Université de Sherbrooke

Épidémiologie moléculaire des entérites à Campylobacter en Estrie

Par
Simon Lévesque
Programme de microbiologie

Thèse présentée à la Faculté de médecine et des sciences de la santé
en vue de l'obtention du grade de philosophiae doctor (Ph.D.) en microbiologie

Sherbrooke, Québec, Canada
Avril 2013

Membres du jury d'évaluation
Sophie Michaud, programme de microbiologie
Eric Frost, programme de microbiologie
Antonio Conconi, programme de microbiologie
Vincent Burrus, département de biologie, faculté des sciences,
Université de Sherbrooke
Marcel Behr, Division of Infectious Diseases and Medical Microbiology,
McGill University

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-95105-7

Our file Notre référence
ISBN: 978-0-494-95105-7

« Dans le cœur de l'homme, il y a une bactérie qui sommeille. »

Bernard Weber

RÉSUMÉ

Épidémiologie moléculaire des entérites à Campylobacter en Estrie

Par
Simon Lévesque
Programme de microbiologie

Thèse présentée à la Faculté de médecine et des sciences de la santé en vue de l'obtention du diplôme de philosophiae doctor (Ph.D.) en microbiologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4

Le Campylobacter est la première cause de gastro-entérites bactériennes dans les pays industrialisés. La grande majorité des cas sont des infections sporadiques dont la source est rarement identifiée. Le Campylobacter fait partie de la flore intestinale normale d'une large diversité d'animaux et peut également se retrouver dans l'eau. Le but de mon projet de recherche était d'étudier l'épidémiologie clinique et moléculaire des infections à Campylobacter en Estrie afin de déterminer les principales sources d'infections sporadiques et de comparer les génotypes des isolats de Campylobacter selon les différentes niches écologiques. Nous avons déterminé le profil de sensibilité aux antibiotiques d'isolats de différentes sources. Nous avons observé un haut taux de résistance à l'érythromycine et à la tétracycline et un faible taux de résistance à la ciprofloxacine chez les isolats de poulet, pouvant refléter l'utilisation de ces antibiotiques dans cet élevage. Le fait que le taux de résistance à l'érythromycine parmi les isolats humains soit significativement moins élevé que chez les isolats de poulet suggérait l'importance d'autres sources de Campylobacter chez l'humain. Afin de déterminer quelle méthode de typage moléculaire serait la mieux adaptée pour notre devis de recherche, nous avons comparé quatre méthodes (AFLP, MLST, typage du gène *fla* et EGCP). Seul le MLST a pu attribuer des isolats humains à des niches écologiques particulières comme le poulet, le lait cru et l'eau. Afin d'optimiser la technique de MLST, nous avons développé un système complémentaire basé sur le HRM, qui est beaucoup plus rapide et moins coûteux que le MLST. Nous avons démontré que le HRM a le potentiel de complémenter les méthodes d'analyses basées sur du séquençage pour l'étude des mutations ponctuelles et de faciliter une vaste gamme d'études basées sur des méthodes génotypiques, telle la détection de mutations ponctuelles qui confèrent de la résistance aux antibiotiques. Nous avons entrepris par la suite une étude cas-cas et un vaste projet d'isolement et de caractérisation moléculaire de souches de Campylobacter en Estrie, afin de véritablement cerner les mécanismes de transmission de la bactérie et de comparer les sources d'infections sporadiques chez les cas acquis en régions rurales vs urbaines. Nous avons confirmé que le poulet était responsable de la majorité des cas de campylobactérioses. Cependant, nos résultats suggèrent que la saisonnalité ainsi que le gradient urbain-rural de la campylobactériose sont dus à l'exposition aux souches bovines, particulièrement chez le groupe d'âge des 15-34 ans via l'exposition professionnelle. Par la détermination des sources d'infections, nous avons établi des pistes d'interventions utilisables par les autorités de santé publique, afin de diminuer l'incidence de la campylobactériose au Québec.

Mots clés : Campylobacter, MLST, épidémiologie moléculaire, résistance aux antibiotiques

SUMMARY

Molecular epidemiology of *Campylobacter* enteritidis in the Eastern Townships

By
Simon Lévesque
Microbiology Program

Thesis presented at the Faculty of medicine and health sciences for the obtention of doctoral degree philosophiae doctor (Ph.D.) in microbiology, Faculty of medicine and health sciences, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4

Campylobacteriosis is the leading notifiable enteric disease in industrialised countries. It colonizes a wide range of animal which in turn spread the disease. The majority of campylobacteriosis cases are sporadic infections for which the source is rarely apparent. The main goal of my research project is to determine contamination sources of *Campylobacter* in the Eastern Townships, to identify the sources and routes of transmission and to establish the main sources of sporadic infections. We determined antimicrobial susceptibility profiles of *Campylobacter* isolates in order to predict which bacterial population will be resistant, caused by antimicrobial selective pressure administered to the host. High levels of resistance of chicken isolates to erythromycin and tetracycline, and low levels of resistance to ciprofloxacin reflect the use of the former antibiotics in animal husbandry. The fact that the erythromycin and tetracycline resistance levels were significantly lower among human isolates suggests that other transmission sources are important for human infection. In order to determine which molecular typing method will be the most relevant for our research design, we compared four typing methods (AFLP, MLST, *fla* typing and PFGE). Only MLST has the potential to link isolates to a particular ecological niche, such as chicken, raw milk and water. In order to optimize MLST, we developed a complementary system based on HRM. We demonstrated that HRM has the potential to complement the analysis methods based on sequencing for SNP and facilitate a wide range of studies based on genotypic methods. We have subsequently undertaken a major project of isolation and molecular characterization of *Campylobacter* in the Eastern Townships, in order to truly understand the mechanisms of transmission of the bacteria and determine the source of sporadic cases. We confirmed that chicken was responsible for the majority of cases of campylobacteriosis. However, we have shown that the urban-rural gradient of campylobacteriosis in the Eastern Townships could be explained by exposure to bovine, especially for the 15-34 year old age group through occupational exposure. By the identification of infection sources, we proposed courses of action that could be used by public health authorities to reduce the incidence of campylobacteriosis in Quebec.

Keywords: *Campylobacter*, MLST, molecular epidemiology, antimicrobial resistance

TABLE DES MATIÈRES

LISTE DES ILLUSTRATIONS

Liste des figures

LISTE DES SIGLES, ABRÉVIATIONS ET SYMBOLES

ADN : Acide désoxyribonucléique

AFLP : *Amplified fragment-length polymorphism*

CC : Complexe clonal ou *Clonal complex*

CMI : Concentration minimale inhibitrice

CVNC : Cellules viable mais non cultivables

EGCP : Électrophorèse sur gel en champ pulsé

HRM : *High-resolution melting*

MADO : Maladie à déclaration obligatoire

MAPAQ : Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec

MLEE : *Multi-locus enzyme electrophoresis*

MLST : *Multi-locus sequence typing*

MRC : Municipalité régionale de comté

PCR : *Polymerase chain reaction*

PCR-RFLP : *PCR-restriction fragment length polymorphism*

PFGE : *Pulsed-field gel electrophoresis*

SGB : Syndrome de Guillain-Barré

SNP : *Single nucleotide polymorphisms*

ST : *Sequence type*

SVR : *Short variable repeat*

T_m : Température de fusion ou *melting temperature*

UFC : Unité formant des colonies

INTRODUCTION

1.1 Historique du Campylobacter

Le Campylobacter fut décrit pour la première fois en 1886 par nul autre que Theodore Escherich qui observa une bactérie de forme spiralée dans le colon d'un enfant décédé d'une maladie nommée à l'époque *cholera infantum* (Skirrow et Butzler, 2000). Il fut cependant incapable de cultiver ce microorganisme. Il fallut attendre jusqu'en 1906 pour que John McFadyean et Stewart Stockman isolent pour la première fois en culture pure un microorganisme « vibrio » provenant de mucus utérin d'une brebis gestante dans un troupeau présentant un taux d'avortement de 33%. En 1919, Theobald Smith et Marian Taylor réisolèrent le même microorganisme lors d'un avortement chez un bovin et le nommèrent *Vibrio fetus* (Skirrow, 2006). Deux autres microorganismes furent subséquemment isolés en 1931 (*Vibrio jejuni*, isolé du jéjunum d'un bovin) et en 1944 (*Vibrio coli*, isolé chez le porc). Ce n'est qu'en 1973, suite à des travaux d'un groupe français, que ces microorganismes furent reconnus comme un groupe de bactérie distinct et renommés Campylobacter. Dû à la grande difficulté à isoler le microorganisme, les premiers isolements de Campylobacter dans les fèces humaines eurent lieu au début des années 1970 et avec l'avènement d'un milieu de culture sélectif pour le Campylobacter, le milieu Skirrow. La communauté scientifique prit alors véritablement conscience de l'importance du Campylobacter comme étant la cause la plus fréquente d'entérites bactériennes acquises en communauté dans le monde (Engberg, 2006).

