicken strains from two Finnish slaughterhouses representing approximately 80% of the total slaughter volume during the study period, all bovine faecal strains isolated at slaughter between January and December 2003 (assuming that the shedding of the subtypes detected at slaughter was similar to that in study II and continued in the herds throughout the year), and all isolates from bovine carcasses during the summer of 2003. Due to the relatively short time-frame of the study in a geographically defined area, we considered PFGE with two restriction enzymes suitable for comparison of the isolates as a highly discriminating subtyping method.

As with the bovine isolates, high genotypic diversity was apparent among the human *C. jejuni* isolates, whereas the number of different subtypes from chickens was small due to the low prevalence of *C. jejuni* in Finnish chicken slaughter batches (Perko-Mäkelä et al. 2002, EFSA 2010c). Only one isolate per chicken slaughter batch was available for comparison. However, isolation of more than one strain from each campylobacter-positive batch would probably not have affected the outcome, because in the majority of Finnish campylobacter-positive chicken flocks, only one *C. jejuni* subtype is present in each growing batch (Hakkinen and Kaukonen 2009).

Isolates representing genotypes indistinguishable from those of chickens or cattle were present in 55.4% of the human infections. Considering the temporal association of chicken isolates, 31.4 % of the human cases could have originated from chickens, similar to the previous estimate from the summer of 1999 (Kärenlampi et al. 2003). The remaining temporally unrelated subtypes that were identical to those from cattle represented 15.4% of the human infections. In addition, subtypes shared only between humans and cattle were present in 3.4% of the human cases. The total proportion of human domestic infections of bovine origin during the summer 2003 in Finland could thus have been approximately 19 %. A previous Finnish MLST study observed a high degree of overlap (61%) between human and chicken isolates, whereas overlap was very low (5.7%) between human and bovine isolates (Kärenlampi et al. 2007). The number of bovine isolates was low in the study, however, and the collections of human isolates represented a different geographical area, and thus probably different sources of infection as well (Schönberg-Norio et al. 2006). In contrast to the study of (Kärenlampi et al.) (2007), which analysed human isolates from a more urban area in southern Finland, our isolates represented the entire country and covered rural areas more extensively. Recent research elsewhere has focused increasingly

on the different exposures among populations in urban and rural areas and has identified, for example, increasing ruminant density and contact with cattle as risk factors (Studahl and Andersson 2000, Kapperud et al. 2003, Nygård et al. 2004).

With one exception, subtypes shared between human and cattle originated from dairy cattle, although *C. jejuni* was more common in beef cattle herds (I). Moreover, none of the *C. jejuni* subtypes isolated from carcasses was present among human isolates, thus supporting the conclusion of the prevalence study (I), which suggests that beef is of minor importance as source of campylobacters in human infections due to the low prevalence of *Campylobacter* spp. on carcasses. Because air-chilling further reduces the contamination of carcasses with campylobacters due to the sensitivity of these organisms to oxygen and drying (Oosterom et al. 1983, Grau 1988), the survival of campylobacters on retail beef is unlikely. Milk, instead, can permit longer survival of *Campylobacter* spp., if failures in milking hygiene lead to faecal contamination with these organisms (Doyle and Roman 1982). Despite the high prevalence of *C. jejuni* in the dairy herds, no *Campylobacter* spp. occurred in the milk samples in study II, which indicates adequate milking hygiene on the participating farms. Milkborne outbreaks are rare in Finland, because up to 97% of milk is delivered to dairies, and the consumption of unpasteurised milk is uncommon (http://www.maataloustilastot.fi/en/node/540). The food-related transmission of bovine *Campylobacter* spp. to humans therefore appears insignificant, whereas occupational and environmental routes require further consideration. In particular, the presence of human pathogenic campylobacters among dairy herds is of concern because of the long life-span of dairy cattle, during which persistent carriers of campylobacters in the herds increase the environmental load of these organisms in rural areas.

6.3.2 Genetic markers in differentiation of the sources of *Campylobacter jejuni* in human infections

Genetic markers revealed higher similarity among human and chicken *C. jejuni* isolates than among human and bovine isolates. The controversy of the PFGE result may partially stem from the different time frames of studies III and IV, and the different geographical origin of human isolates, which were obtained from more urban areas in southern Finland in study III than in study IV. The controversial results may therefore reflect differences in rural and urban exposures (Studahl and Andersson 2000, Schönberg-Norio et al. 2006, Garrett et al. 2007). Nevertheless, the results also indicate differences in the metabolic characteristics of *C. jejuni* strains isolated from chicken and cattle, which supports the previously observed host adaptation of *C. jejuni* (Dingle et al. 2001, Champion et al. 2005, McCarthy et al. 2007).

In our study III, the *ggt* gene, which previously seemed to relate to the prolonged intestinal colonisation of *C. jejuni* in chickens (Barnes et al.

2007) and to the enhanced colonisation of human intestinal tissues due to the acquired ability of *C. jejuni* to utilise glutathione and glutamine as sources of amino acids (Hofreuter et al. 2008), was more common among chicken and human isolates than among bovine *C. jejuni* isolates. Similarly, the subunit of the putative anaerobic DMSO oxidoreductase gene, *dmsA*, was rare among bovine isolates, but occurred frequently among human and chicken isolates in our study. In a previous study, *C. jejuni* colonisation in chickens was associated with the presence of this oxidoreductase (Hiett et al. 2008), which may contribute to the virulence of *C. jejuni* also (Hofreuter et al. 2006).

Another putative oxidoreductase gene, Cj1585, was more common in bovine *C. jejuni* isolates, which may indicate that the Cj1585 type oxidoreductase system is preferential in the oxygen-restricted environment of the bovine intestine. In addition, the bovine isolates were more frequent carriers of the subtilase-type serine protease gene CJJ81176-1367/1371. The presence of the serine protease Cj1371 apparently relates to the tolerance of oxidative stress in *C. jejuni* (Garenaux et al. 2008), but its contribution to the pathogenesis of *C. jejuni* is unknown. In several other pathogens, such as *Vibrio cholerae*, *Shigella dysenteriae* and some VTEC strains, the production of subtilase cytotoxins, which harbour a subunit homologous with subtilase-like serine proteases, appears to be important to virulence (Beddoe et al. 2010). For example, a highly cytotoxic subtilase toxin (SubAB) of some VTEC strains causes in mice lesions that resemble those in patients with HUS (Paton et al. 2004, Wang et al. 2007).

6.4 Antimicrobial susceptibility of bovine *Campylobacter jejuni* isolates

The low prevalence of resistance to antimicrobials among bovine *C. jejuni* isolates is probably a consequence of the prudent veterinarian use of these agents in Finland. We applied the epidemiological cut-off values in the determination of susceptibility, according to the recommendation of EFSA for monitoring purposes. Using the same values, the resistance levels of bovine *C. jejuni* isolates in seven European countries during the period from 2004 to 2007 were substantially higher: the average tetracycline resistance varied between 23% and 33% and nalidixic acid resistance from 23% and 35% (EFSA 2010a). The resistance levels among domestic human *Campylobacter* isolates and chicken isolates have also been low in Finland, and resistant strains occur mainly in travel-related infections (Rautelin et al. 2003, Feodoroff et al. 2009, EFSA 2010a).

7 Conclusions

1. Finnish cattle appeared to be a constant reservoir of *C. jejuni*, the most common *Campylobacter* species in human infections. The level of faecal excretion of *C. jejuni* was usually low, so enrichment is essential for optimal isolation of the organism from bovine faecal samples.

2. Beef cattle appeared to be more frequent carriers of *C. jejuni* than were dairy cattle. The contamination of carcasses was low at slaughter, however, and the isolates from carcasses represented different PFGE types from those in humans. Beef therefore appears to be an insignificant source of campylobacters in human infections.

3. The resistance to antimicrobials was low among bovine *C. jejuni* isolates, and no multiresistance occurred. This is probably due to the prudent use of antimicrobials in Finnish animal production, and indicates a low risk for human infections by resistant strains of bovine origin.

4. Diverse shedding patterns of *C. jejuni* occurred among both dairy cattle herds and individual animals. The same few subtypes of *C. jejuni* were able to persist in a dairy herd for more than one year. Ecological competition in the colonisation of the bovine intestinal tract may occur between different subtypes of *C. jejuni*. In addition, individual animals can be resistant to colonisation. The faecal contamination of water troughs can maintain colonisation in cattle herds during indoor housing. At pasture, however, preventing access to natural waters can limit colonisation. Despite the high percentage of animals in dairy herds shedding *Campylobacter* spp. in their faeces, adequate milking hygiene could prevent the contamination of milk.

5. The distribution of chicken and bovine isolates based on the presence of genetic markers supported the previous observations of the host adaptation of *C. jejuni* strains apparently as a response to different type of oxidative stress and metabolic demands in the intestinal tracts of these animal species. In addition, differences in the genetic markers may suggest differences in the virulence of *C. jejuni* strains from chickens and cattle.

6. The isolates from 55.4% of sporadic domestic human infections during the seasonal peak in 2003 represented identical PFGE subtypes with *C. jejuni* isolates from

chickens and cattle, especially dairy cattle. The proportion of human cases temporally associated with chicken isolates was 31.1%, and approximately 19% of human infections were possibly related to cattle, suggesting an important role for Finnish cattle, besides chickens, as a source of *C. jejuni* in human infections, although common sources of *C. jejuni* in humans, chickens and cattle are also possible. Our results suggest that food is probably a minor route of transmission of bovine *C. jejuni,* and the sources of *C. jejuni* in human infections in rural areas may differ from those in urban areas in Finland.

References

Acik, M.N. and Cetinkaya, B. (2005) The heterogeneity of *Campylobacter jejuni* and *Campylobacter coli* strains isolated from healthy cattle. *Lett Appl Microbiol* **41**, 397-403.

Adak, G.K., Meakins, S.M., Yip, H., Lopman, B.A. and O'Brien, S.J. (2005) Disease risks from foods, England and Wales, 1996-2000. *Emerg Infect Dis* **11**, 365-372.

Anderson, S.A., Yeaton Woo, R.W. and Crawford, L.M. (2001) Risk assessment of the impact on human health of resistant *Campylobacter jejuni* from fluoroquinolone use in beef cattle. *Food Control*, 13-25.

Atabay, H.I. and Corry, J.E. (1998) The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. *J Appl Microbiol* **84**, 733-740.

Bachmann, H.P. and Spahr, U. (1995) The fate of potentially pathogenic bacteria in Swiss hard and semihard cheeses made from raw milk. *J Dairy Sci* **78**, 476-483.

Bae, W., Kaya, K.N., Hancock, D.D., Call, D.R., Park, Y.H. and Besser, T.E. (2005) Prevalence and antimicrobial resistance of thermophilic *Campylobacter* spp. from cattle farms in Washington State. *Appl Environ Microbiol* **71**, 169-174.

Baker, M., Wilson, N., Ikram, R., Chambers, S., Shoemack, P. and Cook, G. (2006) Regulation of chicken contamination is urgently needed to control New Zealand's serious campylobacteriosis epidemic. *N Z Med J* **119**, U2264.

Baker, M.G., Sneyd, E. and Wilson, N.A. (2007) Is the major increase in notified campylobacteriosis in New Zealand real? *Epidemiol Infect* **135**, 163-170.

Barnes, I.H., Bagnall, M.C., Browning, D.D., Thompson, S.A., Manning, G. and Newell, D.G. (2007) Gamma-glutamyl transpeptidase has a role in the persistent colonization of the avian gut by *Campylobacter jejuni*. *Microb Pathog* **43**, 198-207.

Beach, J.C., Murano, E.A. and Acuff, G.R. (2002) Prevalence of *Salmonella* and *Campylobacter* in beef cattle from transport to slaughter. *J Food Prot* **65**, 1687-1693.

Beddoe, T., Paton, A.W., Le Nours, J., Rossjohn, J. and Paton, J.C. (2010) Structure, biological functions and applications of the AB(5) toxins. *Trends Biochem Sci*. **35**, 411-418.

Berry, E.D., Wells, J.E., Archibeque, S.L., Ferrell, C.L., Freetly, H.C. and Miller, D.N. (2006) Influence of genotype and diet on steer performance, manure odor, and carriage of pathogenic and other fecal bacteria. II. Pathogenic and other fecal bacteria. *J Anim Sci* **84**, 2523-2532.

Besser, T.E., Lejeune, J.T., Rice, D.H., Berg, J., Stilborn, R.P., Kaya, K., Bae, W. and Hancock, D.D. (2005) Increasing prevalence of *Campylobacter jejuni* in feedlot cattle through the feeding period. *Appl Environ Microbiol* **71**, 5752-5758.

Birk, T. and Knochel, S. (2009) Fate of food-associated bacteria in pork as affected by marinade, temperature, and ultrasound. *J Food Prot* **72**, 549-555.

Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P. and Blaser, M.J. (1988) Experimental *Campylobacter jejuni* infection in humans. *J Infect Dis* **157**, 472-479.

Blaser, M. and Engberg, J. (2008) Clinical aspects of *Campylobacter jejuni* and *Campylobacter coli* infections. In ed. Nachamkin, I., Szymanski, C.M. and Blaser, M.J. pp. 99-121. Washington, DC, USA: ASM Press.

Boes, J., Nersting, L., Nielsen, E.M., Kranker, S., Enoe, C., Wachmann, H.C. and Baggesen, D.L. (2005) Prevalence and diversity of *Campylobacter jejuni* in pig herds on farms with and without cattle or poultry. *J Food Prot* **68**, 722-727.

Bostan, K., Aydin, A. and Ang, M.K. (2009) Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* species on beef, mutton, and chicken carcasses in Istanbul, Turkey. *Microb Drug Resist* **15**, 143-149.

Broman, T., Palmgren, H., Bergstrom, S., Sellin, M., Waldenstrom, J., Danielsson-Tham, M.L. and Olsen, B. (2002) *Campylobacter jejuni* in black-headed gulls (*Larus ridibundus*): prevalence, genotypes, and influence on *C. jejuni* epidemiology. *J Clin Microbiol* **40**, 4594-4602.

Broman, T., Waldenstrom, J., Dahlgren, D., Carlsson, I., Eliasson, I. and Olsen, B. (2004) Diversities and similarities in PFGE profiles of *Campylobacter jejuni* isolated from migrating birds and humans. *J Appl Microbiol* **96**, 834-843.

Brown, P.E., Christensen, O.F., Clough, H.E., Diggle, P.J., Hart, C.A., Hazel, S., Kemp, R., Leatherbarrow, A.J., Moore, A., Sutherst, J., Turner, J., Williams, N.J., Wright, E.J. and French, N.P. (2004) Frequency and spatial distribution of environmental *Campylobacter* spp. *Appl Environ Microbiol* **70**, 6501-6511.

Busato, A., Hofer, D., Lentze, T., Gaillard, C. and Burnens, A. (1999) Prevalence and infection risks of zoonotic enteropathogenic bacteria in Swiss cow-calf farms. *Vet Microbiol* **69**, 251-263.

Butzler, J.P., Dekeyser, P., Detrain, M. and Dehaen, F. (1973) Related vibrio in stools. *J Pediatr* **82**, 493-495.

Bywater, R., Deluyker, H., Deroover, E., de Jong, A., Marion, H., McConville, M., Rowan, T., Shryock, T., Shuster, D., Thomas, V., Valle, M. and Walters, J. (2004) A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *J Antimicrob Chemother* **54**, 744-754.

Carrique-Mas, J., Andersson, Y., Hjertqvist, M., Svensson, A., Torner, A. and Giesecke, J. (2005) Risk factors for domestic sporadic campylobacteriosis among young children in Sweden. *Scand J Infect Dis* **37**, 101-110.

Centers for Disease Control and Prevention (CDC) (2009) *Campylobacter jejuni* infection associated with unpasteurized milk and cheese--Kansas, 2007. *MMWR Morb Mortal Wkly Rep* **57**, 1377-1379.

Champion, O.L., Gaunt, M.W., Gundogdu, O., Elmi, A., Witney, A.A., Hinds, J., Dorrell, N. and Wren, B.W. (2005) Comparative phylogenomics of the food-borne pathogen *Campylobacter jejuni* reveals genetic markers predictive of infection source. *Proc Natl Acad Sci U S A* **102**, 16043-16048.

Chatre, P., Haenni, M., Meunier, D., Botrel, M.A., Calavas, D. and Madec, J.Y. (2010) Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from cattle between 2002 and 2006 in France. *J Food Prot* **73**, 825-831.

Chaudhuri, R.R., Loman, N.J., Snyder, L.A., Bailey, C.M., Stekel, D.J. and Pallen, M.J. (2008) xBASE2: a comprehensive resource for comparative bacterial genomics. *Nucleic Acids Res* **36**, D543-6.

Clark, C.G., Price, L., Ahmed, R., Woodward, D.L., Melito, P.L., Rodgers, F.G., Jamieson, F., Ciebin, B., Li, A. and Ellis, A. (2003) Characterization of waterborne outbreak-associated *Campylobacter jejuni*, Walkerton, Ontario. *Emerg Infect Dis* **9**, 1232-1241.

CLSI, ed. (2008) *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Third ed. [ISBN Number 1-56238-659-X]. CLSI Document M31-A3.* Wayne, PA, USA: Clinical and Laboratory Standards Institute.

CLSI (2006) *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline. CLSI document M45-A.* Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute.

Colles, F.M., Jones, K., Harding, R.M. and Maiden, M.C. (2003) Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. *Appl Environ Microbiol* **69**, 7409-7413.

de Boer, P., Duim, B., Rigter, A., van Der Plas, J., Jacobs-Reitsma, W.F. and Wagenaar, J.A. (2000) Computer-assisted analysis and epidemiological value of genotyping methods for *Campylobacter jejuni* and *Campylobacter coli*. *J Clin Microbiol* **38**, 1940-1946.

Debruyne, L., Gevers, D. and Vandamme, P. (2008) Taxonomy of the family *Campylobacteraceae*. In *Campylobacter* ed. Nachamkin, I., Szymanski, C.M. and Blaser, M. pp. 3-25. Washington D.C.: ASM Press.

Dekeyser, P., Gossuin-Detrain, M., Butzler, J.P. and Sternon, J. (1972) Acute enteritis due to related vibrio: first positive stool cultures. *J Infect Dis* **125**, 390-392.

Denno, D.M., Keene, W.E., Hutter, C.M., Koepsell, J.K., Patnode, M., Flodin-Hursh, D., Stewart, L.K., Duchin, J.S., Rasmussen, L., Jones, R. and Tarr, P.I. (2009) Tri-county comprehensive assessment of risk factors for sporadic reportable bacterial enteric infection in children. *J Infect Dis* **199**, 467-476.

Desai, M., Logan, J.M., Frost, J.A. and Stanley, J. (2001) Genome sequence-based fluorescent amplified fragment length polymorphism of *Campylobacter jejuni*, its relationship to serotyping, and its implications for epidemiological analysis. *J Clin Microbiol* **39**, 3823-3829.

Devane, M.L., Nicol, C., Ball, A., Klena, J.D., Scholes, P., Hudson, J.A., Baker, M.G., Gilpin, B.J., Garrett, N. and Savill, M.G. (2005) The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. *J Appl Microbiol* **98**, 980-990.

Dilworth, C.R., Lior, H. and Belliveau, M.A. (1988) *Campylobacter* enteritis acquired from cattle. *Can J Public Health* **79**, 60-62.

Dingle, K.E., Colles, F.M., Ure, R., Wagenaar, J.A., Duim, B., Bolton, F.J., Fox, A.J., Wareing, D.R. and Maiden, M.C. (2002) Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. *Emerg Infect Dis* **8**, 949-955.

Dingle, K.E., Colles, F.M., Wareing, D.R., Ure, R., Fox, A.J., Bolton, F.E., Bootsma, H.J., Willems, R.J., Urwin, R. and Maiden, M.C. (2001) Multilocus sequence typing system for *Campylobacter jejuni*. *J Clin Microbiol* **39**, 14-23.

Dingle, K.E., McCarthy, N.D., Cody, A.J., Peto, T.E. and Maiden, M.C. (2008) Extended sequence typing of *Campylobacter* spp., United Kingdom. *Emerg Infect Dis* **14**, 1620-1622.

Dingle, K.E., Van Den Braak, N., Colles, F.M., Price, L.J., Woodward, D.L., Rodgers, F.G., Endtz, H.P., Van Belkum, A. and Maiden, M.C. (2001) Sequence typing confirms that *Campylobacter jejuni* strains associated with Guillain-Barre and Miller-Fisher syndromes are of diverse genetic lineage, serotype, and flagella type. *J Clin Microbiol* **39**, 3346-3349.

Doorduyn, Y., VAN DEN Brandhof, W.E., VAN Duynhoven, Y.T., Breukink, B.J., Wagenaar, J.A. and VAN Pelt, W. (2010) Risk factors for indigenous *Campylobacter jejuni* and *Campylobacter coli* infections in The Netherlands: a case-control study. *Epidemiol Infect*, 1-14.

Doorduyn, Y., Van Pelt, W., Siezen, C.L., Van Der Horst, F., Van Duynhoven, Y.T., Hoebee, B. and Janssen, R. (2008) Novel insight in the association between salmonellosis or campylobacteriosis and chronic illness, and the role of host genetics in susceptibility to these diseases. *Epidemiol Infect* **136**, 1225-1234.

Dorrell, N., Champion, O.L. and Wren, B.W. (2002) Microarray analysis of *Campylobacter jejuni*: to the guts of the problem! *Comp Funct Genomics* **3**, 338-341.

Doyle, M.P. and Roman, D.J. (1982) Prevalence and survival of *Campylobacter jejuni* in unpasteurized milk. *Appl Environ Microbiol* **44**, 1154-1158.

Duim, B., Ang, C.W., van Belkum, A., Rigter, A., van Leeuwen, N.W., Endtz, H.P. and Wagenaar, J.A. (2000) Amplified fragment length polymorphism analysis of *Campylobacter jejuni* strains isolated from chickens and from patients with gastroenteritis or Guillain-Barre or Miller Fisher syndrome. *Appl Environ Microbiol* **66**, 3917-3923.

Duim, B., Wassenaar, T.M., Rigter, A. and Wagenaar, J. (1999) High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with amplified fragment length polymorphism fingerprinting. *Appl Environ Microbiol* **65**, 2369-2375.

EFSA (2010a) Antimicrobial resistance in zoonotic and indicator bacteria from animals and foodin the European Union in 2004-2007. *The EFSA Journal* **8**, 1309.

EFSA (2010b) The Community Summary Report on trends and sources of zoonosis and zoonotic agents and food-borne outbreaks in the European Union in 2008. *The EFSA Journal*, 104-111.

EFSA (2010c) Report of Task Force on Zoonoses Data Collection on the analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Salmonella* on broiler carcasses in the EU, 2008. The EFSA Journal 8(03):1503. *The EFSA Journal* **8**, 1503.

EFSA (2007) Report of the Task Force of Zoonoses Data Collection including a proposal for a harmonizedmonitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys and pigs and *Campylobacter jejuni* and *C. coli* in broilers. *The EFSA Journal*, 1-46.

EFSA Panel on Biological Hazards (BIOHAZ) (2010) Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. *EFSA Journal* **8**, 1437-1526.

Ellis-Iversen, J., Cook, A.J., Smith, R.P., Pritchard, G.C. and Nielen, M. (2009a) Temporal patterns and risk factors for *Escherichia coli* O157 and *Campylobacter* spp, in young cattle. *J Food Prot* **72**, 490-496.

Ellis-Iversen, J., Pritchard, G.C., Wooldridge, M. and Nielen, M. (2009b) Risk factors for *Campylobacter jejuni* and *Campylobacter coli* in young cattle on English and Welsh farms. *Prev Vet Med* **88**, 42-48.

Endtz, H.P., Ruijs, G.J., van Klingeren, B., Jansen, W.H., van der Reyden, T. and Mouton, R.P. (1991) Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *J Antimicrob Chemother* **27**, 199-208.

Engberg, J., Gerner-Smidt, P., Scheutz, F., Moller Nielsen, E., On, S.L. and Molbak, K. (1998) Water-borne *Campylobacter jejuni* infection in a Danish town---a 6-week continuous source outbreak. *Clin Microbiol Infect* **4**, 648-656.

Engberg, J., Neimann, J., Nielsen, E.M., Aerestrup, F.M. and Fussing, V. (2004) Quinolone-resistant *Campylobacter* infections: risk factors and clinical consequences. *Emerg Infect Dis* **10**, 1056-1063.

Englen, M.D., Hill, A.E., Dargatz, D.A., Ladely, S.R. and Fedorka-Cray, P.J. (2007) Prevalence and antimicrobial resistance of *Campylobacter* in US dairy cattle. *J Appl Microbiol* **102**, 1570-1577.

Enokimoto, M., Kubo, M., Bozono, Y., Mieno, Y. and Misawa, N. (2007) Enumeration and identification of *Campylobacter* species in the liver and bile of slaughtered cattle. *Int J Food Microbiol* **118**, 259-263.

Ethelberg, S., Simonsen, J., Gerner-Smidt, P., Olsen, K.E. and Molbak, K. (2005) Spatial distribution and registry-based case-control analysis of *Campylobacter* infections in Denmark, 1991-2001. *Am J Epidemiol* **162**, 1008-1015.

EUCAST (2003) Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth microdilution.EUCAST Discussion Document E. Def 2003, 5.1. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, 1-10.

Evans, M.R., Roberts, R.J., Ribeiro, C.D., Gardner, D. and Kembrey, D. (1996) A milk-borne campylobacter outbreak following an educational farm visit. *Epidemiol Infect* **117**, 457-462.

Fahey, T., Morgan, D., Gunneburg, C., Adak, G.K., Majid, F. and Kaczmarski, E. (1995) An outbreak of *Campylobacter jejuni* enteritis associated with failed milk pasteurisation. *J Infect* **31**, 137-143.

Feodoroff, F.B., Lauhio, A.R., Sarna, S.J., Hänninen, M.L. and Rautelin, H.I. (2009) Severe diarrhoea caused by highly ciprofloxacin-susceptible *Campylobacter* isolates. *Clin Microbiol Infect* **15**, 188-192.

Fitzgerald, C., Stanley, K., Andrew, S. and Jones, K. (2001) Use of pulsed-field gel electrophoresis and flagellin gene typing in identifying clonal groups of *Campylobacter jejuni* and *Campylobacter coli* in farm and clinical environments. *Appl Environ Microbiol* **67**, 1429-1436.

Forbes, K.J., Gormley, F.J., Dallas, J.F., Labovitiadi, O., MacRae, M., Owen, R.J., Richardson, J., Strachan, N.J., Cowden, J.M., Ogden, I.D. and McGuigan, C.C. (2009) *Campylobacter* immunity and coinfection following a large outbreak in a farming community. *J Clin Microbiol* **47**, 111-116.

French, N., Barrigas, M., Brown, P., Ribiero, P., Williams, N., Leatherbarrow, H., Birtles, R., Bolton, E., Fearnhead, P. and Fox, A. (2005) Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. *Environ Microbiol* **7**, 1116-1126.

Gallay, A., De Valk, H., Cournot, M., Ladeuil, B., Hemery, C., Castor, C., Bon, F., Megraud, F., Le Cann, P., Desenclos, J.C. and Outbreak Investigation Team (2006) A large multi-pathogen waterborne community outbreak linked to faecal contamination of a groundwater system, France, 2000. *Clin Microbiol Infect* **12**, 561-570.

Garcia, M.M., Lior, H., Stewart, R.B., Ruckerbauer, G.M., Trudel, J.R. and Skljarevski, A. (1985) Isolation, characterization, and serotyping of *Campylobacter jejuni* and *Campylobacter coli* from slaughter cattle. *Appl Environ Microbiol* **49**, 667-672.

Garenaux, A., Guillou, S., Ermel, G., Wren, B., Federighi, M. and Ritz, M. (2008) Role of the Cj1371 periplasmic protein and the Cj0355c two-component regulator in the *Campylobacter jejuni* NCTC 11168 response to oxidative stress caused by paraquat. *Res Microbiol* **159**, 718-726.

Garrett, N., Devane, M.L., Hudson, J.A., Nicol, C., Ball, A., Klena, J.D., Scholes, P., Baker, M.G., Gilpin, B.J. and Savill, M.G. (2007) Statistical comparison of *Campylobacter jejuni* subtypes from human cases and environmental sources. *J Appl Microbiol* **103**, 2113-2121.

Ghafir, Y., China, B., Dierick, K., De Zutter, L. and Daube, G. (2007) A seven-year survey of *Campylobacter* contamination in meat at different production stages in Belgium. *Int J Food Microbiol* **116**, 111-120.

Gharst, G., Hanson, D. and Kathariou, S. (2006) Effect of direct culture versus selective enrichment on the isolation of thermophilic *Campylobacter* from feces of mature cattle at harvest. *J Food Prot* **69**, 1024-1027.

Giacoboni, G.I., Itoh, K., Hirayama, K., Takahashi, E. and Mitsuoka, T. (1993) Comparison of fecal *Campylobacter* in calves and cattle of different ages and areas in Japan. *J Vet Med Sci* **55**, 555-559.

Gillespie, I.A., O'Brien, S.J. and Bolton, F.J. (2009) Age patterns of persons with campylobacteriosis, England and Wales, 1990-2007. *Emerg Infect Dis* **15**, 2046-2048.

Gilpin, B., Cornelius, A., Robson, B., Boxall, N., Ferguson, A., Nicol, C. and Henderson, T. (2006) Application of pulsed-field gel electrophoresis to identify potential outbreaks of campylobacteriosis in New Zealand. *J Clin Microbiol* **44**, 406-412.

Gilpin, B.J., Scholes, P., Robson, B. and Savill, M.G. (2008a) The transmission of thermotolerant *Campylobacter* spp. to people living or working on dairy farms in New Zealand. *Zoonoses Public Health* **55**, 352-360.

Gilpin, B.J., Thorrold, B., Scholes, P., Longhurst, R.D., Devane, M., Nicol, C., Walker, S., Robson, B. and Savill, M. (2008b) Comparison of *Campylobacter jejuni* genotypes from dairy cattle and human sources from the Matamata-Piako District of New Zealand. *J Appl Microbiol* **105**, 1354-1360.

Grau, F. (1988) *Campylobacter jejuni* and *Campylobacter hyointestinalis* in the intestinal tract and on the carcasses of calves and cattle. *J.Food Prot.* **51**, 857-861.

Grove-White, D.H., Leatherbarrow, A.J., Cripps, P.J., Diggle, P.J. and French, N.P. (2010) Temporal and farm-management-associated variation in the faecal-pat prevalence of *Campylobacter jejuni* in ruminants. *Epidemiol Infect* **138**, 549-558.

Gupta, A., Nelson, J.M., Barrett, T.J., Tauxe, R.V., Rossiter, S.P., Friedman, C.R., Joyce, K.W., Smith, K.E., Jones, T.F., Hawkins, M.A., Shiferaw, B., Beebe, J.L., Vugia, D.J., Rabatsky-Ehr, T., Benson, J.A., Root, T.P., Angulo, F.J. and NARMS Working Group (2004) Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. *Emerg Infect Dis* **10**, 1102-1109.

Hakkinen, M. and Kaukonen, E. (2009) Campylobacters in Finnish broiler flocks: contamination level, diversity, and role of breeders in

transmission. *15th International workshop on Campylobacter, Helicobacter and related organisms.*, 79.

Hald, B. and Madsen, M. (1997) Healthy puppies and kittens as carriers of *Campylobacter* spp., with special reference to *Campylobacter upsaliensis*. *J Clin Microbiol* **35**, 3351-3352.

Hald, B., Pedersen, K., Waino, M., Jorgensen, J.C. and Madsen, M. (2004) Longitudinal study of the excretion patterns of thermophilic *Campylobacter* spp. in young pet dogs in Denmark. *J Clin Microbiol* **42**, 2003-2012.

Hänninen, M.L., Haajanen, H., Pummi, T., Wermundsen, K., Katila, M.L., Sarkkinen, H., Miettinen, I. and Rautelin, H. (2003) Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne outbreaks in Finland. *Appl Environ Microbiol* **69**, 1391-1396.

Hänninen, M.L., Niskanen, M. and Korhonen, L. (1998) Water as a reservoir for *Campylobacter jejuni* infection in cows studied by serotyping and pulsed-field gel electrophoresis (PFGE). *Zentralbl Veterinarmed B* **45**, 37-42.

Hänninen, M.L., Perko-Mäkelä, P., Pitkälä, A. and Rautelin, H. (2000) A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. *J Clin Microbiol* **38**, 1998-2000.

Hänninen, M.L., Perko-Mäkelä, P., Rautelin, H., Duim, B. and Wagenaar, J.A. (2001) Genomic relatedness within five common Finnish *Campylobacter jejuni* pulsed-field gel electrophoresis genotypes studied by amplified fragment length polymorphism analysis, ribotyping, and serotyping. *Appl Environ Microbiol* **67**, 1581-1586.

Harrington, C.S., Thomson-Carter, F.M. and Carter, P.E. (1997) Evidence for recombination in the flagellin locus of *Campylobacter jejuni*: implications for the flagellin gene typing scheme. *J Clin Microbiol* **35**, 2386-2392.

Helms, M., Simonsen, J., Olsen, K.E. and Molbak, K. (2005) Adverse health events associated with antimicrobial drug resistance in *Campylobacter* species: a registry-based cohort study. *J Infect Dis* **191**, 1050-1055.

Heuvelink, A.E., van Heerwaarden, C., Zwartkruis-Nahuis, A., Tilburg, J.J., Bos, M.H., Heilmann, F.G., Hofhuis, A., Hoekstra, T. and de Boer, E. (2009) Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk. *Int J Food Microbiol* **134**, 70-74.

Hiett, K.L., Stintzi, A., Andacht, T.M., Kuntz, R.L. and Seal, B.S. (2008) Genomic differences between *Campylobacter jejuni* isolates identify surface membrane and flagellar function gene products potentially important for colonizing the chicken intestine. *Funct Integr Genomics* **8**, 407-420.

Hoar, B.R., Atwill, E.R., Elmi, C., Utterback, W.W. and Edmondson, A.J. (1999) Comparison of fecal samples collected per rectum and off the ground for estimation of environmental contamination attributable to beef cattle. *Am J Vet Res* **60**, 1352-1356.

Hofreuter, D., Novik, V. and Galan, J.E. (2008) Metabolic diversity in *Campylobacter jejuni* enhances specific tissue colonization. *Cell Host Microbe* **4**, 425-433.

Hofreuter, D., Tsai, J., Watson, R.O., Novik, V., Altman, B., Benitez, M., Clark, C., Perbost, C., Jarvie, T., Du, L. and Galan, J.E. (2006) Unique features of a highly pathogenic *Campylobacter jejuni* strain. *Infect Immun* **74**, 4694-4707.

Hong, J., Kim, J.M., Jung, W.K., Kim, S.H., Bae, W., Koo, H.C., Gil, J., Kim, M., Ser, J. and Park, Y.H. (2007) Prevalence and antibiotic resistance of *Campylobacter* spp. isolated from chicken meat, pork, and beef in Korea, from 2001 to 2006. *J Food Prot* **70**, 860-866.

Hudson, J.A., Nicol, C., Wright, J., Whyte, R. and Hasell, S.K. (1999) Seasonal variation of *Campylobacter* types from human cases, veterinary cases, raw chicken, milk and water. *J Appl Microbiol* **87**, 115-124.

Humphrey, T., O'Brien, S. and Madsen, M. (2007) Campylobacters as zoonotic pathogens: a food production perspective. *Int J Food Microbiol* **117**, 237-257.

Humphrey, T.J. and Beckett, P. (1987) *Campylobacter jejuni* in dairy cows and raw milk. *Epidemiol Infect* **98**, 263-269.

Hussain, I., Shahid Mahmood, M., Akhtar, M. and Khan, A. (2007) Prevalence of *Campylobacter* species in meat, milk and other food commodities in Pakistan. *Food Microbiol* **24**, 219-222.

Inglis, G.D. and Kalischuk, L.D. (2004) Direct quantification of *Campylobacter jejuni* and *Campylobacter lanienae* in feces of cattle by real-time quantitative PCR. *Appl Environ Microbiol* **70**, 2296-2306.

Inglis, G.D., Kalischuk, L.D. and Busz, H.W. (2004) Chronic shedding of *Campylobacter* species in beef cattle. *J Appl Microbiol* **97**, 410-420.

Inglis, G.D., Kalischuk, L.D. and Busz, H.W. (2003) A survey of *Campylobacter* species shed in faeces of beef cattle using polymerase chain reaction. *Can J Microbiol* **49**, 655-661.

Inglis, G.D., Kalischuk, L.D., Busz, H.W. and Kastelic, J.P. (2005) Colonization of cattle intestines by *Campylobacter jejuni* and *Campylobacter lanienae*. *Appl Environ Microbiol* **71**, 5145-5153.

Ishihara, K., Yamamoto, T., Satake, S., Takayama, S., Kubota, S., Negishi, H., Kojima, A., Asai, T., Sawada, T., Takahashi, T. and Tamura, Y. (2006) Comparison of *Campylobacter* isolated from humans and food-producing animals in Japan. *J Appl Microbiol* **100**, 153-160.

ISO (2006) International Standard ISO 10272-1. Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method.

Jacobs, B.C., Van Belkum, A. and Endtz, H.P. (2008) Guillain-Barré syndrome and *Campylobacter* infection. In ed. Nachamkin, I., Szymanski, C.M. and Blaser, M.J. pp. 245-261. Washington, DC, USA: ASM Press.

Jayarao, B.M., Donaldson, S.C., Straley, B.A., Sawant, A.A., Hegde, N.V. and Brown, J.L. (2006) A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. *J Dairy Sci* **89**, 2451-2458.

Jayarao, B.M. and Henning, D.R. (2001) Prevalence of foodborne pathogens in bulk tank milk. *J Dairy Sci* **84**, 2157-2162.

Johnsen, G., Zimmerman, K., Lindstedt, B.A., Vardund, T., Herikstad, H. and Kapperud, G. (2006) Intestinal carriage of *Campylobacter jejuni* and *Campylobacter coli* among cattle from south-western Norway and comparative genotyping of bovine and human isolates by amplified-fragment length polymorphism. *Acta Vet Scand* **48**, 4.

Jones, P.H., Willis, A.T., Robinson, D.A., Skirrow, M.B. and Josephs, D.S. (1981) *Campylobacter* enteritis associated with the consumption of free school milk. *J Hyg (Lond)* **87**, 155-162.

Jore, S., Viljugrein, H., Brun, E., Heier, B.T., Borck, B., Ethelberg, S., Hakkinen, M., Kuusi, M., Reiersen, J., Hansson, I., Engvall, E.O., Lofdahl, M., Wagenaar, J.A., van Pelt, W. and Hofshagen, M. (2010) Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997-2007. *Prev Vet Med* **93**, 33-41.

Kahlmeter, G., Brown, D.F., Goldstein, F.W., MacGowan, A.P., Mouton, J.W., Osterlund, A., Rodloff, A., Steinbakk, M., Urbaskova, P. and Vatopoulos, A. (2003) European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. *J Antimicrob Chemother* **52**, 145-148.

Kalman, M., Szollosi, E., Czermann, B., Zimanyi, M., Szekeres, S. and Kalman, M. (2000) Milkborne campylobacter infection in Hungary. *J Food Prot* **63**, 1426-1429.

Kapperud, G., Espeland, G., Wahl, E., Walde, A., Herikstad, H., Gustavsen, S., Tveit, I., Natas, O., Bevanger, L. and Digranes, A. (2003) Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. *Am J Epidemiol* **158**, 234-242.

Kärenlampi, R., Rautelin, H., Hakkinen, M. and Hänninen, M.L. (2003) Temporal and geographical distribution and overlap of Penner heat-stable serotypes and pulsed-field gel electrophoresis genotypes of *Campylobacter jejuni* isolates collected from humans and chickens in Finland during a seasonal peak. *J Clin Microbiol* **41**, 4870-4872.

Kärenlampi, R., Rautelin, H., Schönberg-Norio, D., Paulin, L. and Hänninen, M.L. (2007) Longitudinal study of Finnish *Campylobacter jejuni* and *C. coli* isolates from humans, using multilocus sequence typing, including comparison with epidemiological data and isolates from poultry and cattle. *Appl Environ Microbiol* **73**, 148-155.

Karlyshev, A.V., Linton, D., Gregson, N.A., Lastovica, A.J. and Wren, B.W. (2000) Genetic and biochemical evidence of a *Campylobacter jejuni* capsular polysaccharide that accounts for Penner serotype specificity. *Mol Microbiol* **35**, 529-541.

King, E.O. (1957) Human infections with *Vibrio fetus* and a closely related vibrio. *J Infect Dis* **101**, 119-128.

Kovats, R.S., Edwards, S.J., Charron, D., Cowden, J., D'Souza, R.M., Ebi, K.L., Gauci, C., Gerner-Smidt, P., Hajat, S., Hales, S., Hernandez Pezzi, G., Kriz, B., Kutsar, K., McKeown, P., Mellou, K., Menne, B., O'Brien, S., van Pelt, W. and Schmid, H. (2005) Climate variability and campylobacter infection: an international study. *Int J Biometeorol* **49**, 207-214.

Kramer, J.M., Frost, J.A., Bolton, F.J. and Wareing, D.R. (2000) *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. *J Food Prot* **63**, 1654-1659.

Kwan, P.S., Barrigas, M., Bolton, F.J., French, N.P., Gowland, P., Kemp, R., Leatherbarrow, H., Upton, M. and Fox, A.J. (2008a) Molecular epidemiology of *Campylobacter jejuni* populations in dairy cattle, wildlife, and the environment in a farmland area. *Appl Environ Microbiol* **74**, 5130-5138.

Kwan, P.S., Birtles, A., Bolton, F.J., French, N.P., Robinson, S.E., Newbold, L.S., Upton, M. and Fox, A.J. (2008b) Longitudinal study of the molecular epidemiology of *Campylobacter jejuni* in cattle on dairy farms. *Appl Environ Microbiol* **74**, 3626-3633.

Lake, I.R., Gillespie, I.A., Bentham, G., Nichols, G.L., Lane, C., Adak, G.K. and Threlfall, E.J. (2009) A re-evaluation of the impact of

temperature and climate change on foodborne illness. *Epidemiol Infect* **137**, 1538-1547.

Lastovica, A.J. and Allos, B.M. (2008) Clinical significance of *Campylobacter* and related species other than *Campylobacter jejuni* and *Campylobacter coli*. In *Campylobacter* ed. Nachamkin, I., Szymanski, C.M. and Blaser, M.J. pp. 123-149. Washington, DC, USA: ASM Press.

Lee, M.D. and Newell, D.G. (2006) *Campylobacter* in poultry: filling an ecological niche. *Avian Dis* **50**, 1-9.

Lehner, A., Schneck, C., Feierl, G., Pless, P., Deutz, A., Brandl, E. and Wagner, M. (2000) Epidemiologic application of pulsed-field gel electrophoresis to an outbreak of *Campylobacter jejuni* in an Austrian youth centre. *Epidemiol Infect* **125**, 13-16.

Levesque, S., Frost, E., Arbeit, R.D. and Michaud, S. (2008) Multilocus sequence typing of *Campylobacter jejuni* isolates from humans, chickens, raw milk, and environmental water in Quebec, Canada. *J Clin Microbiol* **46**, 3404-3411.

Levy, A.J. (1946) A gastroenteritis outbreak probably due to a bovine strain of vibrio. *Yale J. Biol. Med.* **18**, 243.

Lindmark, H., Boqvist, S., Ljungstrom, M., Agren, P., Bjorkholm, B. and Engstrand, L. (2009) Risk factors for campylobacteriosis: an epidemiological surveillance study of patients and retail poultry. *J Clin Microbiol* **47**, 2616-2619.

Lindmark, H., Harbom, B., Thebo, L., Andersson, L., Hedin, G., Osterman, B., Lindberg, T., Andersson, Y., Westoo, A. and Olsson Engvall, E. (2004) Genetic characterization and antibiotic resistance of *Campylobacter jejuni* isolated from meats, water, and humans in Sweden. *J Clin Microbiol* **42**, 700-706.

Lior, H., Woodward, D.L., Edgar, J.A., Laroche, L.J. and Gill, P. (1982) Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. *J Clin Microbiol* **15**, 761-768.

Little, C.L., Richardson, J.F., Owen, R.J., de Pinna, E. and Threlfall, E.J. (2008) Prevalence, characterisation and antimicrobial resistance of *Campylobacter* and *Salmonella* in raw poultrymeat in the UK, 2003-2005. *Int J Environ Health Res* **18**, 403-414.

Louis, V.R., Gillespie, I.A., O'Brien, S.J., Russek-Cohen, E., Pearson, A.D. and Colwell, R.R. (2005) Temperature-driven *Campylobacter* seasonality in England and Wales. *Appl Environ Microbiol* **71**, 85-92.

Lovett, J., Francis, D.W. and Hunt, J.M. (1983) Isolation of *Campylobacter jejuni* from raw milk. *Appl Environ Microbiol* **46**, 459-462.

Lukinmaa, S., Nakari, U.M., Eklund, M. and Siitonen, A. (2004) Application of molecular genetic methods in diagnostics and epidemiology of food-borne bacterial pathogens. *APMIS* **112**, 908-929.

Madden, R.H., Murray, K.A. and Gilmour, A. (2007) Carriage of four bacterial pathogens by beef cattle in Northern Ireland at time of slaughter. *Lett Appl Microbiol* **44**, 115-119.

Manning, G., Dowson, C.G., Bagnall, M.C., Ahmed, I.H., West, M. and Newell, D.G. (2003) Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. *Appl Environ Microbiol* **69**, 6370-6379.

Matsumoto, N., Taniwaki, T., Kinuta, M. and Murase, T. (2008) Isolation of *Campylobacter jejuni* and coliform bacilli from bile and liver obtained from slaughter cattle in Western Japan. *J Food Prot* **71**, 1228-1231.

McCarthy, N.D., Colles, F.M., Dingle, K.E., Bagnall, M.C., Manning, G., Maiden, M.C. and Falush, D. (2007) Host-associated genetic import in *Campylobacter jejuni*. *Emerg Infect Dis* **13**, 267-272.

McNaughton, R.D., Leyland, R. and Mueller, L. (1982) Outbreak of *Campylobacter* enteritis due to consumption of raw milk. *Can Med Assoc J* **126**, 657-658.

Medeiros, D.T., Sattar, S.A., Farber, J.M. and Carrillo, C.D. (2008) Occurrence of *Campylobacter* spp. in raw and ready-to-eat foods and in a Canadian food service operation. *J Food Prot* **71**, 2087-2093.

Meinersmann, R.J., Helsel, L.O., Fields, P.I. and Hiett, K.L. (1997) Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. *J Clin Microbiol* **35**, 2810-2814.

Meinersmann, R.J., Phillips, R.W., Hiett, K.L. and Fedorka-Cray, P. (2005) Differentiation of C*ampylobacter* populations as demonstrated by flagellin short variable region sequences. *Appl Environ Microbiol* **71**, 6368-6374.

Michaud, S., Menard, S. and Arbeit, R.D. (2004) Campylobacteriosis, Eastern Townships, Quebec. *Emerg Infect Dis* **10**, 1844-1847.

Michaud, S., Menard, S., Gaudreau, C. and Arbeit, R.D. (2001) Comparison of SmaI-defined genotypes of *Campylobacter jejuni* examined by KpnI: a population-based study. *J Med Microbiol* **50**, 1075-1081.

Milnes, A.S., Stewart, I., Clifton-Hadley, F.A., Davies, R.H., Newell, D.G., Sayers, A.R., Cheasty, T., Cassar, C., Ridley, A., Cook, A.J., Evans, S.J., Teale, C.J., Smith, R.P., McNally, A., Toszeghy, M., Futter, R., Kay, A. and Paiba, G.A. (2008) Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic

Campylobacter and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. *Epidemiol Infect* **136**, 739-751.

Minihan, D., Whyte, P., O'Mahony, M., Fanning, S., McGill, K. and Collins, J.D. (2004) *Campylobacter* spp. in Irish feedlot cattle: a longitudinal study involving pre-harvest and harvest phases of the food chain. *J Vet Med B Infect Dis Vet Public Health* **51**, 28-33.

Morgan, D., Gunneberg, C., Gunnell, D., Healing, T.D., Lamerton, S., Soltanpoor, N., Lewis, D.A. and White, D.G. (1994) An outbreak of *Campylobacter* infection associated with the consumption of unpasteurised milk at a large festival in England. *Eur J Epidemiol* **10**, 581-585.

Mullner, P., Collins-Emerson, J.M., Midwinter, A.C., Carter, P., Spencer, S.E., van der Logt, P., Hathaway, S. and French, N.P. (2010a) Molecular epidemiology of *Campylobacter jejuni* in a geographically isolated country with a uniquely structured poultry industry. *Appl Environ Microbiol* **76**, 2145-2154.

Mullner, P., Shadbolt, T., Collins-Emerson, J.M., Midwinter, A.C., Spencer, S.E., Marshall, J., Carter, P.E., Campbell, D.M., Wilson, D.J., Hathaway, S., Pirie, R. and French, N.P. (2010b) Molecular and spatial epidemiology of human campylobacteriosis: source association and genotype-related risk factors. *Epidemiol Infect*, 1-12.

Mullner, P., Spencer, S.E., Wilson, D.J., Jones, G., Noble, A.D., Midwinter, A.C., Collins-Emerson, J.M., Carter, P., Hathaway, S. and French, N.P. (2009) Assigning the source of human campylobacteriosis in New Zealand: A comparative genetic and epidemiological approach. *Infect Genet Evol*.

Nakari, U.M., Huovinen, E., Kuusi, M. and Siitonen, A. (2010) Population-based surveillance study of *Campylobacter* infections in Finland. *Epidemiol Infect*, 1-7.

Nakari, U.M., Laaksonen, K., Korkeila, M. and Siitonen, A. (2005) Comparative typing of *Campylobacter jejuni* by heat-stable serotyping and PCR-based restriction fragment length polymorphism analysis. *J Clin Microbiol* **43**, 1166-1170.

National Institute for Health and Welfare (2009) Infectious diseases in Finland in 2008.

National Public Health Institute (2005) Infectious diseases in Finland 1995-2004.

NCFA (Nordic Committee on Food Analysis) (2007) Thermotolerant *Campylobacter*. Detection, semi-quantitative and quantitative determination in foods and drinking water, Method No. 119, 3.

Neimann, J., Engberg, J., Molbak, K. and Wegener, H.C. (2003) A case-control study of risk factors for sporadic campylobacter infections in Denmark. *Epidemiol Infect* **130**, 353-366.

Nicholson, F.A., Groves, S.J. and Chambers, B.J. (2005) Pathogen survival during livestock manure storage and following land application. *Bioresour Technol* **96**, 135-143.

Nielsen, E.M. (2002) Occurrence and strain diversity of thermophilic campylobacters in cattle of different age groups in dairy herds. *Lett Appl Microbiol* **35**, 85-89.

Nielsen, E.M., Engberg, J. and Madsen, M. (1997) Distribution of serotypes of *Campylobacter jejuni* and *C. coli* from Danish patients, poultry, cattle and swine. *FEMS Immunol Med Microbiol* **19**, 47-56.

Nygård, K., Andersson, Y., Rottingen, J.A., Svensson, A., Lindback, J., Kistemann, T. and Giesecke, J. (2004) Association between environmental risk factors and campylobacter infections in Sweden. *Epidemiol Infect* **132**, 317-325.

Nylen, G., Dunstan, F., Palmer, S.R., Andersson, Y., Bager, F., Cowden, J., Feierl, G., Galloway, Y., Kapperud, G., Megraud, F., Molbak, K., Petersen, L.R. and Ruutu, P. (2002) The seasonal distribution of campylobacter infection in nine European countries and New Zealand. *Epidemiol Infect* **128**, 383-390.