1.2 Microbiologie et aspects cliniques des infections à Campylobacter

Le Campylobacter est un petit bâtonnet incurvé ou en forme de « S » de 0,2 à 0,8 µm de large par 0,5 à 5 µm de long. Cette bactérie Gram négatif, non sporulante, à une mobilité en tire bouchon par un seul flagelle polaire nu ou par un flagelle à chaque extrémité (Vandamme, 2000). Ce genre bactérien est considéré comme fastidieux, entre autres, pour son système respiratoire microaérophile nécessitant entre 3 à 15% d'oxygène pour croître (Macfaddin, 2000). La température optimale de croissance se situe entre 30-37°C, sauf pour les Campylobacter thermophiliques (*C. jejuni*, *C. coli* et *C. lari*) qui poussent mieux à 42°C (On, 2005). Le genre Campylobacter compte maintenant 23 espèces et 8 sous-espèces, dont 7 identifiées durant les 4 dernières années (Debruyne *et al.*, 2009). De ce nombre, une dizaine d'espèces et sous-espèces ont été associées avec des maladies chez les animaux ou encore chez l'humain (Chaban *et al.*, 2009).

Le Campylobacter est l'agent bactérien causant le plus de diarrhées dans le monde et représente à lui seul environ 10% de tous les cas d'entérites (EYLES *et al.*, 2003). En 2004 au Canada, le taux de campylobactériose était de 30,22 par 100 000 habitants, ce qui en faisait la quatrième maladie à déclaration obligatoire (MADO) en importance (Agence de santé publique du Canada, 2009). En 2009 au Québec, 2025 cas de campylobactériose étaient déclarés, ce qui est plus important que tous les cas de Salmonelle, Shigelle, *Yersinia enterocolitica* et *Escherichia coli* O157:H7 réunis (Ménard, 2006, MSSS, 2010). Il a été récemment avancé que ce nombre de cas serait substantiellement sous-évalué et que pour chaque cas de campylobactériose déclaré au

Canada chaque année, il y aurait de 23 à 49 cas additionnels non déclarés (Thomas *et al.*, 2006).

Après une période d'incubation de 3 à 5 jours, les principaux symptômes de la campylobactériose sont des crampes abdominales, suivies de diarrhées qui peuvent parfois être sanglantes. Les autres symptômes rencontrés sont la fièvre, les nausées, les vomissements et les maux de tête (Engberg, 2006). La majorité des patients guérissent d'eux-mêmes au bout de quelques jours, mais jusqu'à 20% peuvent avoir une diarrhée prolongée ou encore des complications suite à l'infection et avoir besoin d'un traitement aux antibiotiques (Skirrow et Blaser, 2000). Les antibiotiques habituellement utilisés sont les macrolides, les fluoroquinolones et plus rarement la tétracycline. La principale complication d'une infection à Campylobacter est le développement d'un syndrome de Guillain Barré (SGB) dans environ 1 cas d'infection sur 2000. Le SGB se manifeste par une polyneuropathie démyélinisante sévère caractérisée par une attaque auto-immune de la myéline du système nerveux périphérique (Allos *et al.*, 1998).

La dose infectieuse de *C. jejuni* est relativement faible chez l'humain. Dans une étude sur des volontaires, la dose infectieuse fut estimée à 800 organismes (Black *et al.*, 1988) et Robinson rapporta sa propre expérience suite à l'ingestion de 500 organismes (Robinson, 1981). Cependant, la relation dose-réponse fut récemment mise à jour basée sur deux épidémies de campylobactériose dues à la consommation de lait cru chez des enfants. Les auteurs de cette étude ont suggéré que la probabilité de développer la maladie en ingurgitant de petites doses infectieuses était de 36 fois supérieures qu'estimée précédemment (Teunis *et al.*, 2005). La répartition de

l'incidence de la maladie selon l'âge démontre une distribution unique avec des pics d'incidence chez les 0-4 ans et chez les 15-24 ans. Le premier pic s'explique probablement par l'incidence d'une première infection chez le jeune enfant ou conséquemment aux diagnostics plus fréquents dus au fait que les parents ont tendance à consulter un médecin plus rapidement lorsque leur enfant est malade. Le deuxième pic s'explique cependant difficilement, mais plusieurs l'attribuent au « deuxième sevrage » (i.e. le départ des jeunes de chez leurs parents) qui amènerait des déficiences au niveau de l'hygiène alimentaire chez ce groupe d'âge (Friedman *et al.*, 2000).

Dans la plupart des pays développés, les cas de campylobactériose présentent une distribution saisonnière avec une incidence plus élevée dans les mois les plus chauds (Friedman *et al.*, 2000), ce qui correspond au Canada avec l'été et le début de l'automne. Une étude récente effectuée au Manitoba a soulevé des variations géographiques du Campylobacter associant les plus hauts taux de campylobactériose avec les régions où la densité animale et l'agriculture étaient les plus fortes. Cette différence entre les milieux urbains et ruraux était encore plus évidente chez les 0 à 4 ans. Dans ce groupe d'âge, les garçons vivant en milieu rural développaient 7,3 fois plus de campylobactériose que ceux vivant en milieu urbain, et cette différence était aussi observable chez les filles avec 6,95 fois plus de cas en milieu rural qu'en milieu urbain (Green *et al.*, 2006). En Estrie, des variations géographiques ont également été observées lors d'une étude cas-témoins effectuée en 2001-2002 où la MRC d'Asbestos présentait un risque de campylobactériose 2,4 fois plus élevé que dans le reste de l'Estrie (Michaud *et al.* , 2004).

1.3 Épidémiologie clinique et écologie du Campylobacter

L'épidémiologie du Campylobacter n'est pas encore complètement comprise, mais les sources majeures de contamination sont maintenant connues (FIGURE 1). Cependant, la majorité des infections sont des cas sporadiques dont la source est rarement identifiée (Thomas *et al.*, 2006). Le Campylobacter fait partie de la flore intestinale normale d'une large diversité d'animaux domestiques, d'élevages et sauvages. Parmi les animaux élevés pour la consommation, on retrouve surtout du *C. jejuni* chez les bovins, les poulets et les dindons, tandis que *C. coli* se retrouve en majorité chez le porc. La viande peut devenir contaminée par du Campylobacter suite à un contact fécal durant le processus d'abattage. Le Campylobacter peut survivre aux différentes procédures auxquelles les poulets sont soumis dans les abattoirs et usines de transformation avant d'être mis en vente sur le marché. Le taux de contamination de la viande varie beaucoup selon les pays, mais en général le bœuf et le porc ont de faibles taux comparativement au poulet qui affiche les plus hauts taux de contamination (Engberg, 2006, Moore *et al.*, 2005), possiblement dû à des techniques d'abattage et de refroidissement différentes des carcasses. L'utilisation d'eau en grande quantité tout au long du processus d'abatage des poulets contribuerait également à la dispersion et à la survie du Campylobacter (Jacobs-Reitsma, 2000).