Olson, C.K., Ethelberg, S., van Pelt, W. and Tauxe, R.V. (2008) Epidemiology of *Campylobacter jejuni* infections in industrialized nations. In ed. Nachamkin, I., Szymanski, C.M. and Blaser, M. pp. 163-189. Washington DC, USA: ASM Press.

Oosterom, J., de Wilde, G.J.A., de Boer, E., de Blaauw, L.H. and Karman, H. (1983) Survival of *Campylobacter* during poultry processing and pig slaughtering. *Journal of food protection* **46**, 702-706.

Oporto, B., Esteban, J.I., Aduriz, G., Juste, R.A. and Hurtado, A. (2007) Prevalence and strain diversity of thermophilic campylobacters in cattle, sheep and swine farms. *J Appl Microbiol* **103**, 977-984.

Parisi, A., Lanzilotta, S.G., Addante, N., Normanno, G., Di Modugno, G., Dambrosio, A. and Montagna, C.O. (2007) Prevalence, molecular characterization and antimicrobial resistance of thermophilic campylobacter isolates from cattle, hens, broilers and broiler meat in south-eastern Italy. *Vet Res Commun* **31**, 113-123.

Parkhill, J., Wren, B.W., Mungall, K., Ketley, J.M., Churcher, C., Basham, D., Chillingworth, T., Davies, R.M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A.V., Moule, S., Pallen, M.J., Penn, C.W., Quail, M.A., Rajandream, M.A., Rutherford, K.M., van Vliet, A.H., Whitehead, S. and Barrell, B.G. (2000) The genome sequence of the

food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* **403**, 665-668.

Paton, A.W., Srimanote, P., Talbot, U.M., Wang, H. and Paton, J.C. (2004) A new family of potent AB(5) cytotoxins produced by Shiga toxigenic *Escherichia coli*. *J Exp Med* **200**, 35-46.

Patrick, M.E., Christiansen, L.E., Waino, M., Ethelberg, S., Madsen, H. and Wegener, H.C. (2004) Effects of climate on incidence of *Campylobacter* spp. in humans and prevalence in broiler flocks in Denmark. *Appl Environ Microbiol* **70**, 7474-7480.

Penner, J.L. and Hennessy, J.N. (1980) Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *J Clin Microbiol* **12**, 732-737.

Penner, J.L., Hennessy, J.N. and Congi, R.V. (1983) Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. *Eur J Clin Microbiol* **2**, 378-383.

Perko-Mäkelä, P., Hakkinen, M., Honkanen-Buzalski, T. and Hänninen, M.L. (2002) Prevalence of campylobacters in chicken flocks during the summer of 1999 in Finland. *Epidemiol Infect* **129**, 187-192.

Peterson, M.C. (2003) *Campylobacter jejuni* enteritis associated with consumption of raw milk. *J Environ Health* **65**, 20-1, 24, 26.

Pezzotti, G., Serafin, A., Luzzi, I., Mioni, R., Milan, M. and Perin, R. (2003) Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. *Int J Food Microbiol* **82**, 281-287.

Phillips, D., Jordan, D., Morris, S., Jenson, I. and Sumner, J. (2008) A national survey of the microbiological quality of retail raw meats in Australia. *J Food Prot* **71**, 1232-1236.

Pitkänen, T., Miettinen, I.T., Nakari, U.M., Takkinen, J., Nieminen, K., Siitonen, A., Kuusi, M., Holopainen, A. and Hänninen, M.L. (2008) Faecal contamination of a municipal drinking water distribution system in association with *Campylobacter jejuni* infections. *J Water Health* **6**, 365-376.

Pradhan, A.K., Van Kessel, J.S., Karns, J.S., Wolfgang, D.R., Hovingh, E., Nelen, K.A., Smith, J.M., Whitlock, R.H., Fyock, T., Ladely, S., Fedorka-Cray, P.J. and Schukken, Y.H. (2009) Dynamics of endemic infectious diseases of animal and human importance on three dairy herds in the northeastern United States. *J Dairy Sci* **92**, 1811-1825.

Ragimbeau, C., Schneider, F., Losch, S., Even, J. and Mossong, J. (2008) Multilocus sequence typing, pulsed-field gel electrophoresis,

and fla short variable region typing of clonal complexes of *Campylobacter jejuni* strains of human, bovine, and poultry origins in Luxembourg. *Appl Environ Microbiol* **74**, 7715-7722.

Rahimi, E., Ameri, M. and Kazemeini, H.R. (2010) Prevalence and antimicrobial resistance of *Campylobacter* species isolated from raw camel, beef, lamb, and goat meat in Iran. *Foodborne Pathog Dis* **7**, 443-447.

Rautelin, H. and Hänninen, M.L. (1999) Comparison of a commercial test for serotyping heat-stable antigens of *Campylobacter jejuni* with genotyping by pulsed-field gel electrophoresis. *J Med Microbiol* **48**, 617-621.

Rautelin, H., Vierikko, A., Hänninen, M.L. and Vaara, M. (2003) Antimicrobial susceptibilities of *Campylobacter* strains isolated from Finnish subjects infected domestically or from those infected abroad. *Antimicrob Agents Chemother* **47**, 102-105.

Reich, F., Atanassova, V., Haunhorst, E. and Klein, G. (2008) The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. *Int J Food Microbiol* **127**, 116-120.

Ribot, E.M., Fitzgerald, C., Kubota, K., Swaminathan, B. and Barrett, T.J. (2001) Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. *J Clin Microbiol* **39**, 1889-1894.

Robinson, D.A., Edgar, W.J., Gibson, G.L., Matchett, A.A. and Robertson, L. (1979) *Campylobacter* enteritis associated with consumption of unpasteurised milk. *Br Med J* **1**, 1171-1173.

Robinson, D.A. and Jones, D.M. (1981) Milk-borne campylobacter infection. *Br Med J (Clin Res Ed)* **282**, 1374-1376.

Rosenquist, H., Boysen, L., Galliano, C., Nordentoft, S., Ethelberg, S. and Borck, B. (2009) Danish strategies to control *Campylobacter* in broilers and broiler meat: facts and effects. *Epidemiol Infect* **137**, 1742-1750.

Rotariu, O., Dallas, J.F., Ogden, I.D., MacRae, M., Sheppard, S.K., Maiden, M.C., Gormley, F.J., Forbes, K.J. and Strachan, N.J. (2009) Spatiotemporal homogeneity of *Campylobacter* subtypes from cattle and sheep across northeastern and southwestern Scotland. *Appl Environ Microbiol* **75**, 6275-6281.

Sails, A.D., Swaminathan, B. and Fields, P.I. (2003) Utility of multilocus sequence typing as an epidemiological tool for investigation of outbreaks of gastroenteritis caused by *Campylobacter jejuni*. *J Clin Microbiol* **41**, 4733-4739.

Sammarco, M.L., Ripabelli, G., Fanelli, I., Grasso, G.M. and Tamburro, M. (2010) Prevalence and biomolecular characterization of *Campylobacter* spp. isolated from retail meat. *J Food Prot* **73**, 720-728.

Sandberg, M., Nygård, K., Meldal, H., Valle, P.S., Kruse, H. and Skjerve, E. (2006) Incidence trend and risk factors for campylobacter infections in humans in Norway. *BMC Public Health* **6**, 179.

Schildt, M., Savolainen, S. and Hänninen, M.L. (2006) Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family. *Epidemiol Infect* **134**, 401-405.

Schönberg-Norio, D., Mattila, L., Lauhio, A., Katila, M.L., Kaukoranta, S.S., Koskela, M., Pajarre, S., Uksila, J., Eerola, E., Sarna, S. and Rautelin, H. (2009) Patient-reported complications associated with *Campylobacter jejuni* infection. *Epidemiol Infect*, 1-8.

Schönberg-Norio, D., Sarna, S., Hänninen, M.L., Katila, M.L., Kaukoranta, S.S. and Rautelin, H. (2006) Strain and host characteristics of *Campylobacter jejuni* infections in Finland. *Clin Microbiol Infect* **12**, 754-760.

Schönberg-Norio, D., Takkinen, J., Hänninen, M.L., Katila, M.L., Kaukoranta, S.S., Mattila, L. and Rautelin, H. (2004) Swimming and *Campylobacter* infections. *Emerg Infect Dis* **10**, 1474-1477.

Schouls, L.M., Reulen, S., Duim, B., Wagenaar, J.A., Willems, R.J., Dingle, K.E., Colles, F.M. and Van Embden, J.D. (2003) Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. *J Clin Microbiol* **41**, 15-26.

Schwarz, S., Silley, P., Simjee, S., Woodford, N., van Duijkeren, E., Johnson, A.P. and Gaastra, W. (2010) Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. *J Antimicrob Chemother* **65**, 601-604.

Sebald, E.R. and Véron, M. (1963) Teneur en bases da lÀDN et classification des vibrions. *Annales de L'institut Pasteur (Paris)* **105**, 897-910.

Sheppard, S.K., Dallas, J.F., MacRae, M., McCarthy, N.D., Sproston, E.L., Gormley, F.J., Strachan, N.J., Ogden, I.D., Maiden, M.C. and Forbes, K.J. (2009) *Campylobacter* genotypes from food animals, environmental sources and clinical disease in Scotland 2005/6. *Int J Food Microbiol* **134**, 96-103.

Skirrow, M.B. (2006) John McFadyean and the centenary of the first isolation of *Campylobacter* species. *Clin Infect Dis* **43**, 1213-1217.

Skirrow, M.B. (1977) *Campylobacter* enteritis: a "new" disease. *Br Med J* **2**, 9-11.

Smith, T. and Taylor, M.S. (1919) Some morphological and biochemical characteristics of the spirilla (*Vibrio fetus* n. sp.) associated with disease of the fetal membranes in cattle. *J Exp Med*, 299-312.

Sopwith, W., Ashton, M., Frost, J.A., Tocque, K., O'Brien, S., Regan, M. and Syed, Q. (2003) Enhanced surveillance of *Campylobacter* infection in the North West of England 1997-1999. *J Infect* **46**, 35-45.

Stafford, R.J., Schluter, P., Kirk, M., Wilson, A., Unicomb, L., Ashbolt, R., Gregory, J. and OzFoodNet Working Group (2007) A multi-centre prospective case-control study of campylobacter infection in persons aged 5 years and older in Australia. *Epidemiol Infect* **135**, 978-988.

Stafford, R.J., Schluter, P.J., Wilson, A.J., Kirk, M.D., Hall, G., Unicomb, L. and OzFoodNet Working Group (2008) Population-attributable risk estimates for risk factors associated with *Campylobacter* infection, Australia. *Emerg Infect Dis* **14**, 895-901.

Stanley, K. and Jones, K. (2003) Cattle and sheep farms as reservoirs of *Campylobacter*. *J Appl Microbiol* **94 Suppl**, 104S-113S.

Stanley, K.N., Wallace, J.S., Currie, J.E., Diggle, P.J. and Jones, K. (1998) The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. *J Appl Microbiol* **85**, 472-480.

Stark, K., Niedrig, M., Biederbick, W., Merkert, H. and Hacker, J. (2009) Climate changes and emerging diseases. What new infectious diseases and health problem can be expected? *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* **52**, 699-714.

Stern, N.J., Hiett, K.L., Alfredsson, G.A., Kristinsson, K.G., Reiersen, J., Hardardottir, H., Briem, H., Gunnarsson, E., Georgsson, F., Lowman, R., Berndtson, E., Lammerding, A.M., Paoli, G.M. and Musgrove, M.T. (2003) *Campylobacter* spp. in Icelandic poultry operations and human disease. *Epidemiol Infect* **130**, 23-32.

Strachan, N.J., Gormley, F.J., Rotariu, O., Ogden, I.D., Miller, G., Dunn, G.M., Sheppard, S.K., Dallas, J.F., Reid, T.M., Howie, H., Maiden, M.C. and Forbes, K.J. (2009) Attribution of *Campylobacter* infections in northeast Scotland to specific sources by use of multilocus sequence typing. *J Infect Dis* **199**, 1205-1208.

Studahl, A. and Andersson, Y. (2000) Risk factors for indigenous campylobacter infection: a Swedish case-control study. *Epidemiol Infect* **125**, 269-275.

Svedhem, A., Kaijser, B. and Sjogren, E. (1981) The occurrence of *Campylobacter jejuni* in fresh food and survival under different conditions. *J Hyg (Lond)* **87**, 421-425.

Taboada, E.N., Acedillo, R.R., Carrillo, C.D., Findlay, W.A., Medeiros, D.T., Mykytczuk, O.L., Roberts, M.J., Valencia, C.A., Farber, J.M. and Nash, J.H. (2004) Large-scale comparative genomics meta-analysis of *Campylobacter jejuni* isolates reveals low level of genome plasticity. *J Clin Microbiol* **42**, 4566-4576.

Tam, C.C., Higgins, C.D., Neal, K.R., Rodrigues, L.C., Millership, S.E., O'Brien, S.J. and Campylobacter Case-Control Study Group (2009) Chicken consumption and use of acid-suppressing medications as risk factors for *Campylobacter* enteritis, England. *Emerg Infect Dis* **15**, 1402-1408.

Tam, C.C., Rodrigues, L.C., O'Brien, S.J. and Hajat, S. (2006) Temperature dependence of reported *Campylobacter* infection in England, 1989-1999. *Epidemiol Infect* **134**, 119-125.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H. and Swaminathan, B. (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**, 2233-2239.

Teunis, P., Van den Brandhof, W., Nauta, M., Wagenaar, J., Van den Kerkhof, H. and Van Pelt, W. (2005) A reconsideration of the *Campylobacter* dose-response relation. *Epidemiol Infect* **133**, 583-592.

Tribble, D.R., Baqar, S., Scott, D.A., Oplinger, M.L., Trespalacios, F., Rollins, D., Walker, R.I., Clements, J.D., Walz, S., Gibbs, P., Burg, E.F.,3rd, Moran, A.P., Applebee, L. and Bourgeois, A.L. (2010) Assessment of the duration of protection in *Campylobacter jejuni* experimental infection in humans. *Infect Immun* **78**, 1750-1759.

Unicomb, L.E., Dalton, C.B., Gilbert, G.L., Becker, N.G. and Patel, M.S. (2008) Age-specific risk factors for sporadic *Campylobacter* infection in regional Australia. *Foodborne Pathog Dis* **5**, 79-85.

Unicomb, L.E., Fullerton, K.E., Kirk, M.D. and Stafford, R.J. (2009) Outbreaks of campylobacteriosis in Australia, 2001 to 2006. *Foodborne Pathog Dis* **6**, 1241-1250.

Unicomb, L.E., O'Reilly, L.C., Kirk, M.D., Stafford, R.J., Smith, H.V., Becker, N.G., Patel, M.S., Gilbert, G.L. and Australian Campylobacter Subtyping Study Group (2008) Risk factors for infection with *Campylobacter jejuni* flaA genotypes. *Epidemiol Infect* **136**, 1480-1491.

Vally, H., Hall, G., Scallan, E., Kirk, M.D. and Angulo, F.J. (2009) Higher rate of culture-confirmed *Campylobacter* infections in

Australia than in the USA: is this due to differences in healthcare-seeking behaviour or stool culture frequency? *Epidemiol Infect* **137**, 1751-1758.

van Hees, B.C., Veldman-Ariesen, M.J., de Jongh, B.M., Tersmette, M. and van Pelt, W. (2007) Regional and seasonal differences in incidence and antibiotic resistance of *Campylobacter* from a nationwide surveillance study in The Netherlands: an overview of 2000-2004. *Clin Microbiol Infect* **13**, 305-310.

Vellinga, A. and Van Loock, F. (2002) The dioxin crisis as experiment to determine poultry-related C*ampylobacter* enteritis. *Emerg Infect Dis* **8**, 19-22.

Vierikko, A., Hänninen, M.L., Siitonen, A., Ruutu, P. and Rautelin, H. (2004) Domestically acquired *Campylobacter* infections in Finland. *Emerg Infect Dis* **10**, 127-130.

Vindigni, S.M., Srijan, A., Wongstitwilairoong, B., Marcus, R., Meek, J., Riley, P.L. and Mason, C. (2007) Prevalence of foodborne microorganisms in retail foods in Thailand. *Foodborne Pathog Dis* **4**, 208-215.

Wang, H., Paton, J.C. and Paton, A.W. (2007) Pathologic changes in mice induced by subtilase cytotoxin, a potent new *Escherichia coli* AB5 toxin that targets the endoplasmic reticulum. *J Infect Dis* **196**, 1093-1101.

Wareing, D.R., Ure, R., Colles, F.M., Bolton, F.J., Fox, A.J., Maiden, M.C. and Dingle, K.E. (2003) Reference isolates for the clonal complexes of *Campylobacter jejuni*. *Lett Appl Microbiol* **36**, 106-110.

Wassenaar, T.M. and Newell, D.G. (2000) Genotyping of *Campylobacter* spp. *Appl Environ Microbiol* **66**, 1-9.

Wesley, I.V., Wells, S.J., Harmon, K.M., Green, A., Schroeder-Tucker, L., Glover, M. and Siddique, I. (2000) Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Appl Environ Microbiol* **66**, 1994-2000.

White, A.N., Kinlin, L.M., Johnson, C., Spain, C.V., Ng, V. and Fisman, D.N. (2009) Environmental determinants of campylobacteriosis risk in Philadelphia from 1994 to 2007. *Ecohealth* **6**, 200-208.

WHO (2007) Report of the Second WHO Expert Meeting: Critically important antimicrobials for human medicine: Categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use. http://www.who.int/foodborne_disease/resistance/antimicrobials_humans.pdf.

Whyte, P., McGill, K., Cowley, D., Madden, R.H., Moran, L., Scates, P., Carroll, C., O'Leary, A., Fanning, S., Collins, J.D., McNamara, E., Moore, J.E. and Cormican, M. (2004) Occurrence of *Campylobacter* in retail foods in Ireland. *Int J Food Microbiol* **95**, 111-118.

Wieland, B., Regula, G., Danuser, J., Wittwer, M., Burnens, A.P., Wassenaar, T.M. and Stark, K.D. (2005) *Campylobacter* spp. in dogs and cats in Switzerland: risk factor analysis and molecular characterization with AFLP. *J Vet Med B Infect Dis Vet Public Health* **52**, 183-189.

Wilson, D.J., Gabriel, E., Leatherbarrow, A.J., Cheesbrough, J., Gee, S., Bolton, E., Fox, A., Fearnhead, P., Hart, C.A. and Diggle, P.J. (2008) Tracing the source of campylobacteriosis. *PLoS Genet* **4**, e1000203.

Wingstrand, A., Neimann, J., Engberg, J., Nielsen, E.M., Gerner-Smidt, P., Wegener, H.C. and Molbak, K. (2006) Fresh chicken as main risk factor for campylobacteriosis, Denmark. *Emerg Infect Dis* **12**, 280-285.

Wistrom, J. and Norrby, S.R. (1995) Fluoroquinolones and bacterial enteritis, when and for whom? *J Antimicrob Chemother* **36**, 23-39.

Wong, T.L., Hollis, L., Cornelius, A., Nicol, C., Cook, R. and Hudson, J.A. (2007) Prevalence, numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. *J Food Prot* **70**, 566-573.

Workman, S.N., Mathison, G.E. and Lavoie, M.C. (2005) Pet dogs and chicken meat as reservoirs of *Campylobacter* spp. in Barbados. *J Clin Microbiol* **43**, 2642-2650.

Yang, C., Jiang, Y., Huang, K., Zhu, C. and Yin, Y. (2003) Application of real-time PCR for quantitative detection of *Campylobacter jejuni* in poultry, milk and environmental water. *FEMS Immunol Med Microbiol* **38**, 265-271.

Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D.G., Wagner, D. and Meng, J. (2001) Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. *Appl Environ Microbiol* **67**, 5431-5436.

Zhao, T., Doyle, M.P. and Berg, D.E. (2000) Fate of *Campylobacter jejuni* in butter. *J Food Prot* **63**, 120-122.

I

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2007, p. 3232–3238 Vol. 73, No. 10
0099-2240/07/$08.00+0 doi:10.1128/AEM.02579-06

Prevalence of *Campylobacter* spp. in Cattle in Finland and Antimicrobial Susceptibilities of Bovine *Campylobacter jejuni* Strains▽

Marjaana Hakkinen,[1]* Helmi Heiska,[1] and Marja-Liisa Hänninen[2]

Finnish Food Safety Authority, Mustialankatu 3, Helsinki FI-00790, Finland,[1] *and Department of Food and Environmental Hygiene, University of Helsinki, Helsinki FI-00014, Finland*[2]

Received 9 November 2006/Accepted 12 March 2007

The study investigated the prevalence of *Campylobacter* spp. in Finnish cattle at slaughter and carcass contamination after slaughter. During the period January to December 2003, bovine rectal fecal samples (n = 952) and carcass surface samples (n = 948) from 12 out of 15 Finnish slaughterhouses were examined. In total, campylobacters were detected in 31.1% of fecal samples and in 3.5% of carcass surface samples. *Campylobacter jejuni* was isolated from 19.5%, *Campylobacter coli* from 2.2%, and presumptive *Campylobacter hyointestinalis* from 10.8% of fecal samples. Campylobacters were detected in 4.4% and 37.4% of the fecal samples examined both by direct culture and by enrichment (n = 730), respectively, suggesting a low level of campylobacters in the intestinal content. A slightly increasing trend was observed in the overall prevalence of campylobacters towards the end of summer and autumn. Seventeen different serotypes were detected among the fecal *C. jejuni* isolates using a set of 25 commercial antisera for serotyping heat-stable antigens (Penner) of *C. jejuni* by passive hemagglutination. The predominant serotypes, Pen2 and Pen4-complex, were isolated from 52% of the fecal samples. Subtyping by pulsed-field gel electrophoresis (SmaI) yielded 56 and 20 subtypes out of 330 fecal and 70 carcass *C. jejuni* isolates, respectively. MICs of ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid, and oxytetracycline for 187 *C. jejuni* isolates were determined using a commercial broth microdilution method. Sixteen (9%) of the isolates were resistant to at least one of the antimicrobials tested. Resistance to nalidixic acid was most commonly detected (6%). No multiresistance was observed.

Over the last 20 years thermophilic campylobacters have become the most important human bacterial pathogens in most western European countries (55a). In Finland the number of reported cases has shown an increasing trend over the last 10 years apart from a slight decrease from 2002 to 2003 (35). In Finland, during the seasonal peak from June to September in 2003 approximately 40% of the cases were of Finnish origin (53).

Poultry is generally considered to be the most important single reservoir for campylobacters, mainly *Campylobacter jejuni*. However, there is some evidence based on the temporal occurrence of serotypes and genotypes shared by humans and poultry and on weekly data for poultry and human isolates that suggests that there is a common source of campylobacters instead of direct poultry-human transmission (28, 32). In addition, genotyping data on campylobacters of human and animal origin have raised the question of whether the role of poultry as a source of campylobacter infections has been overestimated (21, 40, 48).

Cattle are also common carriers of campylobacters (23, 25, 49). However, beef is not considered to be an important vehicle of transmission in human infections, because campylobacters are not commonly detected on carcasses or in beef. In surveys of retail beef only 0 to 5% of the samples have tested positive for campylobacters (42, 50, 55). Instead, the importance of raw milk as a risk factor for human campylobacteriosis has been recognized in epidemiological studies (33, 51), and consumption of unpasteurized milk has been associated with campylobacter infections in several outbreaks (12, 30, 47, 51). The environmental load of campylobacters in cattle manure may be a more significant factor in the transmission of infections than contaminated milk or beef (36, 39).

Antimicrobial treatment is not usually required for human campylobacter infections. In cases with severe or prolonged symptoms macrolides or fluoroquinolones have been recommended as treatment. Since the 1990s the increasing resistance of campylobacters to antibiotics, especially to fluoroquinolones, has been reported both among animal isolates and among isolates from human infections (10, 18). Because person-to-person transmission of campylobacters is uncommon and infections are frequently acquired from foods of animal origin, the use of antimicrobials in production animals has been suggested as the cause of the increase in resistance (3, 41). In Finland, products containing macrolides and fluoroquinolones are authorized for bovine use, but their use is limited.

The objective of the present study was to elucidate the role of Finnish cattle as a potential reservoir for thermophilic campylobacters and as a source of antibiotic-resistant *C. jejuni*.

MATERIALS AND METHODS

Sampling. Rectal fecal samples (n = 952) and carcass surface samples (n = 948) from clinically healthy cattle were collected in 12 slaughterhouses in Finland during the period January to December 2003. Sampling was carried out weekly, every second week, or every fourth week. The number of samples and the sampling frequency were calculated from the proportion of the slaughter volumes at each slaughterhouse in 2002. The samples were randomly chosen and taken by meat inspection veterinarians. The plastic sampling jars were filled with 200 to 300 g of fecal material and closed tightly, leaving the air space as small as possible. The carcass surface samples from the same animals were taken before chilling. The brisket, the inner and outer thigh, and the pelvic cavity were

* Corresponding author. Mailing address: Finnish Food Safety Authority, Mustialankatu 3, Helsinki FI-00790, Finland. Phone: 358 2077 24471. Fax: 358 2077 24350. E-mail: marjaana.hakkinen@evira.fi.

▽ Published ahead of print on 16 March 2007.

swabbed with two gauze pads (10 cm by 10 cm) wetted with sterile 0.1% peptone water. Both gauze pads were placed in a sterile plastic bag, the air was squeezed out, and the bag was closed tightly. All samples were sent chilled to the National Veterinary and Food Research Institute (currently the Finnish Food Safety Authority), Helsinki, Finland. The examination started in 1 to 2 days after sampling.

Isolation and identification of campylobacter strains. The fecal samples were examined by enrichment. Ten grams of fecal material was weighed and put into 90 ml of Bolton broth (Campylobacter Enrichment Broth, Lab 135 plus selective supplement X131 [LAB M, Bury, England] plus lysed horse blood) and incubated at 41.5°C for 24 h in a microaerobic incubator (ThermoForma [Thermo Electron Corporation, Marietta, OH]) (O_2, 5%; CO_2, 10%; N_2, 85%). One loopful (10 μl) of enrichment culture was spread onto modified *Campylobacter* charcoal differential agar (mCCDA) plates (Campylobacter Blood Free Selective Medium Lab 112 plus selective supplement X112 [LAB M, Bury, England]), which were incubated in the same conditions. In addition, one loopful (10 μl) of 730 fecal samples was directly cultured on mCCDA. The surface gauze samples were similarly enriched in 225 ml of Bolton broth and spread onto mCCDA.

Two colonies resembling campylobacters from mCCDA plates originating from direct culture and enrichment procedures were subcultured onto brucella agar (BBL, Becton Dickinson, MD) with 5% bovine whole blood treated with sodium citrate (Finnish Food Safety Authority, Helsinki, Finland). At least two isolates from each positive sample were identified to the species level using microscopical examination of motility and cell morphology, catalase and oxidase reactions, hippurate hydrolysis, and susceptibility to nalidixic acid (26). Nalidixic acid-resistant isolates were further examined for indoxyl acetate hydrolysis and susceptibility to cephalotin (26). Hippurate-negative, indoxyl acetate-hydrolyzing isolates were examined for H_2S production in triple sugar iron agar (LAB M, Bury, England) (pH 8) and for urease production to identify *Campylobacter hyointestinalis* strains. The isolates were stored in brucella broth supplemented with 15% glycerol at −70°C.

Serotyping. One to four *C. jejuni* isolates (287 in total) from 176 fecal samples and 21 isolates from carcass samples were serotyped using a set of 25 commercial antisera for the serotyping of heat-stable antigens (Penner) of *C. jejuni* by the passive hemagglutination method (Denka Seiken Co., Ltd., Tokyo, Japan). Tests were performed, and the results were interpreted according to the manufacturer's instructions.

Genotyping by PFGE. A total of 330 and 70 *C. jejuni* isolates from 183 fecal and 33 carcass samples, respectively, were analyzed using pulsed-field gel electrophoresis (PFGE). The agarose plugs were prepared according to the PulseNet protocol (www.cdc.gov/pulsenet/protocols) and stored in Tris-EDTA buffer at 4°C. DNA was digested overnight at 25°C with 20 U of SmaI restriction endonuclease (New England Biolabs Inc., Ipswich, MA) in a final volume of 200 μl containing 2 μl bovine serum albumin (New England Biolabs Inc., Ipswich, MA). PFGE was performed using the CHEF-DRIII pulsed-field electrophoresis system (Bio-Rad, CA). An agarose gel (1%) was prepared in 0.5× Tris-buffered EDTA (Sigma-Aldrich Co, Baltimore, MD). Fragments were separated by electrophoresis for 18 h at 6 V and 14°C with ramped pulse times from 6.8 to 35.4 s. *Salmonella* serotype Braenderup strain H9812 (ATCC BAA-664) was used as the fragment size marker. The gels were stained for 45 min with ethidium bromide (0.5 μg/ml) and photographed under UV illumination. Patterns that differed by at least a single band were considered to be different subtypes. Each subtype was named S1, S2, etc. The criteria presented by Tenover et al. (52) were used to assess how the subtypes were related.

Determination of antimicrobial susceptibility. The MICs of ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid, and oxytetracycline for 187 *C. jejuni* isolates from 183 rectal fecal samples were determined using a commercial broth microdilution method, VetMIC Camp (National Veterinary Institute, Uppsala, Sweden). Epidemiological cutoff values for resistance, based on MIC distributions, were used in the interpretation of results. A *C. jejuni* isolate was considered to be resistant to a specific antimicrobial when its MIC was distinctly higher than those of inherently susceptible *C. jejuni* isolates.

Statistical analysis. The χ^2 test (Excel; Microsoft Corp., Redmond, WA) was performed to investigate the association between month and prevalences of all campylobacters, *C. jejuni*, and *Campylobacter hyointestinalis* subsp. *hyointestinalis*.

RESULTS

Prevalence. Campylobacters were detected in a total of 296 out of 952 (31.1%) rectal fecal samples and in 33 out of 948 (3.5%) carcass surface samples. Campylobacters were detected in 4.4% and 37.4% of the fecal samples examined both by direct culture and by enrichment ($n = 730$), respectively.

TABLE 1. Distribution of campylobacter-positive animals between beef and dairy cattle farms

Herd type	No. of farms	No. of positive farms	% Positive farms	No. of positive animals
Beef cattle	284	122	42.7	154
Dairy cattle	463	133	28.7	142
Total	747	255	34.0	296

C. jejuni was detected in 186 (19.5%) and *Campylobacter coli* in 21 (2.2%) fecal samples. Presumptive *C. hyointestinalis* was isolated from 103 (10.8%) fecal samples, but the isolates from only 93 samples survived after storage at −70°C and all of these could be confirmed as *C. hyointestinalis* subsp. *hyointestinalis*. Two *Campylobacter* species were isolated from 14 samples: *C. jejuni* and *C. hyointestinalis* subsp. *hyointestinalis* in seven and *C. jejuni* and *C. coli* in six samples. The *C. coli* and *C. hyointestinalis* subsp. *hyointestinalis* isolates were detected only after enrichment. *C. jejuni* was detected in 29 (3.1%), *C. coli* in two (0.2%), and presumptive *C. hyointestinalis* in two (0.2%) carcass surface samples. In three cases the isolates from the fecal and carcass samples from the same animal represented different *Campylobacter* species.

Seventy percent of the animals belonged to the age group 1 to 3 years. In this age group the prevalences of *C. jejuni*, *C. hyointestinalis* subsp. *hyointestinalis*, and *C. coli* were 25.6%, 10.0%, and 1.9%, respectively. In the age group that included animals between 3 and 7 years, which represented 25% of the animals, the prevalences were 4.0%, 12.3%, and 3.1%, respectively.

The sampled animals were traced to 747 farms: 411 (43.2%) samples originated from 284 beef cattle farms and 541 (56.8%) samples from 463 dairy cattle farms (Table 1). The proportion of campylobacter-positive beef cattle farms was higher than that of dairy cattle farms. *Campylobacter* isolates originated from all of the 12 abattoirs and from 255 farms. More than one animal (two to five) per farm was sampled on 112 occasions. Animals from 19 farms were all campylobacter positive at the same sampling. In four cases, two *Campylobacter* species were detected in animals from the same farm. Positive and negative animals were detected from 36 farms on the same sampling occasion. Animals from 32 farms were sampled twice. Both samples from six farms were positive, and from 10 farms one of the samples was positive. Samples from 15 farms were campylobacter negative in both samplings. Two or more campylobacter-positive animals were detected from 33 farms either at the same sampling or on different occasions.

Monthly distribution. The monthly distribution of campylobacter-positive fecal samples is presented in Fig. 1. A slightly increasing trend can be seen in the overall prevalence of campylobacters towards the end of summer and late autumn. The prevalence of *C. jejuni* was highest in August and lowest in December. *C. hyointestinalis* subsp. *hyointestinalis* was most frequently isolated in November, and the lowest prevalence was detected in April. A statistical association was observed between month and the overall prevalence of campylobacters,

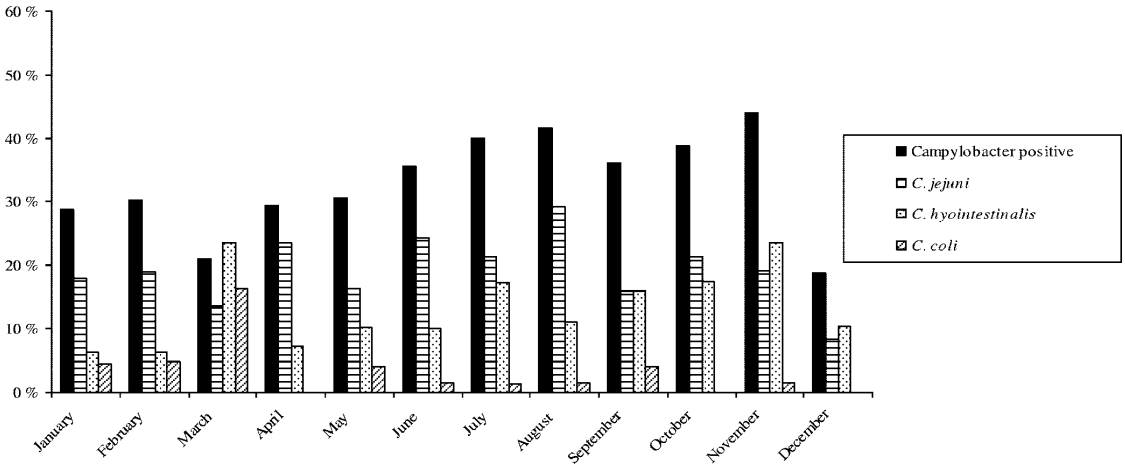

FIG. 1. Monthly distribution of campylobacters in the fecal samples.

but not *C. jejuni* ($P < 0.05$, df = 11). Also the prevalence of *C. hyointestinalis* subsp. *hyointestinalis* was statistically connected with month ($P < 0.01$, df = 11).

Serotyping. Seventeen different serotypes were detected among the fecal *C. jejuni* isolates using the commercial serotyping kit that was employed in this study. Untypeable isolates were obtained from 22 samples (12.5%). The predominant Penner serotypes of *C. jejuni* that were detected in the fecal samples were Pen2 and Pen4-complex, which were isolated from 52% of the fecal samples. In the 79 samples from which two or three isolates were serotyped, the same serotype was detected in 28 samples, two serotypes were detected in nine samples, and three were detected in one sample. Both typeable and untypeable isolates were obtained from the same sample on 17 occasions. Two untypeable isolates were obtained from four fecal samples. In carcass samples Pen2 was detected in 11 and Pen4-complex was detected in 5 out of 21 isolates.

PFGE. Fifty-six different *C. jejuni* subtypes were identified by PFGE among 330 isolates from the fecal samples. The 10 most prevalent subtypes isolated from 103 fecal samples covered 56.3% of *C. jejuni*-positive samples (Table 2). *C. jejuni* isolates from 164 animals (89.6%) were assigned to 21 SmaI subtypes. Unique subtypes were isolated from 30 animals (16.4%). DNA from five isolates was not digestible with SmaI. Multiple isolates were genotyped from 106 fecal samples. Two unrelated SmaI subtypes were observed in six of them. Two and three different but possibly related isolates were observed in two fecal samples.

When several animals from one farm were sampled at the same time, *C. jejuni* isolates from animals originating from the same farm represented indistinguishable SmaI profiles on eight occasions. Closely related subtypes were identified on one occasion and possibly related subtypes on two occasions. Unrelated genotypes were observed in five cases. On the six occasions when positive samples were obtained from the farms that were sampled twice, the *C. jejuni* isolates represented unrelated subtypes.

The *C. jejuni* isolates from carcass surface samples represented 20 different SmaI subtypes. The most frequently isolated subtypes were S1 and S20, which were each detected in four carcasses. Subtypes S9 and S26 were observed in three carcasses. Eleven subtypes were detected only once. Two different SmaI subtypes were isolated from two carcasses. In one case the isolates were possibly related, and in the other case they were unrelated.

On 12 occasions indistinguishable *C. jejuni* PFGE types were detected in the fecal and carcass samples from the same animal. Indistinguishable subtypes isolated from one animal's fecal sample were detected in another animal's carcass sample at six samplings. In eight cases different *C. jejuni* subtypes were obtained from fecal and carcass surface samples on the same sampling occasion.

Sero-/PFGE types. Twenty-three different PFGE subtypes were observed among *C. jejuni* isolates classified as Pen2. The largest group, Pen2/S1, comprised isolates from 19 animals. Isolates belonging to Pen4-complex were split up into 13 PFGE subtypes. The most common was Pen4-complex/S2, which was isolated from 10 animals. The predominant sero-

TABLE 2. Most prevalent PFGE types of *C. jejuni* in fecal and carcass samples

PFGE type	No. of fecal samples	% of positive fecal samples	No. of carcasses	% of positive carcass samples
S1	20	10.9	4	12.1
S2	14	7.7	2	6.1
S3	14	7.7	0	0.0
S5	10	5.5	1	3.0
S6	5	2.7	2	6.1
S7	10	5.5	1	3.0
S9	5	2.7	3	9.1
S10	6	3.3	1	3.0
S11	10	5.5	2	6.1
S13	7	3.8	0	0.0
S14	7	3.8	1	3.0
S20	0	0.0	4	12.1
S26	2	1.1	3	9.1

TABLE 3. Predominant combined sero-/PFGE types of *C. jejuni* isolates from fecal samples

Penner serotype	SmaI subtype	No. of positive samples	% of all positive samples
2	S1	19	10.8
2	S5	10	5.7
2	S11	7	4.0
2	S18	5	2.8
4-complex	S2	10	5.7
4-complex	S3	7	4.0
12	S7	8	4.5
1,44	S6	5	2.8
Total		71	40.3

types/PFGE types are represented in Table 3. Pen2/S1 was also the most common type among isolates from carcass samples comprising isolates from four carcasses. Pen2/S9, Pen2/S34, Pen4-complex/S2, and Pen1,44/S26 were all detected in two carcasses.

Antimicrobial susceptibility of *C. jejuni*. Of the 187 *C. jejuni* isolates that were examined for antimicrobial susceptibility, 16 (9%) were resistant to at least one of the antimicrobials tested (Table 4). Resistance to nalidixic acid was most commonly detected. Six of the 11 nalidixic acid-resistant isolates were also resistant to enrofloxacin. No multiresistance was observed among the isolates.

DISCUSSION

The prevalence of *Campylobacter* spp. in Finnish cattle at slaughter varied monthly between 18.8% and 44.1% during this 1-year study. In several studies performed in other countries prevalences of between 7% and 100% at slaughter have been reported (2, 5, 14, 37, 42, 49). Due to the different study designs regarding various sampling methods and materials, detection methods, etc., the results are not always comparable. The sampling for our survey was carried out in 12 out of 15 Finnish slaughterhouses, which covered 98% of the cattle slaughtered in Finland in 2003. The prevalence of campylobacters in cattle was 4.4% by direct culture and 37.3% by enrichment from the same 730 rectal fecal samples, which suggests that the overall level of campylobacters in the intestinal contents of cattle in Finland was low. This result is in accordance with the reported average most probable number values between 69/g and 6.1×10^2/g in the fecal samples of dairy cattle from other studies (36, 49). Higher numbers of cells have been obtained using real-time PCR for the quantification of campylobacters (25).

The predominance of *C. jejuni* over other *Campylobacter* species has been reported in cattle by Nielsen et al. (37) and by Açik and Çetinkaya (2) and many other studies, whereas *C. hyointestinalis* was the species that was most frequently isolated from cattle at slaughter in the surveys by Grau (17) and Pezzotti et al. (45). In the present study, *C. jejuni* was the most common species in young animals, while in the older age group *C. hyointestinalis* subsp. *hyointestinalis* was most frequently detected. A similar distribution of the *Campylobacter* species among young and adult cattle was reported by Giacoboni et al. (15). In addition to the age of the animals, the choice of method can also influence the diversity of the *Campylobacter* species detected from the samples. In our study, no *C. coli* or *C. hyointestinalis* subsp. *hyointestinalis* isolates were obtained by direct culture. The actual prevalence of *C. hyointestinalis* subsp. *hyointestinalis* in Finnish cattle is probably even higher than observed in this study, where the culture medium and growth conditions were optimized for the selection of the thermophilic *Campylobacter* species. These cultivation methods also exclude more fastidious species like *Campylobacter lanienae*, which proved to be the most prevalent *Campylobacter* species in beef cattle in the studies by Inglis et al. (24, 25), who employed PCR methods for detection.

Significant seasonal variation in the numbers of thermophilic campylobacters in dairy cattle herds but not in beef cattle has been reported by Stanley et al. (49). No evidence of the influence of climatic factors was observed, and the authors suggested that increased fecal excretion of campylobacters was due to hormonal factors or changes in the water supply and diet. In our study the overall patterns of monthly distribution of campylobacters in beef and dairy cattle were similar (data not shown). *C. jejuni* and *C. hyointestinalis* subsp. *hyointestinalis*, however, showed slightly different monthly patterns. The increasing prevalence of *C. jejuni* towards the end of the summer, although not significant, may reflect the continuous challenge during the grazing period (June to September) originating from environmental sources such as drinking water (20, 23) in contrast to the winter period, when the cattle are kept inside in Finland and given tap water to drink. No obvious reason could be found for *C. hyointestinalis* subsp. *hyointestinalis* reaching its highest level in November. In the Nordic countries, a seasonal peak in reported human campylobacter infections as

TABLE 4. Distribution of MICs among *C. jejuni* isolates

Antimicrobial	% Resistant isolates (95% CI[a])	Breakpoint for resistance (mg/liter)	Range of dilutions tested (mg/liter)	% of isolates with MIC[b] (mg/liter):													
				≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
Ampicillin	1.6 (0.3–4.6)	>16	0.5–64					5.9	7.0	41.2	40.1	3.2	1.1	0.5	1.1		
Enrofloxacin	3.2 (1.2–6.9)	>0.5	0.03–4	1.1	8.0	49.2	33.7	4.8	1.1	0.0	1.6	0.5[c]					
Erythromycin	0 (0.0–2.0)	>8	0.12–16			1.1	1.6	22.5	51.9	20.9	2.1						
Gentamicin	0 (0.0–2.0)	>4	0.25–8				3.2	54.0	42.2	0.5							
Nalidixic acid	5.9 (3.0–10.3)	>16	1–128							1.6	15.5	61.0	16.0	3.7	0.0	0.5	1.6[c]
Tetracycline	1.1 (0.1–3.8)	>2	0.25–32				92.0	5.9	0.5	0.5	0.5	0.5					

[a] CI, confidence interval.
[b] MICs equal to or lower than the lowest concentration tested are given as the lowest concentration.
[c] MIC greater than the highest concentration in the range of dilutions tested.

well as in the number of campylobacter-positive broiler flocks has consistently been observed in late summer (22, 35a, 40).

The proportion of campylobacter-positive cattle farms was low, on average 34%, in the present study. In a Danish study *C. jejuni* was present on 83% of dairy farms (36). The low number of samples per farm may explain the low percentage of positive farms in our study. Beef cattle were more frequently colonized by campylobacters than dairy cattle. A similar observation was reported by Beach et al. (5) and Grau (17), who suggested that the diet and high animal density of lot-fed cattle encouraged the intestinal colonization and spread of campylobacters. The variation in the colonization of beef and dairy cattle observed in our study may, however, reflect the age of the animals rather than the type of the herd, because most of the beef cattle were slaughtered at the age of 1 to 2 years, whereas most dairy cattle were slaughtered between the ages of 3 and 7 years. A higher prevalence of campylobacters in young animals has been observed in other studies (17, 36).

The predominant Penner serotypes of *C. jejuni* observed in this study (Pen2, Pen4-complex, Pen1,44, and Pen12) were also common in the human infections originating in Finland during the period July to September 1999 (53), but the most prevalent serotype in the human cases originating in Finland, Pen6,7, was rarely observed in cattle. The Pen4-complex and Pen12 serotypes have also been reported in Finnish poultry, although Pen6,7 was predominant (44). The percentage of fecal samples that yielded untypeable isolates was 12.5%, which is in accordance with the results from other studies, where commercial antisera from the same manufacturer were used for serotyping (44, 46). In our study, Pen2 was most frequently isolated from cattle in June (data not shown), whereas Vierikko et al. (53) reported a peak in the occurrence of the same serotype in humans in Finland in August. It would be interesting to find out whether these two peaks really do follow each other, suggesting that cattle may play a role in human infections. These data, however, originate from different years, and the annual variation cannot be excluded. The second most prevalent serotype from bovine samples, Pen4-complex, showed a different seasonal pattern with a peak in September regarding cattle, but it was at its highest in humans in August (53). These two serotypes were also the most commonly detected in dairy cattle in other studies (9, 27, 36, 37), which suggests that they may be particularly adapted to colonizing the bovine gut. Cocolonization by two serotypes in 8% of animals was reported by Nielsen (36). In the present study concurrent colonization by two *C. jejuni* serotypes was observed in 39% of animals from which two or more isolates were serotyped, assuming that the untypeable isolates represent different serotypes from the identified serotypes in the same sample.

Genotyping by PFGE revealed a high degree of diversity among the bovine *C. jejuni* isolates. This has been seen in other studies with other typing methods as well (2, 6, 48) and also in regard to *C. jejuni* isolates from chickens, sheep, turkeys, water, and human cases (9, 13, 38). A wide variation of SmaI subtypes could be observed among the isolates representing the most common serotype, Pen2. This has been found previously in the Danish study by Nielsen (36). Genomic instability, which enables the adaptation of the organism to variable environmental conditions (19, 54), has been given as the explanation for the diversity. However, significant genomic stability and clonal lineages of certain *C. jejuni* serotypes from a variety of hosts and geographic areas have been reported (29, 31). Despite the small number of isolates from each sample, more than one SmaI subtype was identified from 12% of the fecal samples from which multiple isolates were genotyped. The presence of unrelated subtypes in the samples suggests that there may have been several sources of campylobacters on the farm.

Although the sampling was planned only for investigating the situation at slaughter, the tracing of the animals to their farm also made some considerations possible at the farm level as well. When more than one animal was sampled at a time per farm, most commonly undistinguished or closely related subtypes were isolated from *C. jejuni*-positive samples. The coexistence of two or three unrelated *C. jejuni* subtypes or different *Campylobacter* species in the samples from a farm was observed in few cases. These observations might suggest animal-to-animal transmission or one or a small number of common sources of contamination (6, 36). Closely related isolates were rarely detected on a farm, which may reflect either the genetic instability of the strains or the temporary colonization of the animals. An indication of the latter may also be the detection of campylobacter-positive and -negative samples from the same farms at the same sampling. The observation that only a portion of the animals are simultaneously colonized is possibly due to the intermittent excretion of campylobacters or low numbers of campylobacters in the samples (36).

Campylobacters were not detected in almost half the cases when animals from the same farms were sampled twice. When this and the low prevalence of campylobacters in cattle at slaughter in this study are taken into consideration, it may be possible that cattle farms which are always campylobacter negative do exist. On the other hand, it may also reflect low numbers of campylobacters in the fecal samples.

Campylobacter contamination rates of 0 to 25% of carcasses before chilling and 3% after chilling have been reported in other studies (5, 17, 34). Due to the sensitivity of campylobacters to oxygen and drying, air chilling reduces the contamination of the carcasses (16, 17, 43). In the present survey the contamination level of carcasses was low (3.5%) before chilling, which may reflect the low number of campylobacters in cattle feces but probably indicates good slaughter hygiene as well and suggests that contamination of beef at the retail level is very low. Obviously, during the slaughter process cross-contamination can originate from the feces of the same animal or different animals through the slaughterhouse environment or equipment. The *C. jejuni* serotypes most frequently isolated from carcasses were the same as those isolated from the feces. Comparison of the PFGE subtypes from fecal and carcass samples revealed, however, that some subtypes commonly detected in fecal samples were not isolated from carcasses. This may indicate variation between subtypes regarding tolerance to oxygen and drying. One of the most common subtypes in carcass samples was not, however, isolated in feces. It may be possible that subtypes exist which are poor competitors in the intestines but can survive in the conditions on the surface of the carcass.

The overall prevalence of antimicrobial resistance among bovine fecal *C. jejuni* isolates was low. A small proportion of *C. jejuni* isolates were resistant to ampicillin, tetracycline, and

enrofloxacin. Aminopenicillins, fluoroquinolones, and tetracyclines are used in the treatment of bovine infectious diseases in Finland. No resistance to erythromycin was detected, although macrolides are used in the treatment of bovine infections. Resistance to nalidixic acid was almost twice as common as resistance to enrofloxacin. Similar findings on the resistance of bovine campylobacters to quinolones have been described by Aarestrup et al. (1) and Englen et al. (11). Comparison with resistance data from other countries is complicated by variations in the methodologies and breakpoints that are used to classify the isolates as resistant. Breakpoints recommended for *Enterobacteriaceae* by CLSI (formerly NCCLS) have usually been applied in previous studies, as no internationally agreed clinical or epidemiological breakpoints for antimicrobial resistance of campylobacters have been available. In a recent publication by CLSI (8) criteria are presented for erythromycin (≥32 μg/ml), ciprofloxacin (≥4 μg/ml), and tetracycline (≥16 μg/ml). Interpretation of MICs according to these criteria would have yielded less than 5% total resistance among the bovine *C. jejuni* isolates in the present study, which is substantially lower than that reported from other European countries and the United States (1, 4, 7, 11).

In conclusion, the prevalence of campylobacters in Finnish cattle at slaughter was low and carcass contamination was rare in this survey, indicating that Finnish beef can be considered as a minor source of campylobacters for consumers. The antimicrobial resistance level among bovine *C. jejuni* isolates was also low, and multiresistance was not detected, which may be explained by the prudent use of antimicrobial agents for animals. However, the common occurrence of serotypes Pen2 and Pen4-complex in cattle indicates that there may be an indirect association with human infections.

ACKNOWLEDGMENTS

This work has been supported by the Walter Ehrström Foundation and the Finnish Veterinary Science Foundation.

We thank Kirsi Eklund, Maaret Hyppönen, Mira Kankare, Lea Nygård, and Kaija Pajunen for their excellent technical assistance and Leila Rantala for assistance in interpreting the PFGE profiles.