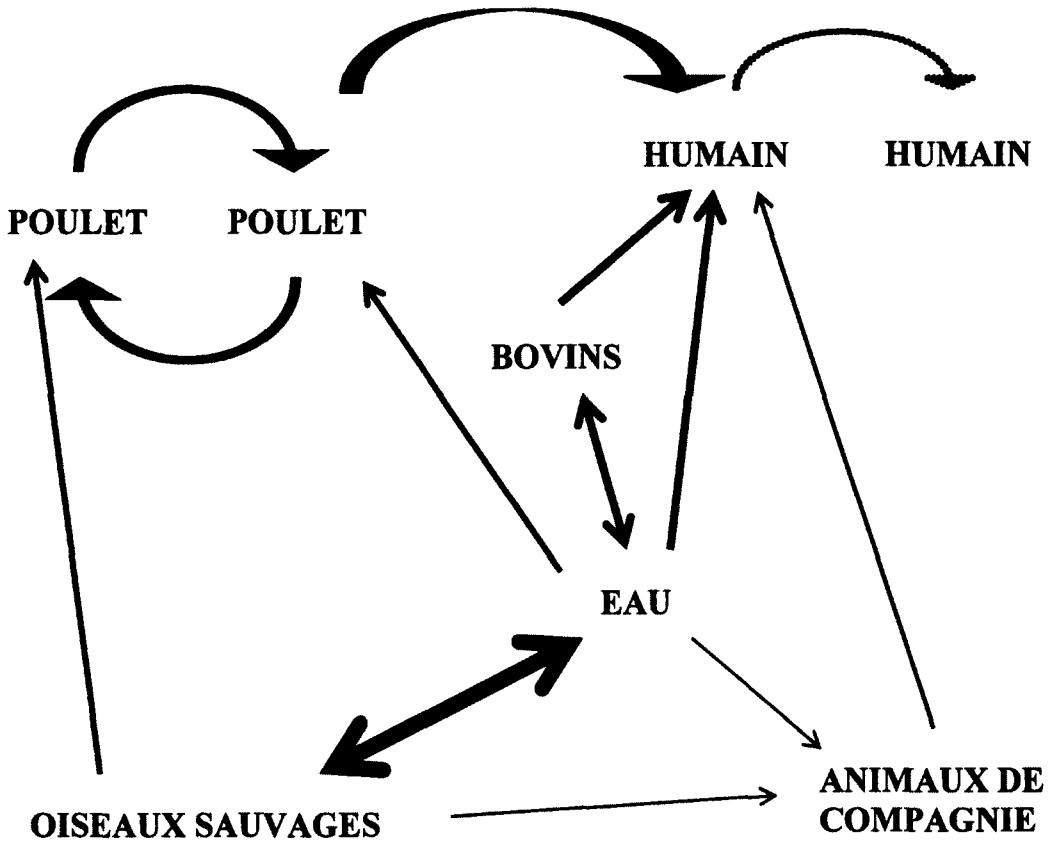


FIGURE 1. Modèle de transmission du *C. jejuni*. Adaptation du modèle proposé en 1999 par Friedman *et al.* (Friedman *et al.*, 2000). L'intensité de la flèche corrèle avec l'importance de la transmission.

1.3.1 Les poulets. La colonisation intestinale des poulets par le Campylobacter se fait assez tôt dans la vie de l'animal et il demeure habituellement asymptomatique jusqu'à son abattage (Gibbens *et al.*, 2001), maintenant un niveau de Campylobacter d'au moins 10^{10} UFC par g de contenu intestinal (Shreeve *et al.*, 2000). La transmission horizontale du Campylobacter à travers l'élevage se fait principalement via la contamination de la nourriture et de l'eau par des matières fécales et par la tendance des poulets à adopter un comportement coprophage. Il a été rapporté que l'insertion d'un poulet contaminé au Campylobacter dans un élevage sans

Campylobacter menait à la contamination de tous les poulets de l'élevage en 3 jours (Shanket *et al.*, 1990).

Le poulet est considéré par la majorité de la communauté scientifique comme étant le réservoir principal de Campylobacter. Sa manipulation et le fait de le consommer mal cuit sont des facteurs de risque importants (Wilson *et al.*, 2008). Une étude effectuée en 2008 en Angleterre estimait que le poulet était la source de 56,5% des cas humains de campylobactériose (Wilson *et al.*, 2008). La crise de la dioxine survenue en 1999 en Belgique fut un excellent contexte pour l'estimation du nombre de campylobactérioses attribuable au poulet. En effet, lors de cette crise, le gouvernement de Belgique ordonna l'arrêt de la vente de poulet sur son territoire et la destruction de tous les produits contenant du poulet. Les autorités de santé publique constatèrent une diminution de 40% des cas de campylobactériose et les auteurs de l'étude attribuèrent donc cette proportion de cas au poulet (Vellinga et Van Loock, 2002). Au Danemark, les autorités ont instauré un vaste plan de contrôle du Campylobacter dans l'industrie du poulet. Suite à des diminutions importantes du Campylobacter dans les élevages de poulet et au retrait de la vente des poulets frais contaminés, la diminution des cas de campylobactériose humaine ne fut que de 12 % (Rosenquist *et al.*, 2009). L'étude cas-témoins estrienne de 2000-2001 avait démontré que seulement 28% des cas explicables par leur modèle étaient associés à la consommation de poulet (Michaud *et al.*, 2004).

1.3.2 Les bovins. Le bovin est également un grand réservoir naturel du Campylobacter. Plusieurs auteurs ont démontré que le tractus intestinal des bovins pouvait être colonisé par une variété d'espèces de Campylobacter, principalement *C.*

jejuni, *C. fetus*, *C. hyointestinalis* et *C. lanienae* (Atabay et Cory, 1998, Guevremont *et al.*, 2008, Inglis *et al.*, 2006). Les bovins sont habituellement des porteurs asymptomatiques, cependant certaines espèces peuvent être pathogènes. *C. fetus* subsp. *fetus* est reconnu pour causer des avortements chez les vaches et *C. fetus* subsp. *veneralis* est responsable d'infertilité chez les taureaux (Atabay et Cory, 1998). Les autres espèces ont déjà été reliées à des entérites chez les veaux et quelques cas de mammites à *C. jejuni* ont déjà été rapportés (Gudmundson et Chirino-Trejo, 1993). Le lait cru contaminé est reconnu comme une source de campylobactériose humaine et est l'une des rares sources de contamination reliées à des épidémies. La contamination du lait cru se fait habituellement par des matières fécales bovines, mais une contamination directe de bactéries dans le lait lors d'une mammite à *C. jejuni* a déjà été rapportée (Orr *et al.*, 1995). Plusieurs épidémies bien documentées sont rapportées dans la littérature (Blaser *et al.*, 1983, Evans *et al.*, 1996, Finch et Blake, 1985, Kornblatt *et al.*, 1985; Wood *et al.*, 1992). La plus grande épidémie de campylobactériose documentée à ce jour impliqua 2500 enfants infectés par du lait à l'école au Royaume-Uni en 1979 (Jones *et al.*, 1981). En Estrie, les données les plus récentes ont démontré que 18% des cas de campylobactériose pouvaient être expliqués par la consommation de lait cru (Michaud *et al.*, 2004). Cependant, il a été avancé que la consommation fréquente de lait cru pouvait amener une certaine immunité contre Campylobacter (Friedman *et al.*, 2000).

1.3.3 Les oiseaux sauvages. Les oiseaux sauvages (goélands, canards, oie, bernaches, moineaux, etc.) sont reconnus par plusieurs études comme un réservoir de Campylobacter (Kinzelman *et al.*, 2008, Quessy et Messier, 1992, Wahlstrom *et al.*, 2003). À ma connaissance, des cas de contamination par contact direct entre un oiseau

sauvage et un humain n'ont jamais été rapportés. L'implication des oiseaux sauvages se situe surtout au niveau de la contamination de l'eau et des plages par leurs excréments qui contiennent du Campylobacter (Kinzelman *et al.*, 2008). Le goéland est notamment reconnu pour être le réservoir principal du *C. lari* (Matsuda *et al.*, 2004, Wheelan *et al.*, 1988).

1.3.4 Les animaux de compagnies. Les animaux de compagnie pour leur part ont été associés comme facteur de risque de développer une campylobactériose et ce principalement chez les jeunes enfants (Salfield et Pugh, 1987, Tenkate et Stafford, 2001). Il est prouvé que les chats et les chiens en santé peuvent être des porteurs asymptomatiques et qu'ils sont les réservoirs principaux du *C. upsaliensis* (Chaban *et al.*, 2009, Moreno *et al.*, 1993). Une moins bonne hygiène et un contact plus étroit que normalement avec les animaux domestiques peuvent en partie expliquer le risque accru de campylobactériose chez les jeunes enfants.

1.3.5 L'eau. La contribution de l'eau comme cas d'infections sporadiques varie beaucoup à travers le monde, mais plusieurs études ont démontré l'importance de ce mode de contamination via l'eau potable ou l'eau de baignade (Friedman *et al.*, 2000). Selon certains auteurs, l'eau pourrait être le plus important réservoir de Campylobacter, non seulement pour son potentiel d'infection chez l'humain, mais aussi pour son potentiel de transmission de la bactérie pour les animaux d'élevage, les animaux et oiseaux sauvages et les animaux de compagnie (Miller et Mandrell, 2005). Le Campylobacter a été isolé dans une multitude de sources d'eau différentes, allant des estuaires marins (Hernandez *et al.*, 1996) à l'eau de rivière et à l'eau potable (St-Pierre *et al.*, 2009). Il a également été démontré que le Campylobacter peut survivre

dans les biofilms formés dans les tuyaux d'aqueducs (Lehtola *et al.*, 2006) et aussi dans l'environnement aquatique (Buswell *et al.*, 1998). Une étude effectuée en Suède a démontré que le risque de campylobactérioses augmentait avec la longueur des tuyaux desservant l'eau potable (Nygard *et al.*, 2004). Il a également été démontré qu'environ 20% de l'eau potable produite était perdue par des fuites le long du système de distribution. Durant des périodes de plus faibles pressions, ces points de fuite peuvent devenir des portes d'entrée pour la contamination bactérienne. Le risque de contamination croisée augmente particulièrement lorsque les tuyaux d'eau potable sont adjacents aux tuyaux d'égout, ce qui est régulièrement le cas pour des considérations pratiques lors de la construction des réseaux.

Les principaux modes de contamination de l'eau environnementale sont : (i) la contamination par les excréments des oiseaux sauvages (principalement les goélands et les oiseaux migrateurs), (ii) le lessivage des terres agricoles suite à l'épandage des lisiers, (iii) les eaux usées provenant des fermes, sites d'élevages et abattoirs et (iv) la contamination par les systèmes d'eau usée des municipalités (Miller et Mandrell, 2005). Pour l'eau potable ou pour l'eau de baignade des plages publiques, aucun test de dépistage n'est présentement disponible pour le Campylobacter. En fait, les laboratoires de références pour la qualité de l'eau se fient à la présence des coliformes fécaux (principalement *E. coli*) pour certifier la qualité de l'eau de consommation et de baignade (Centre d'expertise en analyse environnementale du Québec, 2005). Nous avons démontré l'absence de corrélation entre les coliformes fécaux et le Campylobacter dans l'eau, tant au niveau qualitatif que quantitatif (St-Pierre *et al.*, 2009). Également, une étude conduite au Québec a suggéré que 35% des gastroentérites seraient attribuables à l'eau potable, malgré que les différents points de

production répondaient aux normes (Payment *et al.*, 1991). Il est donc nécessaire de réévaluer les critères et les protocoles pour la surveillance de la qualité de l'eau de consommation et de l'eau à des fins récréatives. Plusieurs problèmes se posent cependant : le Campylobacter est un organisme fastidieux, aucun protocole de détection standardisé n'est disponible, tant par culture que par PCR en temps réel et un débat persiste toujours sur l'existence des colonies viables, mais non cultivables (CVNC) et sur leur pouvoir infectieux.

1.4 La résistance aux antibiotiques

Comme mentionné précédemment, pour la majorité des cas, la campylobactériose est une maladie que le système immunitaire réussit à combattre efficacement. Un traitement aux antibiotiques est donc habituellement réservé pour les cas sévères, les infections systémiques, les individus immunosupprimés et les groupes à risque (Skirrow et Blaser, 2000). Cependant, l'utilisation d'un traitement aux antibiotiques peut être problématique dans certains pays dus à l'émergence de la résistance. Depuis les années 1990, la résistance à la tétracycline, aux fluoroquinolones et un peu plus tard aux macrolides a clairement augmenté, tant dans les pays en développement que dans les pays industrialisés (Engberg *et al.*, 2004, Gaudreau et Gilbert, 1997, Gibrel et Taylor, 2006, Gupta *et al.*, 2004). Conséquemment, la résistance aux antibiotiques chez le Campylobacter acquis via l'alimentation est maintenant reconnue comme un problème émergeant en santé publique (Engberg *et al.*, 2004). La résistance peut apparaitre durant le traitement d'un patient, mais il est bien reconnu que l'utilisation des antibiotiques dans le milieu

vétérinaire contribue à l'augmentation des souches résistantes infectant l'humain (Lindmark, 2004).

1.4.1 Les fluoroquinolones. Les fluoroquinolones sont des agents actifs contre la plupart des pathogènes causant des entérites bactériennes (Nachamkin *et al.*, 2000). Les fluoroquinolones sont des molécules chimiquement modifiées à partir de l'acide nalidixique, une quinolone. Cette classe d'antibiotiques cible deux enzymes essentielles dans la réplication bactérienne : l'ADN gyrase (l'ADN topoisomérase de type II) et l'ADN topoisomérase IV, inhibant la croissance bactérienne (Taylor et Tracz, 2005). Ces deux enzymes sont emprisonnés dans un complexe par les fluoroquinolones, empêchant la réplication de l'ADN et empoisonnant la cellule. L'antibiotique le plus utilisé dans cette classe est la ciprofloxacine. La résistance à une fluoroquinolone ou à l'acide nalidixique procure habituellement une résistance croisée aux autres fluoroquinolones (Trieber et Taylor, 2000).

La résistance aux fluoroquinolones est principalement obtenue par une mutation dans la séquence d'ADN codant pour la sous-unité *gyrA* le l'ADN gyrase (Drlica, 1999). Les mutations ponctuelles Thr-96, Asp-90 ou encore Ala-70 dans le gène *gyrA* peuvent mener à la résistance. La mutation Thr-96 est la mutation la plus fréquente et est celle qui confère les plus hauts niveaux de résistance (CMI > 16-64 µg/ml) (Taylor et Tracz, 2005). Un autre mécanisme de résistance de la bactérie, mais moins important pour la résistance aux fluoroquinolones, est l'efflux de l'antibiotique hors de la cellule via une pompe. L'opéron *cmeABC* code pour une pompe d'efflux ciblant plusieurs antibiotiques dont les fluoroquinolones. Il a été démontré que le fait de perturber le fonctionnement de la pompe CmeABC augmentait l'accumulation

intracellulaire de ciprofloxacine (Lin *et al.*, 2002). La ciprofloxacine est l'un des antibiotiques les plus utilisé dans le monde, tant en médecine humaine qu'en médecine vétérinaire pour des raisons médicales, mais aussi comme facteur de croissance (Acar et Goldstein, 1997). L'augmentation des niveaux de résistance aux fluoroquinolones coïncide avec le début de son utilisation dans le monde animal, particulièrement pour l'élevage du poulet (Smith *et al.*, 1999). Il a été démontré chez des poulets porteurs de Campylobacter sensible aux fluoroquinolones que des bactéries résistantes pouvaient être isolées seulement 24h après l'administration de l'antibiotique (McDermott *et al.*, 2002).

1.4.2 Les macrolides. Les macrolides, dont fait partie l'érythromycine, sont considérés comme des antibiotiques de premier choix contre les entérites à Campylobacter. L'érythromycine est un agent bactériostatique qui inhibe la synthèse des protéines. Il lie de façon réversible la sous-unité 50S du ribosome bactérien 70S et cause la dissociation du peptidyl-tRNA, qui arrête le cycle d'élongation de la synthèse protéique à l'étape de transpeptidation (Nakajima, 1999; Taylor et Tracz, 2005; Trieber et Taylor, 2000). La résistance du Campylobacter aux macrolides est due à la modification de la cible des macrolides sur le ribosome. La mutation ponctuelle A2075G dans les trois copies du gène codant pour l'ARN ribosomal 23S est responsable des hauts niveaux (CMI > 128 µg/ml) de résistance aux macrolides (Payot *et al.*, 2006). Il a été observé dans certaines souches que les trois copies n'ont pas nécessairement besoin de posséder la mutation pour conférer la résistance à la souche (Gibreel *et al.*, 2005). Une autre mutation, moins fréquente, a été rapportée pour des taux plus faibles de résistance (A2074T) (Vacher *et al.*, 2005). Il a cependant été démontré que cette mutation n'était pas portée sur les trois copies du gène 23S et

qu'elle était moins stable après plusieurs passages en culture que la mutation A2075G (Gibreel *et al.*, 2005). La pompe d'efflux CmeABC jouerait aussi un rôle non seulement dans la résistance intrinsèque aux macrolides de la bactérie, mais aussi dans l'acquisition de faible niveau de résistance (Payot *et al.*, 2004). La résistance à l'érythromycine chez les isolats cliniques n'est pas encore un problème majeur en Amérique du Nord, mais l'apparition de taux de plus en plus élevés chez les humains et les animaux en Europe et en Asie est préoccupante (Pezzotti *et al.*, 2003, Pigrau *et al.*, 1997). L'utilisation de l'érythromycine comme facteur de croissance dans l'élevage de poulet et comme outil de prévention thérapeutique dans plusieurs autres types d'élevages au Canada a également de quoi inquiéter (Health Canada, 2002).