REFERENCES

1. **Aarestrup, F. M., E. M. Nielsen, M. Madsen, and J. Engberg.** 1997. Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from humans, pigs, cattle, and broilers in Denmark. Antimicrob. Agents Chemother. **41:**2244–2250.
2. **Açik, M. N., and B. Çetinkaya.** 2005. The heterogeneity of *Campylobacter jejuni* and *Campylobacter coli* strains isolated from healthy cattle. Lett. Appl. Microbiol. **41:**397–403.
3. **Anderson, S. A., R. W. Yeaton Woo, and L. M. Crawford.** 2001. Risk assessment of the impact on human health of resistant *Campylobacter jejuni* from fluoroquinolone use in beef cattle. Food Control **12:**13–25.
4. **Bae, W., K. N. Kaya, D. D. Hancock, D. R. Call, Y. H. Park, and T. E. Besser.** 2005. Prevalence and antimicrobial resistance of thermophilic *Campylobacter* spp. from cattle farms in Washington State. Appl. Environ. Microbiol. **71:** 169–174.
5. **Beach, J. C., E. A. Murano, and G. R. Acuff.** 2002. Prevalence of *Salmonella* and *Campylobacter* in beef cattle from transport to slaughter. J. Food Prot. **65:**1687–1693.
6. **Besser, T. E., J. T. LeJeune, D. H. Rice, J. Berg, R. P. Stilborn, K. Kaya, W. Bae, and D. D. Hancock.** 2005. Increasing prevalence of *Campylobacter jejuni* in feedlot cattle through the feeding period. Appl. Environ. Microbiol. **71:** 5752–5758.
7. **Bywater, R., H. Deluyker, E. Deroover, A. de Jong, H. Marion, M. McConville, T. Rowan, T. Shryock, D. Shuster, V. Thomas, M. Vallé, and J. Walters.** 2004. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. J. Antimicrob. Chemother. **54:**744–754.
8. **Clinical and Laboratory Standards Institute.** 2006. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline M45-A. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **Devane, M. L., C. Nicol, A. Ball, J. D. Klena, P. Scholes, J. A. Hudson, M. G. Baker, B. J. Gilpin, N. Garret, and M. G. Savill.** 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. J. Appl. Microbiol. **98:**980–990.
10. **Endtz, H. P., G. J. Ruijs, B. van Klingeren, W. H. Jansen, T. van der Reyden, and R. P. Mouton.** 1991. Quinolone resistance in campylobacter isolates from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J. Antimicrob. Chemother. **27:**199–208.
11. **Englen, M. D., P. J. Fedorka-Cray, S. R. Ladely, and D. A. Dargatz.** 2005. Antimicrobial resistance patterns of *Campylobacter* from feedlot cattle. J. Appl. Microbiol. **99:**285–291.
12. **Evans, M. R., R. J. Roberts, C. D. Ribeiro, D. Gardner, and D. Kembrey.** 1996. A milk-borne campylobacter outbreak following an educational farm visit. Epidemiol. Infect. **117:**457–462.
13. **Fitzgerald, C., K. Stanley, S. Andrew, and K. Jones.** 2001. Use of pulsed-field gel electrophoresis and flagellin gene typing in identifying clonal groups of *Campylobacter jejuni* and *Campylobacter coli* in farm and clinical environments. Appl. Environ. Microbiol. **67:**1429–1436.
14. **Garcia, M. M., H. Lior, R. B. Stewart, G. M. Ruckerbauer, J. R. R. Trudel, and A. Skljarevski.** 1985. Isolation, characterization, and serotyping of *Campylobacter jejuni* and *Campylobacter coli* from slaughter cattle. Appl. Environ. Microbiol. **49:**667–672.
15. **Giacoboni, G. I., K. Itoh, K. Hirayama, E. Takahashi, and T. Mitsuoka.** 1993. Comparison of faecal *Campylobacter* in calves and cattle of different ages and areas in Japan. J. Vet. Med. Sci. **55:**555–559.
16. **Gill, C. O., and L. M. Harris.** 1982. Contamination of red-meat carcasses by *Campylobacter fetus* subsp. *jejuni*. Appl. Environ. Microbiol. **43:**977–980.
17. **Grau, F. H.** 1988. *Campylobacter jejuni* and *Campylobacter hyointestinalis* in the intestinal tract and on the carcasses of calves and cattle. J. Food Prot. **51:**857–861.
18. **Gupta, A., J. M. Nelson, T. J. Barrett, R. V. Tauxe, S. P. Rossiter, C. R. Friedman, K. W. Joyce, K. E. Smith, T. F. Jones, M. A. Hawkins, B. Shiferaw, J. L. Beebe, D. J. Vugia, T. Rabatsky-Ehr, J. A. Benson, T. P. Root, and J. Angulo.** 2004. Antimicrobial resistance among *Campylobacter* strains, United States, 1997–2001. Emerg. Infect. Dis. **10:**1102–1109.
19. **Hänninen, M.-L., M. Hakkinen, and H. Rautelin.** 1999. Stability of related human and chicken *Campylobacter jejuni* genotypes after passage through chick intestine studied by pulsed-field gel electrophoresis. Appl. Environ. Microbiol. **65:**2272–2275.
20. **Hänninen, M.-L., M. Niskanen, and L. Korhonen.** 1998. Water as a reservoir for *Campylobacter jejuni* infection in cows studied by serotyping and pulsed-field gel electrophoresis (PFGE). J. Vet. Med. B **45:**37–42.
21. **Hänninen, M.-L., P. Perko-Mäkelä, A. Pitkälä, and H. Rautelin.** 2000. A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. J. Clin. Microbiol. **38:**1998–2000.
22. **Hofshagen, M., and H. Kruse.** 2005. Reduction in flock prevalence of *Campylobacter* spp. in broilers in Norway after implementation of an action plan. J. Food Prot. **68:**2220–2223.
23. **Humphrey, T. J., and B. Beckett.** 1987. *Campylobacter jejuni* in dairy cows and raw milk. Epidemiol. Infect. **98:**263–269.
24. **Inglis, G. D., L. D. Kalischuk, and H. W. Busz.** 2003. A survey of *Campylobacter* species shed in faeces of beef cattle using polymerase chain reaction. Can. J. Microbiol. **49:**655–661.
25. **Inglis, G. D., L. D. Kalischuk, and H. W. Busz.** 2004. Chronic shedding of *Campylobacter* species in beef cattle. J. Appl. Microbiol. **97:**410–420.
26. **International Organization for Standardization.** 2006. Microbiology of food and animal feeding stuffs—horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: detection method. International Organization for Standardization, Geneva, Switzerland.
27. **Ishihara, K., T. Yamamoto, S. Satake, S. Takayama, S. Kubota, H. Negishi, A. Kojima, T. Asai, T. Sawada, T. Takahashi, and Y. Tamura.** 2006. Comparison of *Campylobacter* isolated from humans and food-producing animals in Japan. J. Appl. Microbiol. **100:**153–160.
28. **Kärenlampi, R., H. Rautelin, M. Hakkinen, and M.-L. Hänninen.** 2003. Temporal and geographical distribution and overlap of Penner heat-stable serotypes and pulsed-field electrophoresis genotypes of *Campylobacter jejuni* isolates collected from humans and chickens in Finland during a seasonal peak. J. Clin. Microbiol. **41:**4870–4872.
29. **Laturnus, C., J. Jores, I. Moser, P. Schwerk, and L. H. Wieler.** 2005. Long-term clonal lineages within *Campylobacter jejuni* O:2 strains from different geographical regions and hosts. Int. J. Med. Microbiol. **294:**521–524.
30. **Lehner, A., C. Schneck, G. Feierl, P. Pless, A. Deutz, E. Brandl, and M. Wagner.** 2000. Epidemiologic application of pulsed-field gel electrophoresis to an outbreak of *Campylobacter jejuni* in an Austrian youth centre. Epidemiol. Infect. **125:**13–16.
31. **Manning, G., B. Duim, T. Wassenaar, J. A. Wagenaar, A. Ridley, and D. G. Newell.** 2001. Evidence for a genetically stable strain of *Campylobacter jejuni*. Appl. Environ. Microbiol. **67:**1185–1189.

32. **Meldrum, R. J., J. K. Griffiths, R. M. M. Smith, and M. R. Evans.** 2005. The seasonality of human campylobacter infection and *Campylobacter* isolates from fresh, retail chicken in Wales. Epidemiol. Infect. **133:**49–52.
33. **Michaud, S., S. Ménard, and R. D. Arbeit.** 2004. Campylobacteriosis, Eastern Townships, Québec. Emerg. Infect. Dis. **10:**1844–1847.
34. **Minihan, D., P. Whyte, M. O'Mahony, S. Fanning, K. McGill, and J. D. Collins.** 2004. *Campylobacter* spp. in Irish feedlot cattle: a longitudinal study involving pre-harvest and harvest phases of the food chain. J. Vet. Med. B **51:**28–33.
35. **National Public Health Institute.** 2005. Infectious diseases in Finland 1995–2004. National Public Health Institute, Helsinki, Finland.

35a. **National Veterinary Institute.** 2004. Trends and sources of zoonoses and zoonotic agents in humans, foodstuffs, animals and feedingstuffs including information on foodborne outbreaks and antimicrobial resistance in zoonotic agents in 2004. National Veterinary Institute, Uppsala, Sweden. http://www.sva.se/pdf/zoonosis/zoonosrapport_Sverige_2004.pdf.

36. **Nielsen, E. M.** 2002. Occurrence and strain diversity of themophilic campylobacters in cattle of different age groups in dairy herds. Lett. Appl. Microbiol. **35:**85–89.
37. **Nielsen, E. M., J. Engberg, and M. Madsen.** 1997. Distribution of serotypes of *Campylobacter jejuni* and *C. coli* from Danish patients, poultry and swine. FEMS Immunol. Med. Microbiol. **19:**47–56.
38. **Nielsen, E. M., V. Fussing, J. Engberg, N. L. Nielsen, and J. Neimann.** 2006. Most *Campylobacter* subtypes from sporadic infections can be found in retail poultry products and food animals. Epidemiol. Infect. **134:**758–767.
39. **Nygård, K., Y. Andersson, J. A. Røttingen, Å. Svensson, J. Lindbäck, T. Kistemann, and J. Giesecke.** 2004. Association between environmental risk factors and campylobacter infections in Sweden. Epidemiol. Infect. **132:**317–325.
40. **Nylen, G., F. Dunstan, S. R. Palmer, Y. Andersson, F. Bager, J. Cowden, G. Feierl, Y. Galloway, G. Kapperud, F. Megraud, K. Molbak, L. R. Petersen, and P. Ruutu.** 2002. The seasonal distribution of campylobacter infection in nine European countries and New Zealand. Epidemiol. Infect. **128:**383–390.
41. **Olah, P., J. S. Sherwood, L. M. Elijah, M. R. Dockter, C. Doetkott, Z. Miller, and C. M. Logue.** 2004. Comparison of antimicrobial resistance in *Salmonella* and *Campylobacter* isolated from turkeys in the Midwest USA. Food Microbiol. **21:**779–789.
42. **Ono, K., and K. Yamamoto.** 1999. Contamination of meat with *Campylobacter jejuni* in Saitama. Int. J. Food Microbiol. **47:**211–219.
43. **Oosterom, J., G. J. A. de Wilde, E. de Boer, L. H. de Blaauw, and H. Karman.** 1983. Survival of *Campylobacter* during poultry processing and pig slaughtering. J. Food Prot. **46:**702–706.
44. **Perko-Mäkelä, P., M. Hakkinen, T. Honkanen-Buzalski, and M.-L. Hänninen.** 2002. Prevalence of campylobacters in chicken flocks during the summer of 1999 in Finland. Epidemiol. Infect. **129:**187–192.
45. **Pezzotti, G., A. Serafin, I. Luzzi, R. Mioni, M. Milan, and R. Perin.** 2003. Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. Int. J. Food Microbiol. **82:**281–287.
46. **Rautelin, H., and M.-L. Hänninen.** 1999. Comparison of a commercial test for serotyping heat-stable antigens of *Campylobacter jejuni* with genotyping by pulsed-field gel electrophoresis. J. Med. Microbiol. **48:**617–621.
47. **Schildt, M., S. Savolainen, and M.-L. Hänninen.** 2006. Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family. Epidemiol. Infect. **134:**401–405.
48. **Siemer, B. L., C. S. Harrington, E. M. Nielsen, B. Borck, N. L. Nielsen, J. Engberg, and S. L. W. On.** 2004. Genetic relatedness among *Campylobacter jejuni* serotyped isolates of diverse origin as determined by numerical analysis of amplified fragment length polymorphism (AFLP) profiles. J. Appl. Microbiol. **96:**795–802.
49. **Stanley, K. N., J. S. Wallace, J. E. Currie, P. J. Diggle, and K. Jones.** 1998. The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. J. Appl. Microbiol. **85:**472–480.
50. **Stern, N. J., M. P. Hernandez, L. Blankenship, K. E. Deibel, S. Doores, M. P. Doyle, H. Ng, M. D. Pierson, J. N. Sofos, W. H. Sveum, and D. C. Westhoff.** 1985. Prevalence and distribution of *Campylobacter jejuni* and *Campylobacter coli* in retail meats. J. Food Prot. **48:**595–599.
51. **Studahl, A., and Y. Andersson.** 2000. Risk factors for indigenous campylobacter infection: a Swedish case control study. Epidemiol. Infect. **125:**269–275.
52. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
53. **Vierikko, A., M.-L. Hänninen, A. Siitonen, P. Ruutu, and H. Rautelin.** 2004. Domestically acquired *Campylobacter* infections in Finland. Emerg. Infect. Dis. **10:**127–130.
54. **Wassenaar, T. M., B. Geilhausen, and D. G. Newell.** 1998. Evidence of genomic instability in *Campylobacter jejuni* isolated from poultry. Appl. Environ. Microbiol. **64:**1816–1821.
55. **Whyte, P., K. McGill, D. Cowley, R. H. Madden, L. Moran, P. Scates, C. Carroll, A. O'Leary, S. Fanning, J. D. Collins, E. McNamara, J. E. Moore, and M. Cormican.** 2004. Occurrence of *Campylobacter* in retail foods in Ireland. Int. J. Food Microbiol. **95:**111–118.

55a. **World Health Organization.** 2001. The increasing incidence of human campylobacteriosis. Report and proceedings of a WHO consultation of experts. Copenhagen, Denmark, 21 to 25 November 2000. World Health Organization, Geneva, Switzerland. http://whqlibdoc.who.int/hq/2001/who_cds_csr_aph_2001.7.pdf.

II

Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Shedding of *Campylobacter* spp. in Finnish cattle on dairy farms

M. Hakkinen[1] and M.-L. Hänninen[2]

1 Research Department, Finnish Food Safety Authority, Helsinki, Finland
2 Veterinary Faculty, Department of Food and Environmental Hygiene, University of Helsinki, Helsinki, Finland

Keywords
Arcobacter, Campylobacter jejuni, dairy cattle, PFGE.

Correspondence
Marjaana Hakkinen, Research Department, Finnish Food Safety Authority, Mustialankatu 3, FI 00790, Finland.
E-mail: marjaana.hakkinen@evira.fi

2008/1681: received 1 October 2008, revised 2 January 2009 and accepted 21 January 2009

doi:10.1111/j.1365-2672.2009.04269.x

Abstract

Aims: The aim of this study was to determine variation of prevalence throughout a year, colonization levels and genotypes of *Campylobacter jejuni* in Finnish dairy cattle herds.
Methods and Results: Faecal samples and tank milk samples from three dairy cattle herds were taken five times, and swab samples from drinking troughs once during a 1-year sampling period. The samples were enriched in Bolton broth and subsequently spread on mCCDA. Isolates were then subtyped by pulsed-field gel electrophoresis using SmaI. *Campylobacter jejuni* was detected in 169 of the 340 faecal samples and in one drinking trough sample. Prevalences between herds and sampling times varied widely. The faecal levels of *C. jejuni* were mainly low. Between one and four SmaI subtypes were identified from each herd per sampling. Two SmaI subtypes persisted in two of the herds throughout the study.
Conclusions: Dairy cattle can be a long-term reservoir of *C. jejuni* subtypes similar to clinical isolates. Differences in the colonization potential among *C. jejuni* strains as well as in the resistance to campylobacter colonization among animals are possible.
Significance and Impact of the Study: The study provides data on contamination dynamics, colonization levels and the persistence of *C. jejuni* in dairy cattle.

Introduction

The number of reported cases of campylobacteriosis, the most common reported bacterial enteric infection in humans, has steadily increased in most countries. Several studies have identified poultry meat as the most important food item associated with sporadic cases of campylobacter infection (Studahl and Andersson 2000; Neimann *et al.* 2003; Wingstrand *et al.* 2006; Gormley *et al.* 2008). This rising trend in human campylobacteriosis is also evident in the Nordic countries, where the prevalence of campylobacters in poultry flocks is low (Anon. 2007a), thus suggesting other possible sources of these organisms.

Among other food production animals, cattle are identified as common carriers of *Campylobacter jejuni* (Besser *et al.* 2005; Devane *et al.* 2005; Kwan *et al.* 2008), but the occurrences of campylobacters on cattle carcasses and in beef are low (Minihan *et al.* 2004; Whyte *et al.* 2004; Hakkinen *et al.* 2007). Instead, unpasteurized milk has emerged as a risk factor for human campylobacteriosis in epidemiological studies (Studahl and Andersson 2000; Neimann *et al.* 2003) and has caused numerous outbreaks (Evans *et al.* 1996; Lehner *et al.* 2000; Schildt *et al.* 2005). In addition, indirect exposure to cattle faeces through environmental contamination is considered a high risk to humans (Minihan *et al.* 2004; Devane *et al.* 2005; Garrett *et al.* 2007). In a wide water-borne outbreak in Canada, one cattle farm was implicated in the contamination by *C. jejuni* of the municipal drinking water supply (Clark *et al.* 2003).

Longitudinal studies on the persistence of campylobacter colonization among beef cattle have been performed

by, e.g. Minihan *et al.* (2004), Besser *et al.* (2005) and Kwan *et al.* (2008). Stanley *et al.* (1998) carried out a study on seasonal fluctuation in the prevalence and numbers of campylobacters in cattle, including dairy herds. There are also other interesting issues concerning dairy herds because of their longer life span than that of beef cattle. If permanent colonization of dairy cattle by human pathogenic campylobacter genotypes occur, it can maintain the environmental load of pathogenic strains. Few data are available on the persistence of different *C. jejuni* genotypes in dairy herds.

Our study aimed to obtain data on fluctuation in intestinal colonization throughout a year, colonization levels and genotypes of *C. jejuni* in Finnish dairy cattle herds.

Materials and methods

Sampling

Three dairy cattle herds located 60 km apart from each other in Southern Finland were included in the study. *Campylobacter jejuni* was previously detected in pooled faecal samples from each of the herds. The number of animals in herds 1, 2 and 3 was 15, 20 and 90, respectively. Between 17 and 33 samples of fresh, newly avoided faeces per herd were collected from the floor on five sampling occasions during the study: (i) after the grazing period in November 2006, (ii) in the middle of the winter housing period in January–February 2007, (iii) before the new grazing period in April 2007, (iv) during the grazing period in August 2007 and (v) after the grazing period in November 2007. Animals recently treated with antimicrobials were excluded from the sampling. When possible, the individual identification codes of animals were included in the sampling data. Tank milk samples (1000 ml) were also taken on each sampling occasion. In addition, sponge swab (Medical Wire & Equipment, Corsham, Wiltshire, UK) samples from drinking troughs were taken during the last sampling in November 2007. The samples were chilled and transported to the laboratory, and the analyses began later on the same day. The faecal and drinking trough samples were analysed at the Finnish Food Safety Authority Evira, Research Department, Microbiology Unit, and the milk samples were examined at Helsinki University, Veterinary Faculty, Department of Food and Environmental Hygiene.

Isolation, semiquantitative detection and the identification of campylobacters in faecal and sponge swab samples

All the samples were analysed individually. To detect campylobacters, 10 g of faeces were enriched in 90 ml of Bolton broth [Campylobacter Enrichment Broth, Lab 135 + selective supplement X131 (LAB M, Bury, England) + lysed horse blood]. In addition, a 10-fold dilution series up to 10^{-6} was made using 9-ml tubes of Bolton broth for the semiquantitative detection of campylobacters (Anon. 2007b). Sponge swab samples were enriched in 225 ml of Bolton broth. The enrichment cultures were incubated for 24 h at 41·5°C and cultured onto modified charcoal cefoperazone deoxycholate agar [Campylobacter Blood Free Selective Medium Lab 112 + selective supplement X112 (LAB M)] as described in Hakkinen *et al.* (2007).

When possible, a minimum of five typical colonies was isolated per sample, mostly from the highest dilution where growth was observed, but also from lower dilutions, if they contained separate colonies, and especially when colony morphology varied. Isolates were identified at the species level according to ISO 10272-1 (Anon. 2006). To identify *C. hyointestinalis* ssp. *hyointestinalis* strains among hippurate-negative and indoxyl acetate-hydrolysing isolates, H_2S production in TSI agar (LAB M) (pH 8) and urease production were examined. The isolates were stored in Brucella broth supplemented with 15% glycerol at −70°C.

Enumeration of campylobacters in milk samples

The most probable number (MPN) technique was used to enumerate campylobacters in milk samples. Either 10 × 100 ml (November–February) or 10 × 20 ml (April–December) of raw milk was enriched in Bolton broth (100 ml milk + 500 ml Bolton broth or 20 ml milk + 80 ml of Bolton broth). The enrichment cultures were incubated microaerobically at 37°C for 48 h and plated on mCCDA plates which were incubated microaerobically at 37°C for 48 h. Isolates were identified according to ISO 10272-1 (Anon. 2006).

Genotyping by pulsed-field gel electrophoresis (PFGE)

A minimum of two *C. jejuni* isolates per sample were analysed with PFGE using SmaI for the restriction enzyme as described previously by Hakkinen *et al.* (2007). PFGE data were analysed with Bionumerics ver. 5.10 (Applied Maths, Kortrijk, Belgium), with 0·5% optimization and 1·0% tolerance.

Results

In total, *C. jejuni* was detected in 169 of the 340 faecal samples and in one of the sponge swab samples from the drinking troughs. No campylobacters were detected in the milk samples. *Campylobacter coli* and *C. hyointestinalis*

ssp. *hyointestinalis* were detected in 11 (3·2%) and 52 (15·3%) of the faecal samples, respectively. The average prevalence of *C. jejuni* throughout the study was 44%, and the average monthly prevalences were 64%, 43%, 37%, 38% and 45% in November 2006, February 2007, April 2007, August 2007 and November 2007, respectively. In herds 1 and 2, the prevalence of *C. jejuni* was highest in November 2006 and decreased in the winter, whereas in herd 3, the prevalence of *C. jejuni* was high (82–90%) in November 2006, January and April 2007 and only slightly lower in August (Fig. 1). *Campylobacter hyointestinalis* ssp. *hyointestinalis* was detected in samples from herds 2 and 3. In addition, catalase- and urease-negative, H_2S-producing *Campylobacter* sp. was detected in herd 1 throughout the sampling period. Concurrent colonization by *C. jejuni* and *C. hyointestinalis* ssp. *hyointestinalis* occurred in 11 samples from farm 3.

The levels of *C. jejuni* in the faecal samples were low in general (Table 1). In approx. 42% of the positive faecal samples, the highest dilution in which campylobacters were detected was 10^{-2}. Campylobacters were detected from the highest dilution in only four samples. In herd 3, where the prevalence was consistently higher than in the two other herds, high levels of *C. jejuni* were also detected on all sampling occasions except in August 2007. In the animals of herds 1 and 2, high levels were observed only occasionally.

In total, 13 different SmaI subtypes were distinguished among faecal *C. jejuni* isolates (Fig. 2). One of the subtypes, S7, was detected in herds 1 and 2. One to four subtypes were detected from each of the herds on each sampling occasion (Table 2), except in August, when no *C. jejuni* was detected in herd 2. Two *C. jejuni* SmaI subtypes existed in herds 1 and 3 during the entire sampling period (Fig. 3). In August 2007, a few new subtypes emerged in both of the herds. In herd 2, only two *C. jejuni* subtypes occurred throughout the entire sampling period.

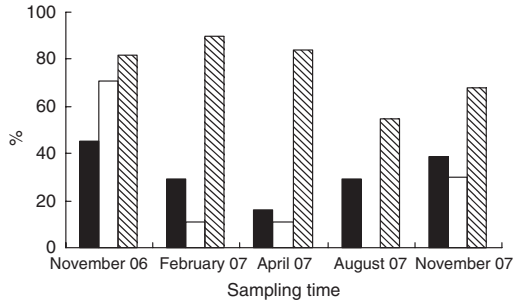

Figure 1 Prevalence of *Campylobacter jejuni* in three dairy cattle herds on different sampling occasions between November 2006 and November 2007. ■ Herd 1; □ Herd 2; and ▨ Herd 3.

From 150 of the positive samples, two isolates per sample were subtyped, and from 19 samples, four to six isolates were subtyped. Two different SmaI subtypes were detected in 3 of the 169 positive samples.

Ten animals from herd 1 were sampled on every sampling occasion. *Campylobacter jejuni* was detected in all the samples of one animal, whereas two of the animals were campylobacter-negative on all sampling occasions (Table 3). Three animals were campylobacter-positive only once: two of them at a low level and one at a high level. One SmaI subtype was consistently isolated from each of the animals that yielded multiple positive samples, with the exception of a previous carrier of subtype S7,

Table 1 Contamination levels of *Campylobacter jejuni* in faecal samples from three dairy cattle herds on different sampling occasions between November 2006 and November 2007

Sample size in enrichment (g)	Number of *Campylobacter jejuni*-positive samples															
	Herd 1					Herd 2					Herd 3					
	Nov 2006	Feb 2007	Apr 2007	Aug 2007	Nov 2007	Nov 2006	Feb 2007	Apr 2007	Aug 2007	Nov 2007	Nov 2006	Feb 2007	Apr 2007	Aug 2007	Nov 2007	Total
10	3	0	0	1	2	3	1	1	0	2	8	3	8	2	8	42
10^{-2}	3	0	1	0	1	5	1	0	0	1	2	3	3	6	3	28
10^{-3}	2	3	1	3	3	1	0	0	0	0	8	3	9	6	3	42
10^{-4}	0	3	1	0	1	2	0	1	0	1	6	11	5	1	2	34
10^{-5}	0	0	0	1	0	1	0	0	0	2	3	9	1	0	1	18
10^{-6}	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	4
Total no. of positive samples	9	6	3	5	7	12	2	2	0	6	27	30	27	16	17	169
Total no. of samples	20	21	19	17	18	17	19	18	19	20	33	33	32	29	25	340

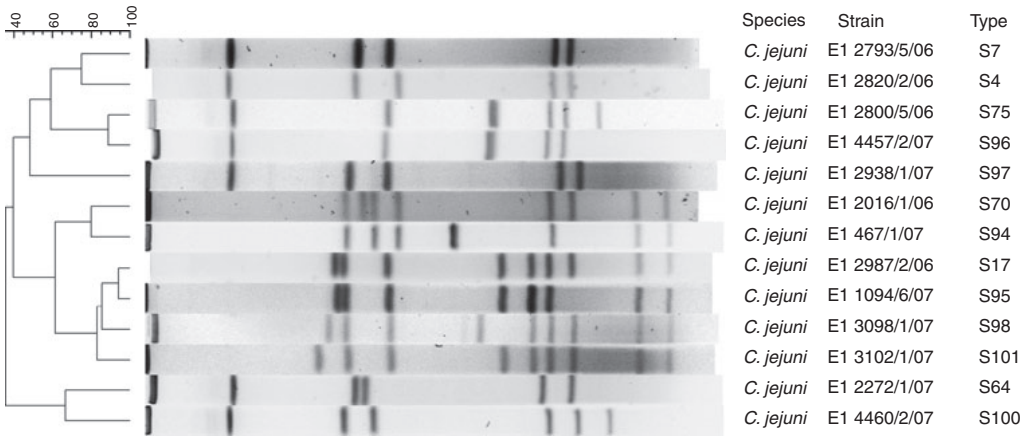

Figure 2 Different SmaI PFGE profiles identified among *Campylobacter jejuni* isolates from three dairy cattle herds during the study period from November 2006 to November 2007.

from which subtype S64 was isolated after two negative samples.