1.4.3 Les tétracyclines. Les tétracyclines sont un groupe d'antibiotiques à large spectre et ont été utilisées contre un grand nombre de pathogènes humains. La tétracycline est un inhibiteur de la synthèse protéique qui agit au niveau du ribosome bactérien. Une fois transportée à l'intérieur de la bactérie par transport actif, la tétracycline se lie de façon réversible à la sous-unité 30S du ribosome bactérien 70S et prévient l'association de l'aminoacyl-tRNA avec le site A du ribosome. Ceci entraîne l'arrêt de l'étape d'élongation de la synthèse protéique (Taylor et Tracz, 2005). La résistance à la tétracycline est modulée par plusieurs gènes *tet*, transférables entre genres bactériens, qui agissent sur l'efflux de l'antibiotique et sur la protection ribosomale. Pour *C. jejuni*, la résistance est principalement modulée par le gène *tetO* qui est porté sur un plasmide transmissible. La protéine TetO protège le ribosome de l'action de l'antibiotique en déplaçant la tétracycline de son site actif sur le ribosome (Connell *et al.*, 2002, Taylor et Tracz, 2005). La très grande prévalence de la résistance à la tétracycline est également due à son utilisation dans le milieu animal

comme facteur de croissance et comme outil thérapeutique pour le contrôle des infections animales (McEwen et Fedorka-Cray, 2002). Cependant, le fait que la résistance à la tétracycline soit portée sur un plasmide augmente sa distribution, non seulement à travers les souches de Campylobacter, mais aussi à travers des différentes espèces et genres bactériens (Taylor et Tracz, 2005).

1.5 L'épidémiologie moléculaire et les différentes méthodes

L'expression « épidémiologie moléculaire » est apparue dans la littérature scientifique au début des années 70. Suivant l'épidémiologie traditionnelle, puis l'épidémiologie clinique, l'épidémiologie moléculaire ajoute un nouveau niveau d'information, intégrant des données d'investigations moléculaires et cellulaires aux données traditionnelles de prévalence, d'incidence et de facteur de risque (Kehoe *et al.*, 2010). L'épidémiologie moléculaire est un outil essentiel pour l'analyse des bactéries pathogènes obtenues durant l'investigation d'éclosions ou d'épidémies ou encore pour le suivi de population bactérienne. La question ultime en typage moléculaire est la suivante : « Est-ce que ces deux isolats bactériens sont identiques ou différents? ». Par contre, une définition plus rigoureuse serait la suivante : « Utilisant une méthode moléculaire bien définie, est-ce que les génotypes de ces deux isolats sont suffisamment similaires pour conclure qu'ils représentent la même souche, ou suffisamment différents pour qu'ils représentent deux souches différentes ? » (Clinical and laboratory standards institute, 2007).

Les techniques d'épidémiologie moléculaire sont donc basées sur la variation génétique des isolats bactériens. Les principales sources de variations génétiques sont

les mutations (insertions et délétions) et la recombinaison. Une large variété de technique de typage moléculaire a été développée au fil des ans pour répondre à ces questions. Cependant, aucune technique de typage moléculaire ne peut détecter toutes les variations génétiques entre deux bactéries, mis à part le séquençage complet des génomes bactériens. Les techniques présentées ici sont les plus utilisées dans l'épidémiologie moléculaire du Campylobacter.

1.5.1 EGCP. L'EGCP (ou *pulsed field gel electrophoresis* [*PFGE*] en anglais) est la méthode la plus répandue et la plus utilisée pour le typage moléculaire des bactéries pathogènes (Tenover *et al.*, 1997). Cette technique d'analyse du génome entier est basée sur la séparation d'un nombre limité de fragments (<25) d'ADN de grande taille (jusqu'à 800 kb) provenant de la digestion enzymatique du génome bactérien par un enzyme de restriction coupant l'ADN peu fréquemment. La technique d'EGCP est illustrée à la Figure 2. La séparation de plus grands fragments d'ADN est possible grâce aux variations de direction et d'intensité du courant électrique durant la migration. En général, l'enzyme *SmaI* est utilisée pour la digestion du génome de Campylobacter et mène à la production de 4 à 20 fragments de 40 à 400 kb (Klena and Konkel, 2005). Il a cependant été démontré que l'enzyme *KpnI* donnait un meilleur pouvoir de discrimination en générant un plus grand nombre de fragments (Michaud *et al.*, 2001). Dans le but de ne pas introduire des cassures aléatoires de l'ADN, une suspension bactérienne est immobilisée dans une carotte d'agarose avant les étapes de lyse cellulaire et de digestion enzymatique. L'EGCP peut donc potentiellement détecter les plasmides que la bactérie peut posséder. Par la suite, la carotte d'agarose contenant le génome bactérien digéré est mise directement dans le gel pour

l'électrophorèse en champ pulsé. Les profils de restriction obtenus sont appelés génotypes, pulsovars ou encore *macrorestriction profile* (Wassenaar et Newell, 2000).

Cette méthode est très largement utilisée, non seulement pour le Campylobacter, mais également pour le *Escherichia coli* O157 :H7, *Listeria monocytogenes*, *Yersinia pestis*, les Salmonelles et les Shigelles via le réseau PULSENET. (Center for disease control and prevention, 2009). C'est une méthode qui offre un excellent pouvoir de discrimination et qui peut détecter jusqu'à 90% des réarrangements chromosomiques obtenus par recombinaison lorsqu'ils altèrent la taille des fragments de plus de 5%. Cette méthode est cependant beaucoup moins sensible aux mutations ponctuelles, qui représentent environ 0,01 à 0,05% des changements de profils génotypiques par EGCP (Clinical and laboratory standards institute, 2007). De plus, cette méthode est fastidieuse, variable entre laboratoires, très dispendieuse en équipement de départ et environ 5% des souches peuvent être non-typables, dépendant des espèces (Spratt, 1999).

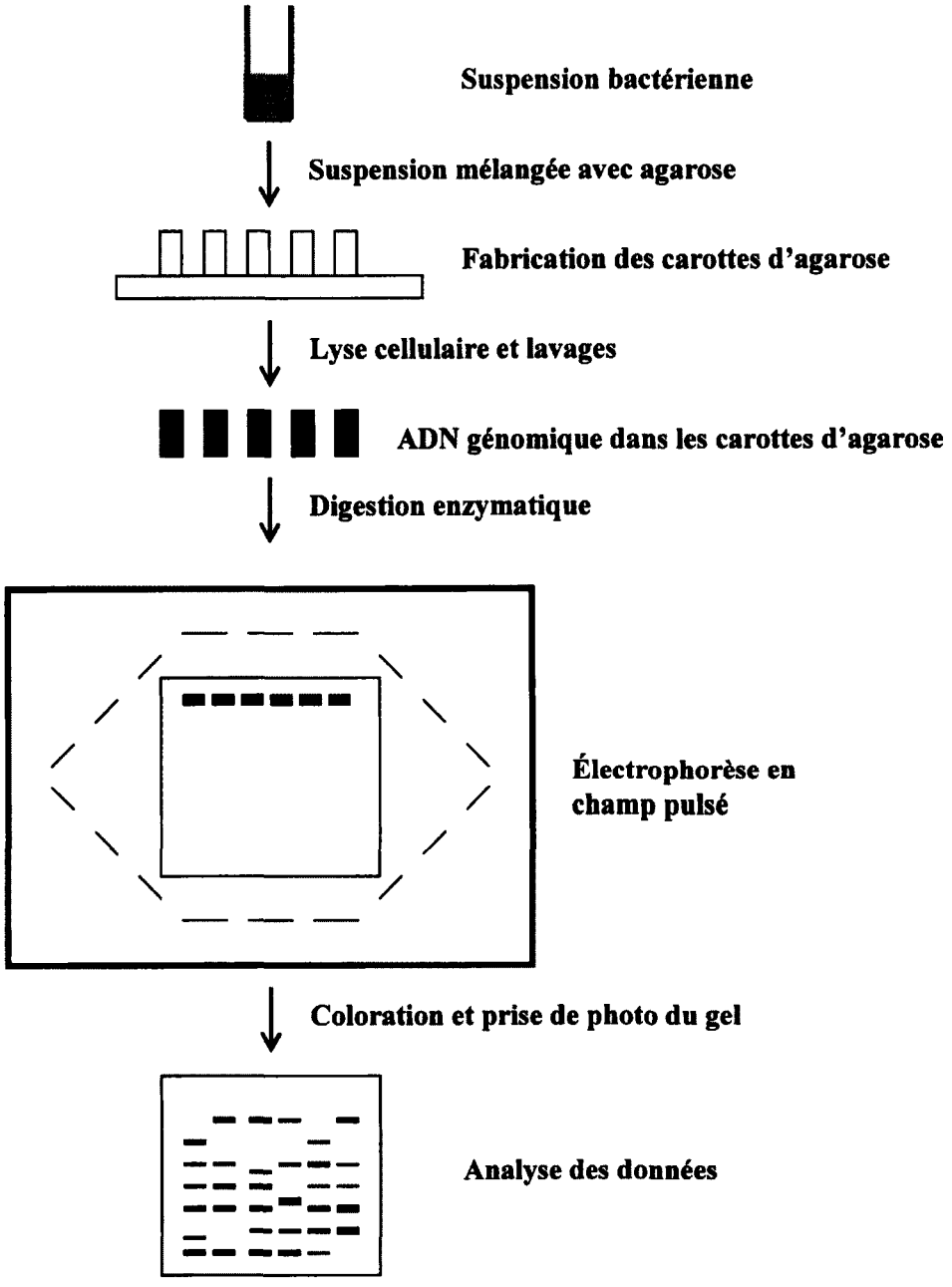


FIGURE 2. Schématisation de la méthode d'EGCP. Inspiré du schéma du réseau PulseNet. (Center for disease control and prevention, 2009).

1.5.2 AFLP. L'AFLP est une technique utilisée tant pour les études phylogénétiques que pour le typage moléculaire. Cette méthode est basée sur la digestion de l'ADN génomique complet de la bactérie avec deux enzymes de

restriction de fréquence de coupure de l'ADN différente (habituellement un enzyme reconnaissant un site de 4 paires de base et un autre de 6 paires de base) (Wassenaar et Newell, 2000). Le protocole complet est illustré dans la Figure 3. Des adaptateurs oligonucléotidiques spécifiques sont par la suite utilisés pour lier les fragments digérés et restaurer des bouts francs aux fragments d'ADN digérés. Ces adaptateurs sont construits de manière à ne pas restaurer les sites de restriction, permettant l'étape de digestion et de ligation des adaptateurs dans la même réaction. Une première amplification par PCR (appelée pré-amplification) est faite avec des amorces couvrant l'adaptateur et le site de restriction, de manière à ce que seuls des fragments flanqués des deux sites de restrictions soient amplifiés. Une seconde amplification (appelée amplification finale) est faite cette fois-ci avec une extension d'un nucléotide au bout 3' des deux amorces. Cette amplification se veut donc sélective sur le nombre de fragments amplifiés. Également, une des deux amorces est habituellement fluorescente pour permettre la détection des fragments dans un séquenceur automatique, ce qui améliore grandement la qualité des résultats comparativement à la migration des profils sur un gel de polyacrylamide (Klena et Konkel, 2005). Cette technique permet donc la séparation d'une cinquantaine de fragments, à un nucléotide près, entre 50 et 500 paires de base (Duim *et al.*, 1999). Cette méthode a pour principal avantage d'être aussi discriminante que le PFGE, d'être plus rapide, moins coûteuse et moins fastidieuse et d'avoir un processus automatisé d'analyse des résultats (Islam *et al.*, 2009, Schouls *el al.*, 2003).

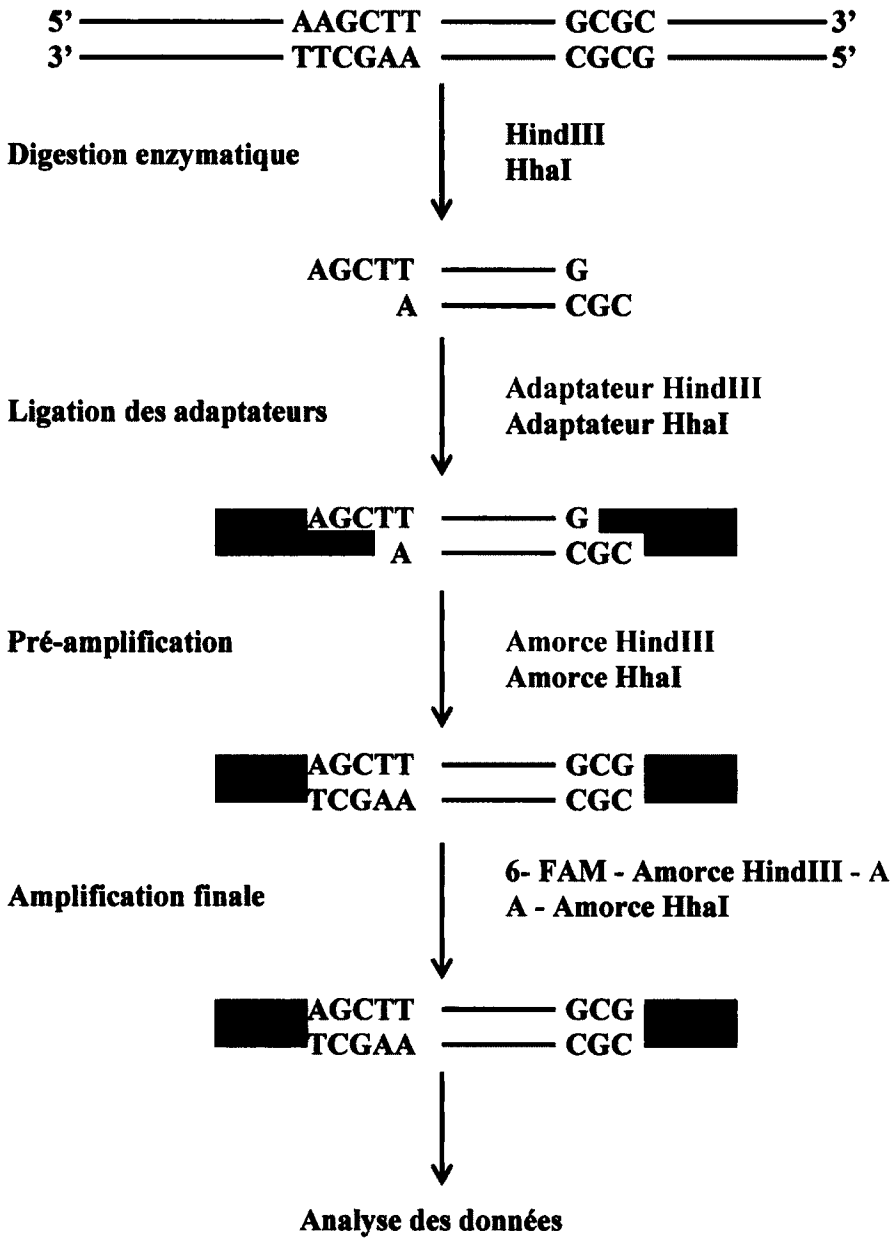


FIGURE 3. Schématisation de la méthode d'AFLP. Inspiré du protocole d'Applied Biosystems (Applied biosystems, 2005).

1.5.3 Typage du flagelle. Le locus des gènes du flagelle de *C. jejuni* contient deux gènes (*flaA* et *flaB*) qui sont en tandem et séparés par environ 170 nucléotides. Dû au fait que ces gènes possèdent des régions hautement conservées et d'autres plus variables, ces gènes sont appropriés pour le typage moléculaire (Meinersmann *et al.*,

1997). Cette variabilité, particulièrement pour le gène *flaA*, a été utilisée pour d'autres méthodes de typage moléculaire basées sur des profils de digestion enzymatique, tel le PCR-RFLP (Nachamkin *et al.*, 1993). Cependant, les méthodes de typages basées sur le séquençage ont plusieurs avantages comparativement aux méthodes basées sur l'analyse de fragments de digestion enzymatique, dont l'absence de variabilité inter-laboratoire et d'ambigüité des résultats obtenus. Une base de données internationale a d'ailleurs été créée pour le partage, l'analyse et la comparaison des profils de séquençage (http://pubmlst.org/campylobacter/). La portion séquencée du gène *flaA* correspond à la région SVR du gène (approximativement entre les nucléotides 450 à 600 du gène). L'analyse des séquences de la région SVR permet de créer des arbres phylogénétiques ayant de meilleurs taux de discrimination que ceux produits avec la séquence complète du gène, en plus d'être plus simples à produire, car une seule paire d'amorces est nécessaire pour l'amplification de ce fragment (Meinersmann *et al.*, 1997).