Campylobacter jejuni was isolated from the drinking trough at farm 3. The isolates represented the most commonly detected SmaI subtypes (S17 and S70) in that herd. No campylobacters were detected in the swab samples from drinking troughs at farms 1 and 2.

No campylobacters were isolated from the milk samples. *Arcobacter butzleri*, identified on the basis of aerobic growth at 25°C, 37°C and 41°C, susceptibility to nalidixic acid and resistance to cephalothin as well as the ability to hydrolyse indoxyl acetate, was detected at a low level in three samples from farm 3 (November 2006, April and May 2007) and in one milk sample from farm 1 (April 2007).

Discussion

In our study, the prevalence of *C. jejuni* varied widely between herds and sampling times. Atabay and Corry (1998), Hänninen *et al.* (1998), Wesley *et al.* (2000) and Nielsen (2002) have reported prevalences of *C. jejuni* from 7% to 38% in dairy herds, which are similar to the results from small herds 1 and 2 in our study. In herd 3 (the largest), the prevalence was high throughout the study and was similar to prevalences reported among beef cattle (Stanley *et al.* 1998; Besser *et al.* 2005). According to Wesley *et al.* (2000), large herd size may contribute to the transmission of infection because of the high number of susceptible hosts that may be continuously challenged by contact with carriers.

Table 2 Occurrence of SmaI PFGE subtypes of *Campylobacter jejuni* in three dairy cattle herds on different sampling occasions between November 2006 and November 2007

Sampling	Herd 1	Herd 2	Herd 3
Nov 2006	S7, S64, S75	S4, S7	S17, S70
Feb 2007	S7, S64, S75	S4	S17, S70, S94
Apr 2007	S7, S64	S4	S17, S70, S95
Aug 2007	S7, S64, S96, S97	–	S17, S70, S98, S101
Nov 2007	S7, S64, S96, S100	S7	S17, S70

In herds 1 and 2, the prevalence of *C. jejuni* decreased during the winter season, when the cattle were housed indoors, which is similar to the results reported by Hänninen *et al.* (1998). In contrast to their observation of an increase in prevalence during the grazing period, the overall prevalence in our study was lowest in August, and herd 2 was campylobacter-negative, although the animals had been grazing since May. Lake water was likely the origin of campylobacters in the study of Hänninen *et al.* (1998), and Humphrey and Beckett (1987) also suggested that natural waters were the main source of campylobacters in cattle. The herds in our study had no access to natural waters, which may explain the low prevalence in summer. Drinking water was obtained from the well in the farm (herds 1 and 2) or from a municipal source (herd 3). Despite the wide distribution of campylobacters among wild birds and other animals and the consequent contamination of the environment, the organism may not survive long enough to infect the grazing cattle, except in water, where prolonged survival of campylobacters has been reported (Cools *et al.* 2003). However, after the grazing period in November 2007, the

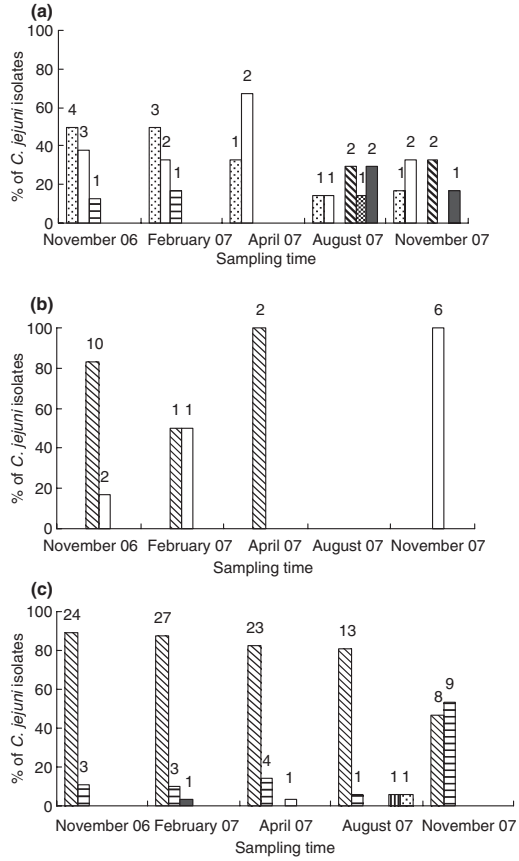

Figure 3 Percentages of *Campylobacter jejuni* SmaI subtypes in three dairy cattle herds on different sampling occasions between November 2006 and November 2007. Herd 1 (a): ▨ S7; □ S64; ▩ S75; ▧ S96; ▤ S97; ■ S100, Herd 2 (b): S4 ▧; □ S7, and Herd 3 (c) ▧ S17; ▤ S70; ■ S94; □ S95; ▥ S98; ▨ S101. The number of isolates representing each subtype is indicated on top of each column.

Table 3 Occurrence of *Campylobacter jejuni* SmaI subtypes in the individual animals of Herd 1 on different sampling occasions between November 2006 and November 2007

Animal no.	Sampling time				
	Nov 2006	Feb 2007	Apr 2007	Aug 2007	Nov 2007
55	Neg*	Neg	Neg	Neg	Neg
107	Neg	Neg	Neg	S100	S100
108	Neg	Neg	Neg	Neg	S96
109	Neg	Neg	Neg	Neg	ND†
124	S64	S64	S64	Neg	Neg
128	Neg	Neg	Neg	ND	S7
129	Neg	Neg	Neg	Neg	ND
130	ND	Neg	Neg	Neg	Neg
131	S64	S64	S64	S64	S64
134	S7	S7	S7	S7	Neg
136	S7	Neg	Neg	Neg	Neg
139	Neg	Neg	Neg	S97	Neg
140	S7	S7	Neg	Neg	S64
141	Neg	Neg	Neg	Neg	Neg

*Not detected.
†Not done.

prevalence was higher in all three herds than it was in August. This difference could reflect a change in the diet, as Stanley *et al.* (1998) suggested regarding seasonal peaks in the excretion of campylobacters coinciding with the beginning of the grazing period in spring and the transition to winter housing in autumn.

Contamination of milk can easily result from a lapse in hygiene, when a herd is campylobacter-positive (Humphrey and Beckett 1987; Schildt *et al.* 2005). The absence of campylobacters in the milk samples indicates that all the farms in the present study employed good milking hygiene and were able to prevent faecal contamination of the milk. Our detection of low-level contamination by *A. butzleri* on four occasions is unexceptional, as other studies focused specifically on *Arcobacter* have detected the organism in raw tank milk as well (Scullion *et al.* 2006). *Arcobacter butzleri* is an environmental contaminant not directly associated with faecal contamination. It has occasionally been isolated from human patients with diarrhoea, but its significance as a food-borne pathogen is under examination (Ho *et al.* 2006).

The prevalence of *C. hyointestinalis* ssp. *hyointestinalis*, a common inhabitant of cattle (Atabay and Corry 1998; Inglis *et al.* 2004; Hakkinen *et al.* 2007), is likely to be higher in the herds than we detected. As the examination focused on *C. jejuni* in this study, the detection method for thermophilic campylobacters was chosen. Atabay and Corry (1998) emphasized the significance of the choice of medium when different *Campylobacter* species are the organisms targeted for detection. *Campylobacter coli* was infrequently detected in our samples. It is a minor species in bovine intestines according to previous studies (Wesley *et al.* 2000; Inglis *et al.* 2004; Hakkinen *et al.* 2007). In most of the samples, only one *Campylobacter* species was detected at a time, as Atabay and Corry (1998) also reported. Catalase-negative *Campylobacter* sp. isolates from farm 1 were probably *C. sputorum* biovar *sputorum* (Vandamme and On 2001). Atabay and Corry (1998) isolated similar unidentified catalase- and urease-negative, H_2S-producing campylobacters from cattle faeces.

We determined *C. jejuni* levels in the faecal samples by using a semiquantitative method. Consequently, our results are not fully comparable to counts from other

studies. However, *C. jejuni* levels in the positive samples were generally low. In 13% of the samples, *C. jejuni* was detected in dilution 10^{-5} or higher, indicating levels that are closer to counts reported from beef cattle and calves (Stanley *et al.* 1998; Nielsen 2002; Inglis *et al.* 2004) than the average concentrations of $1{\cdot}2 \times 10^2$ CFU g^{-1} and 69 MPN g^{-1} in dairy cattle reported by Nielsen (2002) and Stanley *et al.* (1998), respectively. The *C. jejuni* levels in our study varied depending on the herd and sampling time. Stanley *et al.* (1998) observed a clear seasonality, with spring and autumn peaks in the number of thermophilic campylobacters in the faeces of dairy herds. As a result of the small number of herds and only a 1-year sampling period, such conclusions cannot be drawn from our study, where opposite trends in prevalences as well as in *C. jejuni* levels in positive animals occurred among the herds.

The detection of only few PFGE subtypes in each herd may reflect a small number of sources of *C. jejuni* and transmission of the organism between animals in the herd rather than the introduction of new types from various sources. Of the seven and six different SmaI subtypes of *C. jejuni* identified in herds 1 and 3, respectively, two subtypes persisted in each of the herds throughout the 1-year sampling period. In August and November 2007, new subtypes also emerged, possibly from the environment during grazing. Until the end of the study, however, these new subtypes were unable to exclude the original ones and seemed to be only temporarily excreted, as three of the four new types found in August were no longer detectable in November. The persistent subtypes may represent genotypes especially adapted to colonizing bovine intestines. Two of them, S7 and S64, were identical with the SmaI types commonly detected in human campylobacter infections of domestic origin as well as in chicken flocks in Finland (M. Hakkinen *et al.* unpublished data) and seem able to colonize diverse hosts. The other two persistent subtypes may represent host-specific *C. jejuni* genotypes in cattle, which may not pose a significant health risk to humans (Kärenlampi *et al.* 2007; McCarthy *et al.* 2007). In herd 2, only two subtypes (S4 and S7) were detected during the entire sampling period. Subtype S7 was found only on two occasions: the first and last samplings. This may, however, indicate that this subtype existed in the herd permanently, perhaps at concentrations below the detection limit or in the intestines of individual animals from which samples were not obtained on all occasions.

Only one *C. jejuni* subtype was detected in most of the samples, when two to six isolates per sample were analysed with PFGE. This is consistent with sero- and genotyping results from other studies (Hänninen *et al.* 1998; Nielsen 2002). The results of individual animals suggest that the shedding of campylobacters is principally intermittent, but the amount of campylobacters excreted can be occasionally high. One of the animals in herd 1 could be considered as a permanent carrier, as the same subtype of *C. jejuni* was isolated on all sampling occasions from its faeces. Hänninen *et al.* (1998) have reported similar observations on the persistence of *C. jejuni* in the intestines of an individual animal. On the contrary, some of the animals in our study were campylobacter-negative on all sampling occasions, suggesting that permanently campylobacter-negative animals may also exist, although a higher frequency of sampling and an extended sampling period could also have yielded some positive samples from these individuals. However, animal-related factors may exist that render some individuals more resistant than others to campylobacter colonization.

No samples of drinking water were examined in this study. However, persons living on the farms consumed water drawn from the same sources with no intestinal disturbances. The detection of the same subtypes of *C. jejuni* from the faecal samples and the surface sample from the drinking trough of herd 3 suggests that the trough was contaminated by faeces and could circulate campylobacters among the animals of the herd. Water trough surface contaminated by campylobacters was implicated as a risk factor in the study by Minihan *et al.* (2004).

The results of this study suggest that permanent or long-term shedding of the same subtypes of *C. jejuni* occurs in dairy cattle. In addition, after initial colonization of the gut by one subtype, no other subtype may be able to exclude it at a later stage. Kwan *et al.* (2008) have also presented results suggesting ecological competition between campylobacter strains in bovine gut. The decreasing prevalence of campylobacters in herds without access to natural water sources during summer grazing can be considered indirect evidence of the significance of drinking water in the transmission of campylobacters. The ability of different *C. jejuni* subtypes to colonize bovine intestines may vary, and individual resistance to *Campylobacter* colonization may differ between animals in a cattle herd as well. Moreover, at least some subtypes common in human infections may be permanent or long-term colonizers in the bovine gut.

Acknowledgements

This study was partially funded by the Ministry of Agriculture and Forestry and Walter Ehrström Foundation. The authors would like to thank Kirsi-Maria Eklund, Sari Maljanen and Mirva Tahvanainen for their technical assistance. They would also like to thank the farmers for their cooperation.

References

Anonymous (2006) *International Standard ISO 10272-1. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for Detection and Enumeration of Campylobacter spp. Part 1: Detection Method*. Geneva, Switzerland: International Organization for Standardization (ISO).

Anonymous (2007a) *The Community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006*. *EFSA J* **130**, 106–352.

Anonymous (2007b) *Thermotolerant Campylobacter. Detection, Semi-quantitative and Quantitative Determination in Foods and Drinking Water*, Method No. 119, 3. Oslo, Norway: Nordic Committee on Food Analysis.

Atabay, H.I. and Corry, J.E.L. (1998) The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. *J Appl Microbiol* **84**, 733–740.

Besser, T.E., LeJeune, J.T., Rice, D.H., Berg, J., Stilborn, R.P., Kaya, K., Bae, W. and Hancock, D.D. (2005) Increasing prevalence of *Campylobacter jejuni* in feedlot cattle through the feeding period. *Appl Environ Microbiol* **71**, 5752–5758.

Clark, G.C., Price, L., Ahmed, R., Woodward, D., Melito, P.L., Rodgers, F.G., Jamieson, F., Ciebin, B. *et al.* (2003) Characterization of water-borne outbreak-associated *Campylobacter jejuni*, Walkerton, Ontario. *Emerg Infect Dis* **9**, 1232–1241.

Cools, I., Uyttendale, M., Caro, C., D'Haese, E., Nelis, H.J. and Debevere, J. (2003) Survival of *Campylobacter jejuni* strains of different origin in drinking water. *J Appl Microbiol* **94**, 886–892.

Devane, M.L., Nicol, C., Ball, A., Klena, J.D., Scholes, P., Hudson, J.A., Baker, M.G., Gilpin, B.J. *et al.* (2005) The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. *J Appl Microbiol* **98**, 980–990.

Evans, M.R., Roberts, R.J., Ribeiro, C.D., Gardner, D. and Kembrey, D. (1996) A milk-borne campylobacter outbreak following an educational farm visit. *Epidemiol Infect* **117**, 457–462.

Garrett, N., Devane, M.L., Hudson, J.A., Nicol, C., Ball, A., Klena, J.D., Scholes, P., Baker, M.G. *et al.* (2007) Statistical comparison of *Campylobacter jejuni* subtypes from human cases and environmental sources. *J Appl Microbiol* **103**, 2113–2121.

Gormley, F.J., MacRae, M., Forbes, K.J., Ogden, I.D., Dallas, J.F. and Strachan, N.J.C. (2008) Has retail chicken played a role in the decline of human campylobacteriosis. *Appl Environ Microbiol* **74**, 383–390.

Hakkinen, M., Heiska, H. and Hänninen, M.-L. (2007) Prevalence of *Campylobacter* spp. in cattle in Finland and antimicrobial susceptibility of bovine *Campylobacter jejuni* strains. *Appl Environ Microbiol* **73**, 3232–3238.

Hänninen, M.-L., Niskanen, M. and Korhonen, L. (1998) Water as a reservoir for *Campylobacter jejuni* infection in cows studied by serotyping and pulsed-field gel electrophoresis. *J Vet Med B* **45**, 37–42.

Ho, H.T., Lipman, L.J. and Gaastra, W. (2006) Arcobacter, what is known and unknown about a potential foodborne zoonotic agent! *Vet Microbiol* **115**, 1–13.

Humphrey, T.J. and Beckett, B. (1987) *Campylobacter jejuni* in dairy cows and raw milk. *Epidemiol Infect* **98**, 263–269.

Inglis, G.D., Kalischuk, L.D. and Busz, H.W. (2004) Chronic shedding of *Campylobacter* species in beef cattle. *J Appl Microbiol* **97**, 410–420.

Kärenlampi, R., Rautelin, H., Schönberg-Norio, D., Paulin, L. and Hänninen, M.-L. (2007) Longitudinal study of Finnish *Campylobacter jejuni* and *C. coli* isolates from humans, using multilocus sequence typing, including comparison with epidemiological data and isolates from poultry and cattle. *Appl Environ Microbiol* **73**, 148–155.

Kwan, P.L.S., Birtles, A., Bolton, F.J., French, N.P., Robinson, S.E., Newbold, L.S., Upton, M. and Fox, A.J. (2008) Longitudinal study of the molecular epidemiology of *Campylobacter jejuni* in cattle on dairy farms. *Appl Environ Microbiol* **74**, 3626–3633.

Lehner, A., Schneck, C., Feierl, G., Pless, P., Deutz, A., Brandl, E. and Wagner, M. (2000) Epidemiologic application of pulsed-field gel electrophoresis to an outbreak of *Campylobacter jejuni* in an Austrian youth centre. *Epidemiol Infect* **125**, 13–16.

McCarthy, N., Colles, F.M., Dingle, K.E., Bagnall, M.C., Manning, G., Maiden, M.C.J. and Falush, D. (2007) Host-associated genetic import in *Campylobacter jejuni*. *Emerg Infect Dis* **13**, 267–272.

Minihan, D., Whyte, P., O'Mahony, M., Fanning, S., McGill, K. and Collins, J.D. (2004) *Campylobacter* spp. in Irish feedlot cattle: a longitudinal study involving pre-harvest and harvest phases of the food chain. *J Vet Med B* **51**, 28–33.

Neimann, J., Engberg, J., Mølbak, K. and Wegener, H.C. (2003) A case–control study of risk factors for sporadic campylobacter infections in Denmark. *Epidemiol Infect* **130**, 353–366.

Nielsen, E.M. (2002) Occurrence and strain diversity of thermophilic campylobacters in cattle of different age groups in dairy herds. *Lett Appl Microbiol* **35**, 85–89.

Schildt, M., Savolainen, S. and Hänninen, M.-L. (2005) Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family. *Epidemiol Infect* **134**, 401–405.

Scullion, R., Harrington, C.S. and Madden, R.H. (2006) Prevalence of *Arcobacter* spp. in raw milk and retail raw meats in Northern Ireland. *J Food Prot* **69**, 1986–1090.

Stanley, K.N., Wallace, J.S., Currie, J.E., Diggle, P.J. and Jones, K. (1998) The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. *J Appl Microbiol* **85**, 472–480.

Studahl, A. and Andersson, Y. (2000) Risk factors for indigenous campylobacter infection: a Swedish case control study. *Epidemiol Infect* **125**, 269–275.

Vandamme, P. and On, S.L.W. (2001) Recommendations of the subcommittee on the taxonomy of *Campylobacter* and related bacteria. *Int J Syst Evol Microbiol* **51**, 719–721.

Wesley, I.V., Wells, S.J., Harmon, K.M., Green, A., Schroeder-Tucker, L., Glover, M. and Siddique, I. (2000) Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Appl Environ Microbiol* **66**, 1994–2000.

Whyte, P., McGill, K., Cowley, D., Madden, R.H., Moran, L., Scates, P., Carroll, C., O'Leary, A. *et al.* (2004) Occurrence of *Campylobacter* in retail foods in Ireland. *Int J Food Microbiol* **95**, 111–118.

Wingstrand, A., Neimann, J., Engberg, J., Nielsen, E.M., Gerner-Schmidt, P., Wegener, H. and Mølbak, K. (2006) Fresh chicken as main risk factor for campylobacteriosis, Denmark. *Emerg Infect Dis* **12**, 280–284.

III

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 2009, p. 1208–1210 Vol. 75, No. 4
0099-2240/09/$08.00+0 doi:10.1128/AEM.01879-08

Bovine *Campylobacter jejuni* Strains Differ from Human and Chicken Strains in an Analysis of Certain Molecular Genetic Markers▽

Manuel Gonzalez,[1] Marjaana Hakkinen,[2] Hilpi Rautelin,[3,4] and Marja-Liisa Hänninen[1]*

Department of Food and Environmental Hygiene, University of Helsinki,[1] Microbiology Unit, Research Department, Finnish Food Safety Authority,[2] Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki,[3] and HUSLAB, Helsinki University Central Hospital Laboratory,[4] Helsinki, Finland

Received 13 August 2008/Accepted 14 December 2008

The association of four new genetic markers with a chicken, bovine, or human host was studied among 645 *Campylobacter jejuni* isolates. The γ-glutamate transpeptidase gene and *dmsA* were common in human and chicken isolates but uncommon among bovine isolates. In the *t* test, bovine isolates differed significantly ($P < 0.05$) from human and chicken isolates.

Campylobacter jejuni is a zoonotic human enteric pathogen with a large number of animal hosts (12, 19). Campylobacteriosis is a leading cause of human bacterial gastroenteritis in many industrialized countries (19). Epidemiological studies indicate that exposure to improperly cooked chicken meat, handling of raw chicken meat, and drinking unpasteurized milk are important risk factors for campylobacteriosis (12, 15, 19, 20).

The role of different animal sources in human infections is not well characterized. Molecular typing methods applied for fingerprinting of *C. jejuni* strains have shown overlapping genotypes between animal and human isolates (5, 16, 17, 21). Population biological studies using multilocus sequence typing (6) have revealed that a host-*C. jejuni* interaction may leave a signature in the bacterial genome. As a consequence, e.g., chicken- or cattle-associated populations can be assigned to their hosts (18). We investigated host association of *C. jejuni* isolates from cattle, chickens, and humans using PCR detection of four new genetic markers developed under our study. Using comparative genomics (3), four genetic markers—i.e., *ggt*, the γ-glutamyl transpeptidase gene; *dmsA* (Cju34), a subunit of the putative tripartite anaerobic dimethyl sulfoxide (DMSO) oxidoreductase (DMSO/trimethylamine *N*-oxide reductase) gene; Cj1585c, coding for a putative oxidoreductase; and CJJ81176-1371, a putative serine protease gene—were selected from the genomes of *C. jejuni* strains 81-176 (10), RM1221, and NCTC 11168. *ggt* is in the genome of 81-176 but not in the genome of NCTC 11168 or RM1221 (10). Gene Cj1585c of NCTC 11168 is replaced in 81-176 by a cluster of four genes (*dmsA*, *dmsB*, *dmsC*, and *dmsD*) (10). The presence of these four genes in a total of 645 *C. jejuni* isolates from bovine fecal samples ($n = 131$) (8), chicken cecal or meat samples ($n = 205$), and human patients ($n = 309$) (16, 17) was examined by PCR to find their suitability for host association studies. PCR primers designed for the amplification of the fragments are shown in Table 1. Twelve PCR products for each gene fragment were sequenced. The sequences of each gene were shown to be rather conserved (95.5 to 100% similarity within each gene) because only a few nucleotide positions (from 2 to 9) were found to be variable.

TABLE 1. PCR primers used amplification of the fragments of the four marker genes

Gene (product)	Primer sequence		Product size (bp)
	Forward	Reverse	
ggt (γ-glutamyl transpeptidase)	TTTTAGCCATATC CGCTGCT	AGCTGGAGTACCA GGAA	339
dmsA[a]	GATAGGGCATTG CGATGAGT	CTTGCTAGCCCAAT CAGGAG	238
Cj1585c (oxidoreductase)	TGTTGTGGGTTT GCTGGATA	TTGCTTCACTGCAT TCATCC	202
CJJ81176-1367/1371 (serine protease)	TGCAAAGCAGGG CTAAGAAT	TTATGGAGCTGGG GTGTTTC	318

[a] Cju34.

Statistical analyses were performed using SPSS software. The χ^2 test was used to test for similarity in the frequencies of marker genes within the isolates from different hosts. In addition, we used the paired two-tailed Student's *t* test for analysis of host associations for the combined set of four genes.

Frequencies of the genes are shown in Table 2. Similarly, the results of the paired two-tailed *t* test on the significance of the frequencies of the combined four genes from different hosts are shown in Table 2. These results indicated significant ($P < 0.05$) association of bovine and chicken isolates with their host source, but a high similarity was observed between the chicken and human isolates ($P = 0.9949$). Annual frequencies of the genes are presented for human isolates in Table 3 and for chicken isolates in Table 4. The analysis of the annual frequencies of the four genes combined showed that the human isolates were similar in 1996 and 2002 and 2002 and 2003, but differed between 1996 and 2003 (Table 3). The chicken isolates were similar in all study years (Table 4). These results revealed that these genes associated with metabolism and energy production (*ggt*, oxidoreductases) (2, 11, 22), colonization (*ggt*) (2, 11), or unknown function (serine protease genes) are not randomly distributed among the isolates from different hosts but show a host association.