1.5.4 MLST. La première technique utilisée pour l'étude génétique des populations bactériennes fut le MLEE. Cette technique, basée sur la détection de variations dans plusieurs loci, permettait de mesurer la variabilité à l'intérieur de ces différents loci et d'établir le degré de diversité génétique d'une population bactérienne afin d'identifier des regroupements clonaux de bactéries (Dingle et Maiden, 2005). Le même principe fut appliqué pour le MLST, mais au lieu d'analyser la mobilité électrophorétique d'enzymes du métabolisme bactérien, la comparaison de séquences d'ADN de gènes métaboliques fut l'innovation de cette méthode. Par l'utilisation de séquences, le MLST venait surmonter les lacunes du MLEE, soit la difficulté de comparer les données entre les laboratoires et le fait que seuls les changements

affectant les profils de migration étaient détectés (Maiden *et al.*, 1998). De ce fait, le MLST devint la méthode de référence pour l'analyse des populations génétiques bactériennes (Dingle *et al.*, 2002). Pour *C. jejuni*, un système MLST fut développé au début des années 2000, basé sur le séquençage d'environ 500 paires de base de 7 gènes métaboliques de la bactérie. Suite au séquençage des gènes, les séquences sont comparées sur une base de données internationale (http://pubmlst.org/campylobacter/) afin de leur attribuer un numéro d'allèle. La combinaison des 7 numéros d'allèle (un pour chaque gène séquencé) forme le profil allélique ou *sequence type* (ST). Par la suite, les différents ST sont comparés entre eux et regroupés en complexes clonaux (CC). Chaque complexe clonal est formé d'isolats partageant 4 allèles ou plus avec le profil allélique du génotype central. Le génotype central est l'ancêtre commun de qui tous les membres du complexe clonal sont issus. Les avantages de cette méthode sont le haut pouvoir de discrimination, sa reproductibilité, l'absence d'ambigüité d'interprétation des résultats, la facilité de comparaison des données entre laboratoires et d'échange de résultats via internet (Dingle *et al.*, 2001). Le MLST a permis d'observer que *C. jejuni* était génétiquement très diversifié et avait une population faiblement clonale, notamment dû à de nombreux échanges génétiques intra et inter espèce (Dingle *et al.*, 2001, Djordjevic *et al.*, 2007, Meinersmann *et al.*, 1997). Le MLST est la seule méthode de typage moléculaire dont certains génotypes (complexes clonaux) ont été reliés à des régions géographiques particulières (Duim *et al.*, 2003, McTavish *et al.*, 2008) ou encore à des niches écologiques particulières, tels les oiseaux sauvages, le sable de plage, l'eau, les poulets, les porcs, les bovins ou les moutons (Carter *et al.*, 2009, Colles *et al.*, 2003, Dingle *et al.*, 2001, Manning *et al.*, 2003, McTavish *et al.*, 2009).

1.6 Objectifs de recherche

Mes objectifs de recherche étaient les suivants :

1) Étudier les niveaux et la distribution de la résistance aux antibiotiques des isolats de *C. jejuni* obtenus des différentes niches écologiques afin de déterminer les niveaux de résistances des isolats de l'Estrie et de déterminer la provenance des isolats humains résistants.
2) Vérifier si les niveaux de résistance aux antibiotiques à l'intérieur d'une niche écologique en particulier sont attribuables à des génotypes bien précis.
3) Déterminer si les résultats des différentes méthodes de typage moléculaire pour le Campylobacter sont comparables.
4) Développer un système plus performant pour effectuer le MLST.
5) Déterminer si la contamination de l'eau en Estrie par du Campylobacter est principalement due aux élevages de bovins ou à une autre source et si l'eau est une source significative de campylobactérioses.
6) Vérifier l'hypothèse selon laquelle les sources d'infection à Campylobacter seraient différentes en milieu rural qu'en milieu urbain.
7) Analyser les différents génotypes des isolats de Campylobacter obtenus des différentes niches écologiques et comparer la variabilité des génotypes en circulation chez l'humain.

CHAPITRE 1

AVANT-PROPOS DE L'ARTICLE

Titre

Comparison of antimicrobial resistance of *Campylobacter jejuni* isolated from humans, chickens, raw milk and environmental water in Quebec

Auteurs

Simon Lévesque, Eric Frost et Sophie Michaud.

État de l'article à ce jour

Publié dans la revue Journal of Food Protection, Vol. 70, No. 3, 2007, Pages 729–735.

Le formulaire « Autorisation d'intégration d'un article écrit en collaboration à un mémoire ou une thèse » a été signé par chaque co-auteure ou co-auteur conformément à la « Directive relative au dépôt des essais, des mémoires et des thèses » de l'Université de Sherbrooke.

RÉSUME DE L'ARTICLE

Cette étude avait pour but la comparaison des profils de résistance à l'érythromycine, la ciprofloxacine et la tétracycline parmi 384 isolats de *Campylobacter jejuni* isolés chez l'humain (245), de poulets frais entiers achetés dans les épiceries (56), du lait cru (33) et de l'eau environnementale (41), obtenus entre 2000 et 2003 au Québec. La résistance à la ciprofloxacine fut significativement plus fréquente pour les isolats humains acquis à l'étranger par rapport à ceux acquis localement (50% versus 5,9% ; p<0,0001). La résistance à la ciprofloxacine fut presque absente pour les isolats d'eau, de poulet et de lait cru. Par contre, la résistance à l'érythromycine fut significativement plus courante chez les isolats de poulet que chez les isolats humains acquis localement (16% versus 3,0%, respectivement ; p<0,001). Aucune résistance à l'érythromycine ne fut trouvée parmi les isolats d'eau, de lait cru et humains acquis à l'étranger. La résistance à la tétracycline fut significativement plus courante pour les isolats de poulet et les isolats humains acquis localement (58,9% et 45,8%, respectivement) que ceux de lait cru et d'eau (9,1% et 7,3%, respectivement ; p<0,001). La résistance à la tétracycline fut observée dans 44,4% des isolats humains acquis à l'étranger. Aucun isolat humain ne fut doublement résistant à la ciprofloxacine et à l'érythromycine. Un isolat de poulet fut résistant aux trois antibiotiques. Nos résultats suggèrent que, de 2000 à 2003 au Québec, la résistance aux antibiotiques est demeurée stable parmi les isolats humains acquis localement et pourrait même avoir diminuée. Cependant, le haut taux de résistance à l'érythromycine observé parmi les isolats de poulet est préoccupant, dû au risque de

transmission de ces isolats à l'humain. Des études additionnelles sont nécessaires pour suivre la tendance des taux de résistances aux antibiotiques des isolats de *C. jejuni* obtenus de la nourriture, de l'environnement et chez les humains, aussi bien que le suivi de l'utilisation des antibiotiques dans le milieu vétérinaire.

Contribution de l'étudiant

J'ai contribué totalement à l'obtention et à l'analyse de tous les résultats décrits dans l'article. J'ai également écrit entièrement la première ébauche du manuscrit et participé à la correction avant publication.

COMPARISON OF ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* ISOLATED FROM HUMANS, CHICKENS, RAW MILK AND ENVIRONMENTAL WATER IN QUEBEC

Simon Lévesque, Eric Frost, Ph.D., Sophie Michaud, M.D., M.P.H.

Department of Microbiology and Infectious Diseases, Faculté de Médecine de l'Université de Sherbrooke, Québec, Canada.

Corresponding author: Sophie Michaud, M.D., M.P.H., C.S.P.Q., F.R.C.P.C.
Department of Microbiology and Infectious Diseases
Faculté de Médecine de l'Université de Sherbrooke
3001, 12e avenue Nord
Sherbrooke, Québec J1H 5N4
Phone: (819) 564-5321
Fax: (819) 564-5392
E-mail: Sophie.Michaud@USherbrooke.ca

Running head: Antimicrobial resistance of *Campylobacter jejuni*

Key words: Campylobacter, antimicrobial resistance, humans, chickens, raw milk, water, quinolones, macrolides, tetracycline.

Word count (text): 3109

ABSTRACT

This study compares the occurrence of antimicrobial resistance to erythromycin, ciprofloxacin and tetracycline among 384 *Campylobacter jejuni* isolates from humans (245), fresh whole retail chickens (56), raw milk (33), and environmental water (41), collected between 2000 and 2003 in Québec, Canada. Resistance to ciprofloxacin was significantly more frequent in human isolates acquired abroad than in those acquired locally (50% versus 5.9%; $p<0.001$); ciprofloxacin resistance was almost absent in water, chicken, and raw milk isolates. In contrast, resistance to erythromycin was significantly more common in chicken than in locally-acquired human isolates (16% versus 3.0%, respectively; $p<0.001$); no erythromycin resistance was found among water, raw milk and human isolates acquired abroad. Resistance to tetracycline was significantly more common in chicken and human isolates acquired locally (58.9% and 45.8%, respectively) than in raw milk and water isolates (9.1% and 7.3%, respectively, $p<0.001$). Tetracycline resistance was also observed in 44.4% of human isolates acquired abroad. No human isolate was resistant to both ciprofloxacin and erythromycin, but one chicken isolate was resistant to all three antimicrobial agents. Our results suggest that from 2000 to 2003 in Québec, antimicrobial resistance has remained stable among locally-acquired *C. jejuni* human clinical isolates, and might even have decreased. However, the high erythromycin resistance rate observed among chicken isolates is concerning, due to the risk of transmission of such isolates to humans. Additional studies are needed to monitor trends in antimicrobial resistance among food, environment and human *C. jejuni* isolates, as well as antibiotic use in animals.