* Corresponding author. Mailing address: Department of Food and Environmental Hygiene, P.O. Box 66, 0014 University of Helsinki, Finland. Phone: 358-9-19157113. Fax: 358-9-19157101. E-mail: marja-liisa.hanninen@helsinki.fi.

▽ Published ahead of print on 19 December 2008.

TABLE 2. Frequency of the four marker genes *ggt*, Cj1585c, *dmsA* (Cju34), and CJJ81176-1371 in 645 human, chicken, and cattle *C. jejuni* isolates

Marker gene (product)	No. of isolates with gene/total no. of isolates (%)			*P* value for source[a]:		
	Human	Chicken	Bovine	Human/chicken	Chicken/bovine	Human/bovine
					χ^2 test	
ggt (γ-glutamyl transpeptidase)	169/309 (54.7)	75/205 (36.6)	11/131 (8.4)	<0.05	<0.05	<0.05
Cj1585c (oxidoreductase)	99/309 (32)	49/205 (23.9)	83/131 (62.6)	<0.05	<0.05	<0.05
dmsA[b]	256/309 (82.8)	151/205 (73.3)	18/131 (13.7)	<0.05	<0.05	<0.05
CJJ81176-1367/1371 (serine protease)	117/309 (37.8)	74/205 (36.1)	96/131 (73.3)	0.68	<0.05	<0.05
					t test[c]	
				0.9949	0.0087	0.0122

[a] $P < 0.05$ represents significant difference.
[b] *dmsA* (Cju34) is a subunit of the putative tripartite anaerobic DMSO oxidoreductase gene.
[c] Significance ($P < 0.05$) of the frequency of the combined four genes by paired two-tailed *t* test.

The intestinal environments of cattle and chicken are quite different, which may select isolates with variable characteristics, e.g., related to energy metabolism, adaptation to lower or higher oxygen contents or amino acid metabolism. *C. jejuni* colonization in dairy cattle can be persistent, as shown by the studies in which the same genotype was isolated for up to 1 year (1, 13, 14). The life cycle of cattle is several years, providing a long potential time span for the adaptation of *C. jejuni* with its host. The life cycle of chickens, in contrast, is much shorter, 5 weeks or more. Our results suggested that host adaptation of certain *C. jejuni* strains is evident. The *dmsA* subunit was more often detected among chicken and human isolates than among bovine isolates (Table 2). In addition, *dmsA*-positive chicken isolates occurred with similar high annual frequency in 2003, 2006, and 2007 (Table 4), indicating that this characteristic is most probably important in colonization. The occasional significant annual fluctuation seen in the frequency of *dmsA*-positive human isolates may reflect variation in the infection sources (Table 3). In a recent study (9), *dmsB* was one of the genes present in *C. jejuni* strain A 74/C, shown to be robust colonizer in chickens, but absent from *C. jejuni* 11168(GS), a poorly colonizing strain (7). The *C. jejuni* NCTC 11168, 81116, and 81-176 strains have another putative DMSO oxidoreductase gene (homologous to Cj0264c) that differs from Cju34. In opposition, the Cj1585c-type oxidoreductase was more frequently present in isolates from cattle than in those from chickens or humans (Table 2). Analyses of *C. jejuni* genomes have predicted a branched complex electron transport chain capable of utilizing multiple electron donors and acceptors (22), and our results suggest flexibility in the oxidoreductase systems as well.

ggt (γ-glutamyl transpeptidase) has been shown to be important in the persistent colonization of *C. jejuni* in chickens (2), and recent studies (11) further extend the significance of this gene in the glutamine and glutathione metabolism and colonization of *C. jejuni*. In our study, the frequency of the *ggt*-positive human and chicken isolates was high (Table 2) and the frequencies remained similar over the study years (Tables 3 and 4). These results further reveal the importance of γ-glutamyl transpeptidase in colonization and pathogenesis. In contrast, a low frequency of *ggt*-positive isolates (8.4%) was found among bovine isolates (Table 2), suggesting that this type of metabolism is not crucial for colonization of the bovine gut. Similar variable frequencies to those in our study were found in the study by Barnes et al. (2).

The genomes of NCTC 11168, RM1221, and 81-176 have a subtilase-type serine protease gene homologous to CJJ81176-1367, which is located close to the CJJ81176-1371 gene in the genome of 81-176 (10). The G+C composition of this gene is 29%, whereas the G+C composition of CJJ81176-1371 is 36%, indicating that these genes most probably have different evolutionary origins. In our study, the serine gene was common among bovine isolates (Table 2) and less common among chicken and human isolates. The primers we used may amplify both types of the subtilase genes. Proteases in *C. jejuni* have a role in stress tolerance (4). Whether the serine protease is important in the pathogenesis of campylobacteriosis remains to be elucidated.

TABLE 3. Frequency of the four marker genes *ggt*, Cj1585c, *dmsA* (Cju34), and CJJ81176-1367/1371 in 309 *C. jejuni* isolates from humans

Marker gene (product)	No. of isolates with gene/total no. of isolates (%)			*P* value for yr:		
	1996	2002	2003	1996–2002	1996–2003	2002–2003
					χ^2 test	
ggt (γ-glutamyl transpeptidase)	52/97 (53.6)	57/111 (51.3)	60/101 (59.4)	0.74	0.41	0.24
Cj1585c (oxidoreductase)	27/97 (27.8)	25/111 (22.5)	47/101 (46.5)	0.38	<0.05	<0.05
dmsA[a]	69/97 (71.3)	101/111 (91)	86/101 (85.1)	<0.05	<0.05	0.19
CJJ81176-1367/1371 (serine protease)	34/97 (35.1)	37/111 (33.3)	46/101 (45.5)	0.79	0.13	0.07
					t test[b]	
				0.4506	0.0003	0.052

[a] *dmsA* (Cju34) is a subunit of the putative tripartite anaerobic DMSO oxidoreductase gene.
[b] Significance ($P < 0.05$) of the frequency of the combined four genes.

TABLE 4. Presence of the four marker genes *ggt*, Cj1585c, *dmsA* (Cju34), and CJJ81176-1367/1371 in 205 *C. jejuni* isolates from chickens

Marker gene (product)	No. of isolates with gene/total no. of isolates (%)			*P* value for yr:		
	2003	2006	2007	2003–2006	2003–2007	2006–2007
					χ^2 test	
ggt (γ-glutamyl transpeptidase)	16/37 (43.2)	29/71 (40.8)	30/97 (30.9)	0.81	0.19	0.18
Cj1585c (oxidoreductase)	15/37 (40.5)	6/71 (8.5)	28/97 (28.9)	<0.05	0.21	<0.05
dmsA[a]	30/37 (81.1)	49/71 (69)	72/97 (74.2)	0.15	0.38	0.46
CJJ81176-1367/1371 (serine protease)	20/37 (54.1)	23/71 (32.4)	31/97 (31.9)	<0.05	<0.05	0.95
					t test[b]	
				0.074	0.095	0.317

[a] *dmsA* (Cju34) is a subunit of the putative tripartite anaerobic DMSO oxidoreductase gene.
[b] Significance ($P < 0.05$) of the frequency of the combined four genes.

The genetic markers associated with metabolism, colonization, or an unknown protease function allowed assignment of the chicken or bovine source of *C. jejuni*. These results suggest that metabolic diversity is an important adaptive factor in host adaptation.

We acknowledge financial support from the Academy of Finland (Elvira) and EU project no. 036272 (Biotracer).

REFERENCES

1. **Bae, W., K. N. Kaya, D. D. Hancock, D. R. Call, Y. H. Park, and T. E. Besser.** 2005. Prevalence and antimicrobial resistance of thermophilic *Campylobacter* spp. from cattle farms in Washington State. Appl. Environ. Microbiol. **71:** 169–174.
2. **Barnes, I. H., M. C. Bagnall, D. D. Browning, S. A. Thompson, G. Manning, and D. G. Newell.** 2007. Gamma-glutamyl transpeptidase has a role in the persistent colonization of the avian gut by *Campylobacter jejuni*. Microb. Pathog. **43:**198–207.
3. **Chaudhuri, P. C., and M. J. Pallen.** 2006. *x*BASE, a collection of online databases for bacterial comparative genomics. Nucleic Acids Res. **34:**D335–D337.
4. **Cohn, M. T., H. Ingmer, F. Mulholland, K. Jørgensen, J. M. Wells, and L. Brøndsted.** 2007. Contribution of conserved ATP-dependent proteases of *Campylobacter jejuni* to stress tolerance and virulence. Appl. Environ. Microbiol. **73:**7803–7813.
5. **Denis, M., B. Chidaine, M. J. Laisney, I. Kempf, K. Rivoal, F. Mégraud, and P. Fravalo.** 2008. Comparison of genetic profiles of *Campylobacter* strains isolated from poultry, pig and *Campylobacter* human infections in Brittany, France. Pathol. Biol. (Paris). doi:10.1016/j.patbio.2008.04007.
6. **Dingle, K. E., F. M. Colles, D. R. A. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J. Bootsma, R. J. L. Willems, R. Urwin, and M. C. J. Maiden.** 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J. Clin. Microbiol. **39:**14–23.
7. **Gaynor, E. C., S. Cawthraw, G. Manning, J. K. MacKichan, S. Falkow, and D. G. Newell.** 2004. The genome-sequenced variant of *Campylobacter jejuni* NCTC 11168 and the original clonal clinical isolate differ markedly in colonization, gene expression, and virulence-associated phenotypes. J. Bacteriol. **186:**503–517.
8. **Hakkinen, M., H. Heiska, and M.-L. Hänninen.** 2007. Prevalence of *Campylobacter* spp. in cattle in Finland and antimicrobial susceptibility of bovine *Campylobacter jejuni* strains. Appl. Environ. Microbiol. **73:**3232–3238.
9. **Hiett, K. L., A. Stintzi, T. M. Andacht, R. L. Kuntz, and B. S. Seal.** 2008. Genomic differences between *Campylobacter jejuni* isolates identify surface membrane and flagellar function gene products potentially important for colonizing the chicken intestine. Funct. Integr. Genomics **8:**407–420.
10. **Hofreuter, D., J. Tsai, R. O. Watson, V. Novik, B. Altman, M. Benitez, C. Clark, C. Perbost, T. Jarvie, L. Du, and J. E. Galan.** 2006. Unique features of a highly pathogenic *Campylobacter jejuni* strain. Infect. Immun. **74:**4694–4707.
11. **Hofreuter, D., V. Novik, and J. E. Galan.** 2008. Metabolic diversity in *Campylobacter jejuni* enhances specific tissue colonization. Cell Host Microbe **4:**425–433.
12. **Humphrey, T., S. O'Brien, and M. Madsen.** 2007. Campylobacters as zoonotic pathogens: a food production perspective. Int. J. Food Microbiol. **117:**237–257.
13. **Inglis, G. D., L. D. Kalischuk, and H. W. Busz.** 2004. Chronic shedding of *Campylobacter* species in beef cattle. J. Appl. Microbiol. **97:**410–420.
14. **Johnsen, G., K. Zimmerman, B. A. Lindstedt, T. Vardund, H. Herikstad, and G. Kapperud.** 2006. Intestinal carriage of *Campylobacter jejuni* and *Campylobacter coli* among cattle from south-western Norway and comparative genotyping of bovine and human isolates by amplified-fragment length polymorphism. Acta Vet. Scand. **48:**4–9.
15. **Kapperud, G., G. Espeland, E. Wahl, A. Walde, H. Herikstad, S. Gustavsen, I. Tveit, O. Natås, L. Bevanger, and A. Digranes.** 2003. Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. Am. J. Epidemiol. **158:**234–242.
16. **Kärenlampi, R., H. Rautelin, M. Hakkinen, and M.-L. Hänninen.** 2003. Temporal and geographical distribution and overlap of Penner heat-stable serotypes and pulsed-field electrophoresis genotypes of *Campylobacter jejuni* isolates collected from humans and chickens in Finland during a seasonal peak. J. Clin. Microbiol. **41:**4870–4872.
17. **Kärenlampi, R., H. Rautelin, D. Schönberg-Norio, L. Paulin, and M.-L. Hänninen.** 2007. Longitudinal study of Finnish *Campylbacter jejuni* and *Campylobacter coli* isolates from humans, using multilocus sequence typing, including comparison with epidemiological data and isolates from poultry and cattle. Appl. Environ. Microbiol. **73:**148–155.
18. **McCarthy, N. D., F. M. Colles, K. E. Dingle, M. C. Bagnall, G. Manning, M. C. Maiden, and D. Falush.** 2007. Host-associated genetic import in Campylobacter jejuni. Emerg. Infect. Dis. **13:**267–272.
19. **Olson, K. C., S. Ethelberg, W. van Pelt, and R. V. Tauxe.** 2008. Epidemiology of *Campylobacter jejuni* infections in industrialized nations, p. 163–189. *In* I. Nachamkin, C. M. Szymanski, and M. J. Blaser (ed.), *Campylobacter*, 3rd ed. American Society for Microbiology, Washington, DC.
20. **Schönberg-Norio, D., J. Takkinen, M.-L. Hänninen, M.-L. Katila, S. S. Kaukoranta, L. Mattila, and H. Rautelin.** 2004. Swimming and Campylobacter infections. Emerg. Infect. Dis. **10:**1474–1477.
21. **Wassenaar, T. M., and D. G. Newell.** 2000. Genotyping of *Campylobacter* spp. Appl. Environ. Microbiol. **66:**1–9.
22. **Weingarten, R. A., J. L. Grimes, and J. W. Olson.** 2008. Role of *Campylobacter jejuni* respiratory oxidases and reductases in host colonization. Appl. Environ. Microbiol. **74:**1367–1375.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 2009, p. 5244–5249 Vol. 75, No. 16
0099-2240/09/$08.00+0 doi:10.1128/AEM.00374-09

Chickens and Cattle as Sources of Sporadic Domestically Acquired *Campylobacter jejuni* Infections in Finland▽

Marjaana Hakkinen,[1,2]* Ulla-Maija Nakari,[3] and Anja Siitonen[3]

Research Department, Finnish Food Safety Authority Evira, Helsinki, Finland[1]; Department of Food and Environmental Hygiene, FI-00014 University of Helsinki, Helsinki, Finland[2]; and Unit of Gastrointestinal Infections, National Institute for Health and Welfare, Helsinki, Finland[3]

Received 16 February 2009/Accepted 12 June 2009

A substantial sampling among domestic human campylobacter cases, chicken process lots, and cattle at slaughter was performed during the seasonal peak of human infections. *Campylobacter jejuni* isolates ($n = 419$) were subtyped using pulsed-field gel electrophoresis with SmaI, and isolates representing overlapping types ($n = 212$) were further subtyped using KpnI for restriction. The SmaI/KpnI profiles of 55.4% (97/175) of the human isolates were indistinguishable from those of the chicken or cattle isolates. The overlapping SmaI/KpnI subtypes accounted for 69.8% (30/43) and 15.9% (32/201) of the chicken and cattle isolates, respectively. The occurrence of identical SmaI/KpnI subtypes with human *C. jejuni* isolates was significantly associated with animal host species ($P < 0.001$). A temporal association of isolates from chickens and patients was possible in 31.4% (55/175) of the human infections. Besides chickens as sources of *C. jejuni* in the sporadic infections, the role of cattle appears notable. New approaches to restrict the occurrence of campylobacters in other farm animals may be needed in addition to hygienic measures in chicken production. However, only about half of the human infections were attributable to these sources.

The incidence of human enteric infections caused by campylobacters is highest in the summer months, showing a consistent peak at the end of July in Finland (www.ktl.fi/attachments/suomi/julkaisut/julkaisusarja_b/2008/2008b09.pdf), as well as in other Nordic countries (16, 33). Almost 70% of campylobacter infections detected in July and August in Finland are domestically acquired, whereas the annual average proportion of domestic cases is about 30%, and most of them are caused by *Campylobacter jejuni* (30). The prevalence of campylobacters in Finnish broiler flocks peaks simultaneously with the human cases (7), and similar sero- and genotypes have been reported among human and poultry strains isolated in Finland and in other countries (5, 8, 21–23). Several epidemiological studies have identified the handling and consumption of raw or undercooked poultry meat as a major risk factor for campylobacter enteritis (for example, see references 18, 20, and 41), whereas opposite conclusions about the significance of the consumption of chicken meat were drawn from the Swedish case-control study among young children (2) and an extensive Danish register-based study (6).

Data derived from the genotyping studies of *C. jejuni* isolates from human infections and animals support the current suggestion that poultry is the most important single source of sporadic campylobacteriosis (12, 22, 29). However, several reports on genotype comparisons suggest that poultry may be a less significant source of campylobacters than generally thought, and other animal reservoirs should also be considered notable sources of campylobacters pathogenic to humans (3, 8, 17, 27, 31). Studies of the temporal occurrence of campylobacters in human infections and poultry flocks have revealed that the peak in prevalence, as well as some of the overlapping sero- and genotypes, is detected in humans prior to being detected in poultry (21, 28).

Although cattle are well-known carriers of campylobacters, the survival of these fragile organisms in beef is poor (39, 42). In recent years, some authors (1, 4, 10) have raised the question of an indirect association between cattle and human cases. In a Finnish study combining data from the multilocus sequence typing of campylobacters isolated from production animals and from epidemiological studies of human cases, significant associations emerged between certain sequence-type complexes from human infections and contact with cattle, the consumption of unpasteurized milk, or the tasting or consumption of raw minced meat (23).

The aim of this study was to investigate the contributions of poultry and cattle as sources of human *C. jejuni* infections in Finland by comparing over a limited time frame the macrorestriction profiles obtained from pulsed-field gel electrophoresis (PFGE) analysis of a geographically representative collection of *C. jejuni* isolates from domestically acquired sporadic human infections, chicken process lots, and cattle.

MATERIALS AND METHODS

Isolates. We studied a total of 419 isolates. Human *C. jejuni* isolates ($n = 175$) were collected from June to August 2003, during the seasonal peak of human cases. The isolates represented all domestic *C. jejuni* strains isolated in 9 of 25 clinical microbiology laboratories located in nine hospital districts across the country. They were isolated from the fecal samples of patients using modified charcoal cefoperazone deoxycholate agar. One isolate per patient was submitted to the National Public Health Institute (KTL; currently, the National Institute for Health and Welfare [THL]) for further investigation, and an isolate was defined as domestic if the patient had no history of traveling abroad within 10 days before the onset of symptoms or 17 days before the specimen was taken. Only isolates from sporadic infections were included.

Bovine fecal ($n = 186$) and carcass ($n = 15$) isolates were obtained from

* Corresponding author. Mailing address: Finnish Food Safety Authority Evira, Mustialankatu 3, FI-00790 Helsinki, Finland. Phone: 358 2077 24471. Fax: 358 2077 24350. E-mail: marjaana.hakkinen@evira.fi.
▽ Published ahead of print on 19 June 2009.

samples of 952 cattle in a survey carried out by the National Veterinary and Food Research Institute (currently, the Finnish Food Safety Authority Evira) at 12 of 15 Finnish slaughterhouses in 2003 (13). Altogether, 71 of the bovine fecal isolates originated from dairy cattle and 115 from beef cattle. Because most of the isolates originated from different farms and because long-term carriage of the same genotype of *C. jejuni* in a herd was considered likely, fecal isolates over the entire year were included in the study. Isolates from 262 carcass samples taken only between May and August 2003 were included, because those isolated during the rest of the year could not have been associated with human infections during the summer.

Isolates from chickens ($n = 43$) were obtained from cecal samples taken at slaughter. Two of three Finnish broiler slaughterhouses participated in this study. All 955 process lots slaughtered between May and August 2003 were sampled. One loopful (10 μl) of cecal contents of three to five chickens from each process lot was directly cultured on modified charcoal cefoperazone deoxycholate agar. One isolate from each campylobacter-positive process lot was submitted to Evira for further investigation.

Identification and genotyping of isolates. The identification of isolates was based on standard biochemical tests (19). The human isolates were genotyped at THL and the bovine and chicken isolates at Evira by PFGE using SmaI for restriction as described by Hakkinen et al. (13).

All isolates representing overlapping SmaI subtypes were additionally subtyped using KpnI for restriction. DNA was digested for a minimum of 4 h at 37°C with 20 U of KpnI restriction endonuclease (New England Biolabs, Inc., Ipswich, MA) in a final volume of 200 μl containing 2 μl of bovine serum albumin (New England Biolabs, Inc., Ipswich, MA). PFGE data were analyzed with Bionumerics V5.10 (Applied Maths, Kortrijk, Belgium) at 0.5% optimization and 1.0% tolerance. Patterns differing by at least a single band were considered different subtypes. Subtypes obtained by SmaI and KpnI restriction were named S1, S2, etc., and K1, K2, etc., respectively.

Evaluation of the temporal association among isolates. The temporal association of the SmaI/KpnI subtypes among isolates from chickens and patients was evaluated using the criteria presented by Kärenlampi et al. (21).

Statistical methods. The χ^2 test was performed to investigate the association between human *C. jejuni* genotypes and animal reservoirs as well as their association with the type of cattle herds. A P value of <0.05 indicated statistical significance.

RESULTS

We identified 109 different SmaI subtypes among the 419 *C. jejuni* isolates investigated. Forty-three subtypes were distinguished among the 175 isolates from human infections, 15 subtypes among the 43 isolates from chickens, and 61 subtypes among the 201 isolates from cattle (data not shown). Of these, 26, 10, and 36 occurred only once in human, chicken, and bovine samples, respectively; 18 isolates from humans and 1 from chickens were untypeable by SmaI.

Fourteen SmaI subtypes of *C. jejuni* (32.6% of all 43 human subtypes) representing 114 (65.1%) of 175 human isolates were indistinguishable from those of chicken or bovine isolates (Table 1). In total, 36 (83.7%) of 43 chicken isolates and 62 (30.8%) of 201 isolates from cattle represented SmaI subtypes shared with humans.

Further subtyping of 212 *C. jejuni* isolates (114 human, 36 chicken, and 62 cattle isolates), representing the 14 overlapping SmaI subtypes, with KpnI as a restriction enzyme yielded 44 subtypes, 17 of which were shared between human and animal isolates (Table 1). The combined type S6/K12 predominated among isolates from human patients (12%) and occurred in both chickens and cattle (Table 1; Fig. 1).

Of the combined SmaI/KpnI subtypes, 12 were present only in humans, 4 only in chickens, and 12 only in cattle. In total, the SmaI/KpnI profiles of 97 (55.4%) human isolates were indistinguishable from those of chicken or cattle isolates. The overlapping combined SmaI/KpnI subtypes accounted for 69.8% (30/43) and 15.9% (32/201) of the chicken and cattle isolates, respectively. The occurrence of identical SmaI/KpnI subtypes with human *C. jejuni* isolates was significantly associated with animal host species ($P < 0.001$).

A total of 17 of the 71 (23.9%) fecal isolates from dairy cattle and 15 (13.0%) of the 115 fecal isolates from beef cattle represented the overlapping SmaI/KpnI subtypes with human isolates. The occurrence of identical SmaI/KpnI subtypes with

TABLE 1. SmaI/KpnI subtypes of *Campylobacter jejuni* in domestically acquired sporadic human infections, chickens, and cattle in Finland between June and August 2003[a]

PFGE subtype (SmaI/KpnI)	No. (%) of isolates from:		
	Humans	Chicken	Cattle
S1/K13	0 (0.0)	0 (0.0)	6 (2.9)
S1/K21	0 (0.0)	0 (0.0)	1 (0.5)
S1/K22	0 (0.0)	0 (0.0)	5 (2.4)
S1/K23	0 (0.0)	0 (0.0)	1 (0.5)
S1/K24	0 (0.0)	0 (0.0)	4 (1.9)
S1/K25	0 (0.0)	0 (0.0)	1 (0.5)
S1/K26	0 (0.0)	0 (0.0)	5 (2.4)
S1/K33	1 (0.0)	0 (0.0)	0 (0.0)
S4/K28	0 (0.0)	1 (2.3)	3 (1.5)
S4/K29	**1 (0.6)**	**1 (2.3)**	**1 (0.5)**
S4/K31	0 (0.0)	2 (4.7)	0 (0.0)
S4/K32	0 (0.0)	1 (2.3)	0 (0.0)
S5/K27	**1 (0.6)**	0 (0.0)	**10 (4.9)**
S6/K12	**21 (12.0)**	**2 (4.7)**	**7 (3.4)**
S7/K1	**12 (6.9)**	**2 (4.7)**	**7 (3.4)**
S7/K2	**4 (2.3)**	**2 (4.7)**	**2 (1.0)**
S7/K3	**17 (9.7)**	**2 (4.7)**	**1 (0.5)**
S7/K36	2 (1.1)	0 (0.0)	0 (0.0)
S22/K14	0 (0.0)	0 (0.0)	1 (0.5)
S22/K15	0 (0.0)	0 (0.0)	1 (0.5)
S22/K16	**1 (0.6)**	0 (0.0)	**1 (0.5)**
S38/K17	0 (0.0)	0 (0.0)	1 (0.5)
S38/K34	1 (0.6)	0 (0.0)	0 (0.0)
S54/K8	0 (0.0)	0 (0.0)	1 (0.5)
S54/K9	0 (0.0)	1 (2.3)	0 (0.0)
S54/K10	**6 (3.4)**	**2 (4.7)**	0 (0.0)
S54/K11	**3 (1.7)**	**1 (2.3)**	0 (0.0)
S54/K42	1 (0.6)	0 (0.0)	0 (0.0)
S54/K43	2 (1.1)	0 (0.0)	0 (0.0)
S64/K19	**7 (4.0)**	**1 (2.3)**	**1 (0.5)**
S64/K35	2 (1.1)	0 (0.0)	0 (0.0)
S66/K18	**4 (2.3)**	0 (0.0)	**1 (0.5)**
S74/K4	**5 (2.9)**	**8 (18.6)**	0 (0.0)
S74/K5	**8 (4.6)**	**4 (9.3)**	**1 (0.5)**
S74/K6	0 (0.0)	1 (2.3)	0 (0.0)
S74/K7	**2 (1.1)**	**2 (4.7)**	0 (0.0)
S74/K37	1 (0.6)	0 (0.0)	0 (0.0)
S74/K38	1 (0.6)	0 (0.0)	0 (0.0)
S74/K39	1 (0.6)	0 (0.0)	0 (0.0)
S74/K40	1 (0.6)	0 (0.0)	0 (0.0)
S76/K20	**3 (1.7)**	**1 (2.3)**	0 (0.0)
S76/K6	1 (0.6)	0 (0.0)	0 (0.0)
S77/K30	**1 (0.6)**	**1 (2.3)**	0 (0.0)
S77/K41	3 (1.7)	0 (0.0)	0 (0.0)
S78/K6	**1 (0.6)**	**1** (2.3)	0 (0.0)
Overlapping combined subtypes	97 (55.4)	30 (69.8)	32 (15.9)
Overlapping SmaI types	114 (65.1)	36 (83.7)	62 (30.8)
Total no. of isolates	175	43	201

[a] Overlapping subtypes between human and animal isolates appear in bold.

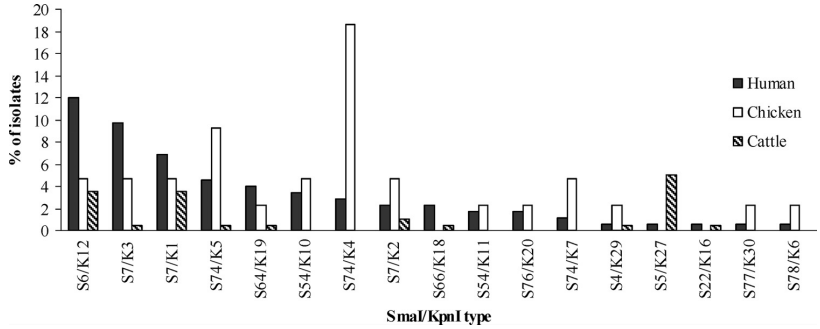

FIG. 1. Distribution of 17 *Campylobacter jejuni* SmaI/KpnI subtypes among isolates from domestically acquired human infections, chickens, and cattle in Finland between June and August 2003.

human *C. jejuni* isolates in cattle was not significantly related to herd type ($P = 0.056$). All bovine subtypes overlapping those of humans occurred among isolates from dairy cattle, with the exception of S22/K16, isolated only from beef cattle (Fig. 2).

A temporal association of the SmaI/KpnI subtypes among isolates from chickens and patients was possible in 55 (31.4%) of 175 human infections (Table 2). Isolates from 12 (6.9%) human infections temporally associated with chicken isolates represented SmaI/KpnI subtypes that failed to occur in cattle.

DISCUSSION

In this study, we compared the DNA macrorestriction profiles of *C. jejuni* isolates from domestic human infections, chickens, and cattle covering the whole of Finland over a time frame of three summer months with the aim of estimating the attribution of these animal sources to human infections. A total of 419 *C. jejuni* isolates were genotyped with PFGE using SmaI and KpnI as restriction enzymes.

The *C. jejuni* isolates from food production animals were collected from 12 cattle slaughterhouses and 2 chicken slaughterhouses, representing 98% of the cattle and 85% of the chicken slaughter volume in Finland in 2003, respectively. The human clinical *C. jejuni* isolates of domestic origin represented 54% of all isolates collected by 9 of 25 Finnish clinical laboratories during a three-month period from June to August 2003. The total number of campylobacter infections reported during the same time period in Finland was 1,281, including infections contracted abroad (http://www3.ktl.fi/stat/).

The summer months were chosen as the time period to examine because of the pronounced seasonality of human campylobacteriosis and because the proportion of domestically acquired human cases in Finland is highest during the summer months (23; www.ktl.fi/attachments/suomi/julkaisut/julkaisusarja_b/2005/2005b13.pdf). Furthermore, the occurrence of campylobacter in Finnish chicken process lots and, consequently, in retail poultry meat peaks in July and August (7, 15, 24). A comparison of *C. jejuni* isolates from retail chicken meat would have focused specifically on the genotypes to which consumers are exposed. On the other hand, by sampling at slaughter, we could obtain samples from more than

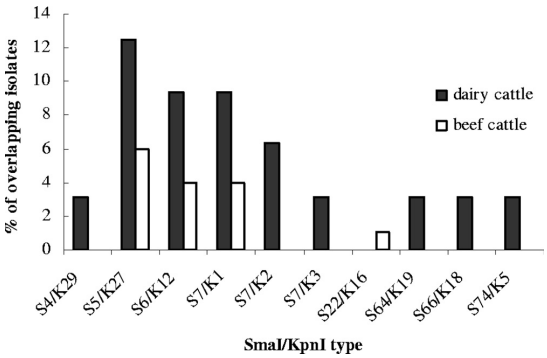

FIG. 2. Distribution of the most prevalent SmaI/KpnI subtypes of human *Campylobacter jejuni* isolates among fecal isolates from Finnish dairy and beef cattle.

TABLE 2. Temporal association between human and broiler *Campylobacter jejuni* isolates during the seasonal peak in Finland from June to August 2003

SmaI/KpnI subtype	No. of human isolates temporally associated with isolates from positive broiler flocks/total no. of human isolates			
	June	July	August	Total
S4/K29	0/0	0/1	0/0	0/1
S6/K12	0/0	7/7	14/14	21/21
S7/K1	0/1	8/8	3/3	11/12
S7/K2	0/0	0/4	0/0	0/4
S7/K3	0/2	1/6	9/9	10/17
S54/K10	0/0	0/6	0/0	0/6
S54/K11	0/0	0/1	1/2	1/3
S64/K19	0/0	0/5	1/2	1/7
S74/K4	0/0	5/5	0/0	5/5
S74/K5	0/0	0/8	0/0	0/8
S74/K7	0/0	0/0	2/2	2/2
S76/K20	0/0	0/0	3/3	3/3
S77/K30	0/0	0/0	1/1	1/1
S78/K6	0/0	0/1	0/0	0/1
Total	0/3	21/52	34/36	55/91
Total no. of human isolates per month	11	106	58	175

80% of all process lots during the sampling period and, therefore, probably a better overall view of the situation. As Nielsen et al. (31) observed, the same *C. jejuni* subtypes that colonize the intestines of chickens can be detected in retail samples of chicken meat. Due to the small number of cecal samples per process lot, we may have excluded some positive lots if the contamination rate of chickens in the process lot was less than 50%. In recent years, after the implementation of the Finnish campylobacter monitoring program for poultry in 2004, slightly higher prevalences (5.9 to 7.4%) of campylobacters have been reported during the summer months, probably due to the higher number of cecal samples (10 ceca per process lot [7]), than the prevalence in this study, which is in accordance with that previously reported by Perko-Mäkelä et al. (35). In general, the prevalence of campylobacters in Finnish chicken process lots is lower than in most other countries, where prevalences from 15% to 87% have been reported (7, 29, 38). The proportion of slaughtering by each slaughterhouse in the preceding year was taken into account in the randomized sampling of cattle (13). The bovine fecal isolates collected throughout the entire year were included in our study, as evidence suggests that the long-term excretion of the same *C. jejuni* genotypes occurs both in dairy herds (14, 26) and in the farm environment (8).

As in several previous studies that have used different genotyping methods (8, 11, 26, 31), we obtained a wide variety of different *C. jejuni* subtypes with PFGE typing using SmaI and KpnI restriction enzymes. All different SmaI subtypes among multiple isolates from each bovine sample were included in our study. However, more than one SmaI subtype was present in less than 10% of the samples from cattle (13).

A few SmaI/KpnI subtypes predominated among the human isolates; the five most frequently detected comprised 37% of all the human isolates. Two subtypes predominated among the chicken strains, accounting for 27% of the chicken isolates. Isolates representing the most prevalent bovine SmaI subtypes (13), except S1, underwent no further analysis using KpnI restriction, because no identical SmaI types occurred among the human isolates. The predominant SmaI subtype in cattle, S1, was divided into seven KpnI subtypes, indicating that bovine isolates may be more evenly distributed among different subtypes than those from humans and chickens. This may reflect the diversity of sources of campylobacters in different geographical areas of Finland, where cattle farms are situated all over the country and chicken production is concentrated in the western part. Kwan et al. (26) and French et al. (9) have previously shown that the transmission of *C. jejuni* genotypes occurs over distances of only ca. 1 km at maximum in farmland area.

In a study by Kärenlampi et al. (22), the degree of overlap was 61% between human and chicken isolates and 5.7% between human and bovine isolates. Our observation of a higher overlap between isolates from humans and cattle (15.9%) may be due to the higher number of bovine isolates in our study but may also indicate differences in the sources of infection between rural and urban areas. Our isolates were collected from across the country, excluding the capital city of Helsinki, and thus covered rural areas more extensively than did the human isolates analyzed by Kärenlampi et al. (22) from the Helsinki district in the southern part of Finland. As Ethelberg et al. (6) and Garrett et al. (10) have suggested, the relative importance of poultry as a source of campylobacters may be lower in infections among the rural population. However, a higher percentage of chicken isolates (69.8%), compared with that of bovine strains (15.9%), represented SmaI/KpnI subtypes detected in human infections in our study.

SmaI/KpnI subtypes of *C. jejuni* isolated from chickens and cattle, including shared subtypes, were detected in 52% and 42% of human cases, respectively. Gilpin et al. (11) reported a similar overlap between bovine isolates and human infections. A similar percentage of overlap between campylobacters from chickens and humans, but much higher (83%) between those from cattle and humans, was observed in a study by Nielsen et al. (31). In our study, subtypes shared by chickens and cattle were isolated in 40% of the human cases and could have originated from either of the two animal reservoirs or from some source common to all three of the hosts. Half of the human infections in our study could not be explained by these animal reservoirs, which may indicate the existence of additional sources for campylobacteriosis besides chickens and cattle, as has been suggested previously (2, 23). On the contrary, based on English data, Wilson et al. (40) estimated that meat production animals and poultry are the sources of campylobacters in 97% of sporadic infections.

Hopkins et al. (17) concluded that genotypically similar *C. jejuni* strains are rather able to colonize a range of hosts instead of being host specific. Besides the SmaI/KpnI subtypes shared by all three of the hosts in our study, seven *C. jejuni* subtypes were shared between only humans and poultry and three between only humans and cattle. These subtypes could represent human pathogenic genotypes adapted to chickens and cattle. On the other hand, numerous subtypes were identified among strains isolated only from cattle and some only from chickens but not from human infections. This observation reinforces previous suggestions that probably not all *C. jejuni* types are pathogenic to humans, but nonpathogenic host-specific types may also exist in animal carriers (8, 9, 17, 23, 27, 34). In addition, the most prevalent of the shared *C. jejuni* subtypes in cattle, S5/K27, was detected in only one patient. This type could represent subtypes that are adapted to a specific animal host and that only occasionally cause disease in humans.

The temporal distribution of isolates from human infections and the appearance of indistinguishable SmaI/KpnI subtypes in chicken process lots indicate that up to 31% of the human cases of campylobacteriosis could have been mediated by chickens during the study period. Kärenlampi et al. (21) have presented a similar estimate. *C. jejuni* isolates from 27 (15.4%) human infections not temporally related to chickens were indistinguishable from bovine isolates. Taking into account the three subtypes shared only between humans and cattle (S5/K27, S22/K16, and S66/K18), which occurred in 3.4% of the human cases, an estimated 19% of the Finnish human infections could have been caused by *C. jejuni* strains originating from cattle in the summer of 2003. This estimate should be considered with caution, however, because indistinguishable genotypes may also exist in other animal or environmental sources not included in this study. In addition, some of the human infections temporally associated with chicken isolates could also have been caused by similar bovine campylobacters. However, this study confirms the conclusion of several authors

from other countries (9, 10, 25, 26, 31, 32) that cattle, in addition to chickens, can be an important source of *C. jejuni* for human sporadic infections.

The low-level occurrence of campylobacters in bovine carcasses and beef has been reported in several retail and slaughterhouse surveys (13, 31, 38). Therefore, beef is generally not considered significant in the transmission of campylobacteriosis. Our results support this conclusion, as none of the *C. jejuni* strains isolated from bovine carcasses represented similar SmaI/KpnI subtypes to those of human isolates during the summer of 2003. Direct contact with cattle, fecally contaminated drinking and swimming waters, and raw milk have been suggested as routes of occupational and recreational exposure of rural populations to bovine *C. jejuni* (6, 10, 11). Drinking dug-well water and swimming in natural waters have been identified as risk factors for domestically acquired human campylobacteriosis in Finland (37), and significant associations have been shown between particular sequence-type complexes from human infections and contact with cattle as well as the consumption of unpasteurized milk (23). Most milk is delivered to dairies (ca. 97% in 2003), and the consumption of unpasteurized milk is low in Finland (http://www.matilda.fi/servlet/page?_pageid=501,193&_dad=portal30&_schema=PORTAL30&784_MATILDA_JULKAISUT_4484043.docid=906&784_MATILDA_JULKAISUT_4484043.versio=1170260951). However, occasional failures in milking hygiene can lead to the contamination of milk by campylobacters and cause family outbreaks on dairy cattle farms (36). In Sweden, ruminant density has proven to be more important than poultry-related factors for human campylobacter infections in rural areas (32). The situation may be similar in Finland, where the prevalence of campylobacters in chickens is low (7, 35) and cattle are common carriers of campylobacters (13).

Due to our substantial sampling over a limited time frame, we could estimate the relative contribution of two well-known reservoirs of campylobacters, chickens and cattle, to human campylobacter infections in Finland during the summer of 2003. Although chickens can be considered the most important single source of *C. jejuni* in sporadic, domestically acquired infections, the contribution of cattle appeared notable. Due to overlapping subtypes among chicken and bovine strains, isolates from human infections cannot be directly connected to specific animal sources through PFGE typing without additional epidemiological investigation. Besides hygienic measures in chicken production, new approaches to restrict the occurrence of campylobacters in other farm animals may be needed. However, only about half of the domestic human cases could have originated from the sources examined in our study, and the other half remained unexplained.

ACKNOWLEDGMENTS

This work received partial funding from the Finnish Veterinary Science Foundation.

We thank Kirsi-Maria Eklund, Maaret Hyppönen, Sami-Petteri Karjalainen, and Kristiina Kopisto for their technical assistance in subtyping the isolates and Marja-Liisa Hänninen for her comments on the manuscript. We also thank the Finnish broiler industry for their cooperation.

REFERENCES

1. **Brown, P. E., O. F. Christensen, H. E. Clough, P. J. Diggle, C. A. Hart, S. Hazel, R. Kemp, A. J. H. Leatherbarrow, A. Moore, J. Sutherst, J. Turner, N. J. Williams, E. J. Wright, and N. P. French.** 2004. Frequency and spatial distribution of environmental *Campylobacter* spp. Appl. Environ. Microbiol. **70:**6501–6511.
2. **Carrique-Mas, J., Y. Andersson, M. Hjertqvist, Å. Svensson, A. Torner, and J. Giesecke.** 2005. Risk factors for domestic sporadic campylobacteriosis among young children in Sweden. Scand. J. Infect. Dis. **37:**101–110.
3. **Colles, F. M., K. Jones, R. M. Harding, and M. C. J. Maiden.** 2003. Genetic diversity of *Campylobacter jejuni* isolates from farm animals and farm environment. Appl. Environ. Microbiol. **69:**7409–7413.
4. **Devane, M. L., C. Nicol, A. Ball, J. D. Klena, P. Scholes, J. A. Hudson, M. G. Baker, B. J. Gilpin, N. Garret, and M. G. Savill.** 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. J. Appl. Microbiol. **98:**980–990.
5. **Dingle, K. E., F. M. Colles, R. Ure, J. A. Wagenaar, B. Duim, F. J. Bolton, A. J. Fox, D. R. A. Wareing, and M. C. J. Maiden.** 2002. Molecular characterization of *Campylobacter jejuni* clones: a basis of epidemiologic investigation. Emerg. Infect. Dis. **8:**949–955.
6. **Ethelberg, S., J. Simonsen, P. Gerner-Schmidt, K. E. P. Olsen, and K. Mølbak.** 2005. Spatial distribution and registry-based case-control analysis of *Campylobacter* infections in Denmark, 1991–2001. Am. J. Epidemiol. **162:**1008–1015.
7. **European Food Safety Authority.** 2007. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. EFSA J. **130:**106–352.
8. **Fitzgerald, C., K. Stanley, S. Andrew, and K. Jones.** 2001. Use of pulsed-field gel electrophoresis and flagellin gene typing in identifying clonal groups of *Campylobacter jejuni* and *Campylobacter coli* in farm and clinical environments. Appl. Environ. Microbiol. **67:**1429–1436.
9. **French, N., M. Barrigas, P. Brown, P. Ribiero, N. Williams, H. Leatherbarrow, R. Birtles, E. Bolton, P. Fearnhead, and A. Fox.** 2005. Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. Environ. Microbiol. **7:**1116–1126.
10. **Garrett, N., M. L. Devane, J. A. Hudson, C. Nicol, A. Ball, J. D. Klena, P. Scholes, M. G. Baker, B. J. Gilpin, and M. G. Savill.** 2007. Statistical comparison of *Campylobacter jejuni* subtypes from human cases and environmental sources. J. Appl. Microbiol. **103:**2113–2121.
11. **Gilpin, B. J., B. Thorrold, P. Scholes, R. D. Longhurst, M. Devane, C. Nicol, S. Walker, B. Robson, and M. Savill.** 2008. Comparison of *Campylobacter jejuni* genotypes from dairy cattle and human sources from the Matamata-Piako District of New Zealand. J. Appl. Microbiol. **105:**1354–1360.
12. **Gormley, F. J., M. MacRae, K. J. Forbes, I. D. Ogden, J. F. Dallas, and J. C. Strachan.** 2008. Has retail chicken played a role in the decline of human campylobacteriosis? Appl. Environ. Microbiol. **74:**383–390.
13. **Hakkinen, M., H. Heiska, and M.-L. Hänninen.** 2007. Prevalence of *Campylobacter* spp. in cattle in Finland and antimicrobial susceptibility of bovine *Campylobacter jejuni* strains. Appl. Environ. Microbiol. **73:**3232–3238.
14. **Hakkinen, M, and M.-L. Hänninen.** 26 March 2009. Shedding of *Campylobacter* spp. in Finnish cattle on dairy farms. J. Appl. Microbiol. doi:10.1111/j.1365-2672.2009.04269.
15. **Hänninen, M.-L., P. Perko-Mäkelä, A. Pitkälä, and H. Rautelin.** 2000. A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. J. Clin. Microbiol. **38:**1998–2000.
16. **Hofshagen, M., and H. Kruse.** 2005. Reduction in flock prevalence of *Campylobacter* spp. in broilers in Norway after implementation of an action plan. J. Food Prot. **68:**2220–2223.
17. **Hopkins, K. L., M. Desai, J. A. Frost, J. Stanley, and J. M. J. Logan.** 2004. Fluorescent amplified fragment length polymorphism genotyping of *Campylobacter jejuni* and *Campylobacter coli* strains and its relationship with host specificity, serotyping, and phage typing. J. Clin. Microbiol. **42:**229–235.
18. **Hopkins, R. S., R. Olmsted, and G. R. Istre.** 1984. Endemic *Campylobacter jejuni* infection in Colorado: identified risk factors. Am. J. Public Health **74:**249–250.
19. **International Organization for Standardization.** 2006. International standard ISO 10272-1. Microbiology of food and animal feeding stuffs—horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: detection method. International Organization for Standardization, Geneva, Switzerland.
20. **Kapperud, G., E. Skjerve, N. H. Bean, S. M. Ostroff, and J. Lassen.** 1992. Risk factors for sporadic *Campylobacter* infections in southeastern Norway. J. Clin. Microbiol. **30:**3117–3121.
21. **Kärenlampi, R., H. Rautelin, M. Hakkinen, and M.-L. Hänninen.** 2003. Temporal and geographical distribution and overlap of Penner heat-stable serotypes and pulsed-field electrophoresis genotypes of *Campylobacter jejuni* isolates collected from humans and chickens in Finland during a seasonal peak. J. Clin. Microbiol. **41:**4870–4872.
22. **Kärenlampi, R., H. Rautelin, and M.-L. Hänninen.** 2007. Evaluation of genetic markers and molecular typing methods for prediction sources of *Campylobacter jejuni* and *C. coli* infections. Appl. Environ. Microbiol. **73:**1683–1685.
23. **Kärenlampi, R., H. Rautelin, D. Schönberg-Norio, L. Paulin, and M.-L. Hänninen.** 2007. Longitudinal study of Finnish *Campylobacter jejuni* and *C.*

coli isolates from humans, using multilocus sequence typing, including comparison with epidemiological data and isolates from poultry and cattle. Appl. Environ. Microbiol. **73:**148–155.
24. **Katzav, M., P. Isohanni, M. Lund, M. Hakkinen, and U. Lyhs.** 2008. PCR assay for the detection of *Campylobacter* in marinated and non-marinated poultry products. Food Microbiol. **25:**908–914.
25. **Kwan, P. L. S., M. Barrigas, F. J. Bolton, N. P. French, P. Gowland, R. Kemp, H. Leatherbarrow, M. Upton, and A. J. Fox.** 2008. Molecular epidemiology of *Campylobacter jejuni* populations in dairy cattle, wildlife, and the environment in a farmland area. Appl. Environ. Microbiol. **74:**5130–5138.
26. **Kwan, P. L. S., A. Birtles, F. J. Bolton, N. P. French, S. E. Robinson, L. S. Newbold, M. Upton, and A. J. Fox.** 2008. Longitudinal study of the molecular epidemiology of *Campylobacter jejuni* in cattle on dairy farms. Appl. Environ. Microbiol. **74:**3626–3633.
27. **Manning, G., C. G. Dowson, M. C. Bagnall, I. H. Ahmed, M. West, and D. G. Newell.** 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl. Environ. Microbiol. **69:**6370–6379.
28. **Meldrum, R. J., J. K. Griffiths, R. M. M. Smith, and M. R. Evans.** 2005. The seasonality of human campylobacter infection and *Campylobacter* isolates from fresh, retail chicken in Wales. Epidemiol. Infect. **133:**49–52.
29. **Nadeau, É., S. Messier, and S. Quessy.** 2002. Prevalence and comparison of genetic profiles of *Campylobacter* strains isolated from poultry and sporadic cases of campylobacteriosis in humans. J. Food Prot. **65:**73–78.
30. **National Public Health Institute.** 2005. Infectious diseases in Finland, 1995–2004. National Public Health Institute, Helsinki, Finland.
31. **Nielsen, E. M., V. Fussing, J. Engberg, and N. L. Nielsen.** 2006. Most *Campylobacter* subtypes from sporadic cases can be found in retail poultry products and food animals. Epidemiol. Infect. **134:**758–767.
32. **Nygård, K., Y. Andersson, J. A. Røttingen, Å. Svensson, J. Lindbäck, T. Kistemann, and J. Giesecke.** 2004. Association between environmental risk factors and campylobacter infections in Sweden. Epidemiol. Infect. **132:**317–325.
33. **Nylen, G., F. Dunstan, S. R. Palmer, Y. Andersson, F. Bager, J. Cowden, G. Feierl, Y. Galloway, G. Kapperud, F. Megraud, K. Mølbak, L. R. Petersen, and P. Ruutu.** 2002. The seasonal distribution of campylobacter infection in nine European countries and New Zealand. Epidemiol. Infect. **128:**383–390.
34. **On, S. L. W., E. M. Nielsen, J. Engberg, and M. Madsen.** 1998. Validity of SmaI-defined genotypes of *Campylobacter jejuni* examined by SalI, KpnI and BamHI polymorphism; evidence of identical clones infecting humans, poultry, and cattle. Epidemiol. Infect. **120:**231–237.
35. **Perko-Mäkelä, P., M. Hakkinen, T. Honkanen-Buzalski, and M.-L. Hänninen.** 2002. Prevalence of campylobacters in chicken flocks during the summer of 1999 in Finland. Epidemiol. Infect. **129:**187–192.
36. **Schildt, M., S. Savolainen, and M.-L. Hänninen.** 2005. Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family. Epidemiol. Infect. **133:**1–5.
37. **Schönberg-Norio, D., J. Takkinen, M.-L. Hänninen, S.-S. Kaukoranta, L. Mattila, and H. Rautelin.** 2004. Swimming and *Campylobacter* infections. Emerg. Infect. Dis. **10:**1474–1477.
38. **Stern, N. J., K. L. Hiett, G. A. Alfredsson, K. G. Kristinsson, J. Reiersen, H. Hardardottir, H. Briem, E. Gunnarsson, F. Georgsson, R. Lowman, E. Berndtson, A. M. Lammerding, G. M. Paoli, and M. T. Musgrove.** 2003. *Campylobacter* spp. in Icelandic poultry operations and human disease. Epidemiol. Infect. **130:**23–32.
39. **Whyte, P., K. McGill, D. Cowley, R. H. Madden, L. Moran, P. Scates, C. Carroll, A. O'Leary, S. Fanning, J. D. Collins, E. McNamara, J. E. Moore, and M. Cormican.** 2004. Occurrence of *Campylobacter* in retail foods in Ireland. Int. J. Food Microbiol. **95:**111–118.
40. **Wilson, D. J., E. Gabriel, A. H. J. Leatherbarrow, J. Cheesbrough, S. Gee, E. Bolton, A. Fox, P. Fearnhead, C. A. Hart, and P. Diggle.** 2008. Tracing the source of campylobacteriosis. PLoS Genet. **4:**1–9.
41. **Wingstrand, A., J. Neimann, J. Engberg, E. M. Nielsen, P. Gerner-Schmidt, H. Wegener, and K. Mølbak.** 2006. Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg. Infect. Dis. **12:**280–284.
42. **Zhao, C., B. Ge, J. de Villena, R. Sudler, E. Yeh, S. Zhao, D. White, D. Wagner, and J. Meng.** 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. Appl. Environ. Microbiol. **67:**5431–5436.