Word count: 247

Campylobacter jejuni is the leading reported cause of bacterial gastroenteritis in developed countries *(3)*, but knowledge of the epidemiology of this organism is still developing. Most clinical cases appear as isolated, sporadic infections for which the source is rarely apparent. In addition to gastroenteritis, *C. jejuni* can cause post-infectious manifestations, including Guillain-Barré syndrome, an acute, immune-mediated disorder considered to be the most serious secondary complication *(46)*.

Campylobacter colonizes a wide range of hosts, including domestic animals and wild birds, and thus a substantial burden of campylobacter is excreted via animal fecal material *(3)*. Transmission to humans occurs by ingestion of contaminated foods of animal origin or contaminated water, and by direct contact with infected animals. In general, the occurrence of human campylobacter gastroenteritis has been largely attributed to the consumption of contaminated food animal products, especially poultry, because of the high prevalence of campylobacter in these animals *(2)*. A growing body of evidence, however, suggests that other vehicles such as environmental water, and unpasteurized milk may be important sources of these organisms, but the relative contributions of these and other potential sources, such as domestic pets, and wild birds to human infection are currently not known *(42)*.

Most cases of enteritis are self-limited and do not require treatment; antimicrobial therapy is indicated in case of severe and prolonged gastroenteritis, septicaemia or other invasive manifestations, as well as for immune-suppressed patients. Macrolides and fluoroquinolones are considered the first- and second-line drugs for treatment of complicated campylobacter infections; tetracycline is used as an alternative agent. However, since the 1990s, resistance to tetracycline, fluoroquinolones and, to a lesser extent, macrolides, has clearly increased both in

developed and developing countries *(11, 17, 21, 25)*, and antimicrobial resistance in campylobacter from food animals is now recognized as an emerging public health problem *(11)*. Resistance may arise during treatment of humans, but it is also believed that the use of these antibiotics in the veterinary field contributes to increased resistance among strains infecting humans *(37)*. As campylobacter may be transferred from animals to humans via food or water, the emergence of multidrug resistance to fluoroquinolones and macrolides in campylobacter strains from the food chain has raised concerns that the treatment of human infections will be compromised.

Comparison of the distribution of antimicrobial resistance of campylobacter isolates from different sources could help understand the source of transmission of antimicrobial-resistant campylobacter to humans. *C jejuni* antimicrobial resistance profiles have rarely been studied in environmental water *(37)* or in cattle *(1, 4, 13, 35, 52, 57)*, and relatively few data exist on the antimicrobial resistance of human *(16, 17, 22, 28)* and chicken *(29, 34, 35)* isolates in Canada. This study compares the occurrence of antimicrobial resistance to erythromycin, ciprofloxacin and tetracycline among 384 *C. jejuni* isolates from humans (254), fresh whole retail chickens (56), raw milk (33), and environmental water (41), collected between 2000 and 2003 in Québec.

MATERIALS AND METHODS

Human isolates originated from fecal samples of clinical cases of diarrhoea submitted to the Eastern Townships hospital microbiology laboratories between 2000 and 2003 (153 in 2000-2001, 32 in 2002, and 69 in 2003). For subjects with recurrent infections, only the first *C. jejuni* isolate was included in the study. Human isolates were considered to be acquired internationally if the subject had travelled abroad during the entire 10-day period before the onset of symptoms. Isolates for which this information was not available were excluded from the study. The 18 international isolates were acquired in France (3), Mexico (2), Peru (2), Spain (1), Tunisia (1), Eastern Europe (1), Dominican Republic (1), Chad (1), Bolivia (1), Indonesia (1), Togo or France or Holland (1), Mexico or Africa (1), Bolivia or Argentina (1) and one isolate was from an unspecified origin.

The raw milk isolates were cultured in Québec province in 2000-2001; they were graciously provided to us by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ). The chicken isolates were cultured from fresh whole retail chickens purchased in grocery stores in the Eastern Townships (37 isolates in 2000-2001 and 19 in 2003) *(42, 43)*. The water isolates were obtained from river and tributary water samples collected in the Eastern Townships between May 13 and August 12, 2003 *(36)*. In brief, each sample represented about 500 ml of water collected in a sterile Nalgene bottle that was transported on ice, held at 4°C and tested within 24 h. The water was filtered through a sterile 0.45 µm membrane filter; some samples required pre-filtration with a 1.5µm membrane. The filters were transferred into a Whirl-Pak bag containing 100 ml of Park-Sanders enrichment broth with 0.5 ml of Supplement A (0.2% vancomycin and 0.2% trimethoprim lactate) and 5 ml of Supplement B (0.064% sodium cefoperazone in brucella broth) *(9)*, incubated for 4 h

at 37°C in a microaerobic atmosphere, and then at 42°C for an additional 44 h. One ml of the suspension was transferred into a second Park-Sanders broth (10 ml) and incubated at 42°C for 24 h in a microaerobic atmosphere. Then, 100 µl of the final enrichment broth was plated on Karmali agar and incubated in a microaerobic atmosphere at 42°C for 48 h. Plates that were negative for campylobacter at that time were re-incubated for an additional 24 h. Isolates were identified to the species level by routine phenotyping methods *(45)*, and by two PCR methods *(9, 14)*. Only *C. jejuni* isolates were included in the study. The hippurate gene was detected by PCR in all included isolates; hippurate-negative campylobacter in which hippuricase gene could be detected by polymerase chain reaction were identified as *C. jejuni (58)*.

Agar dilution antimicrobial susceptibility tests were performed with ciprofloxacin (INC Biomedicals, inc., Aurora, Oh), erythromycin and tetracycline (Sigma Chemical CO., St. Louis, Mo), according to CLSI standards *(49)*. The concentrations of the antibiotics tested were 0.06-256 µg/ml, and a control plate without antibiotic was inoculated at the beginning and at the end of the procedure. Inocula were prepared from overnight growth on blood agar plates by suspending each culture in Mueller-Hinton broth (Quelab laboratories inc., Montreal, Qc) at a density adjusted to a 0.5 McFarland turbidity standard. A final inoculum of about 10^4 CFU was delivered with a 1-mm Cathra replicator onto Mueller-Hinton agar plates supplemented with 5% defibrinated sheep blood (Oxoid inc., Nepean, On). Plates were incubated in a microaerobic atmosphere at 42°C for 24 h. The breakpoints used for campylobacter resistance were: ciprofloxacin, $\geq$ 4 mg/L; erythromycin, $\geq$ 8 mg/L; and tetracycline, $\geq$ 16 mg/L *(18)*. Of note, although the CLSI recently proposed a breakpoint of 32 mg/L for erythromycin *(7)* we chose to use the prior commonly-used

breakpoint of 8 mg/L for erythromycin to compare our results with previous studies more easily.

C. jejuni ATCC 33560, one local *C. jejuni* isolate (001B-34)and one local *C. coli* isolate (001A-18) were used as control strains *(26)*. The 001A-18 isolate was resistant tetracycline and erythromycin and (MICs of 128 mg/L and >256 mg/L, respectively), and susceptible to ciprofloxacin (MIC of 0.12 mg/L). The 001B-34 isolate was resistant to ciprofloxacin, erythromycin and tetracycline, with MICs of 64 mg/L, >256 mg/L and >256 mg/L, respectively.

Data were analyzed with Statistix for Windows version 7.1 (Analytical Software, Tallahassee, Fl.) using Chi-square and Fisher's exact two-tailed tests and a significance level of 5%.

RESULTS

Among the 384 isolates tested, 25 (6.5%) were resistant to ciprofloxacin, 16 (4.2%) were resistant to erythromycin, and 155 (40.4%) were resistant to tetracycline (Figure 1). Twenty (5.2%) isolates, all from humans or chickens, were resistant to two of the three antibiotics: 10 were resistant to both ciprofloxacin and tetracycline, and 10 were resistant to both erythromycin and tetracycline. No human isolate was resistant to both ciprofloxacin and erythromycin, but one chicken isolate was resistant to all three antimicrobial agents.

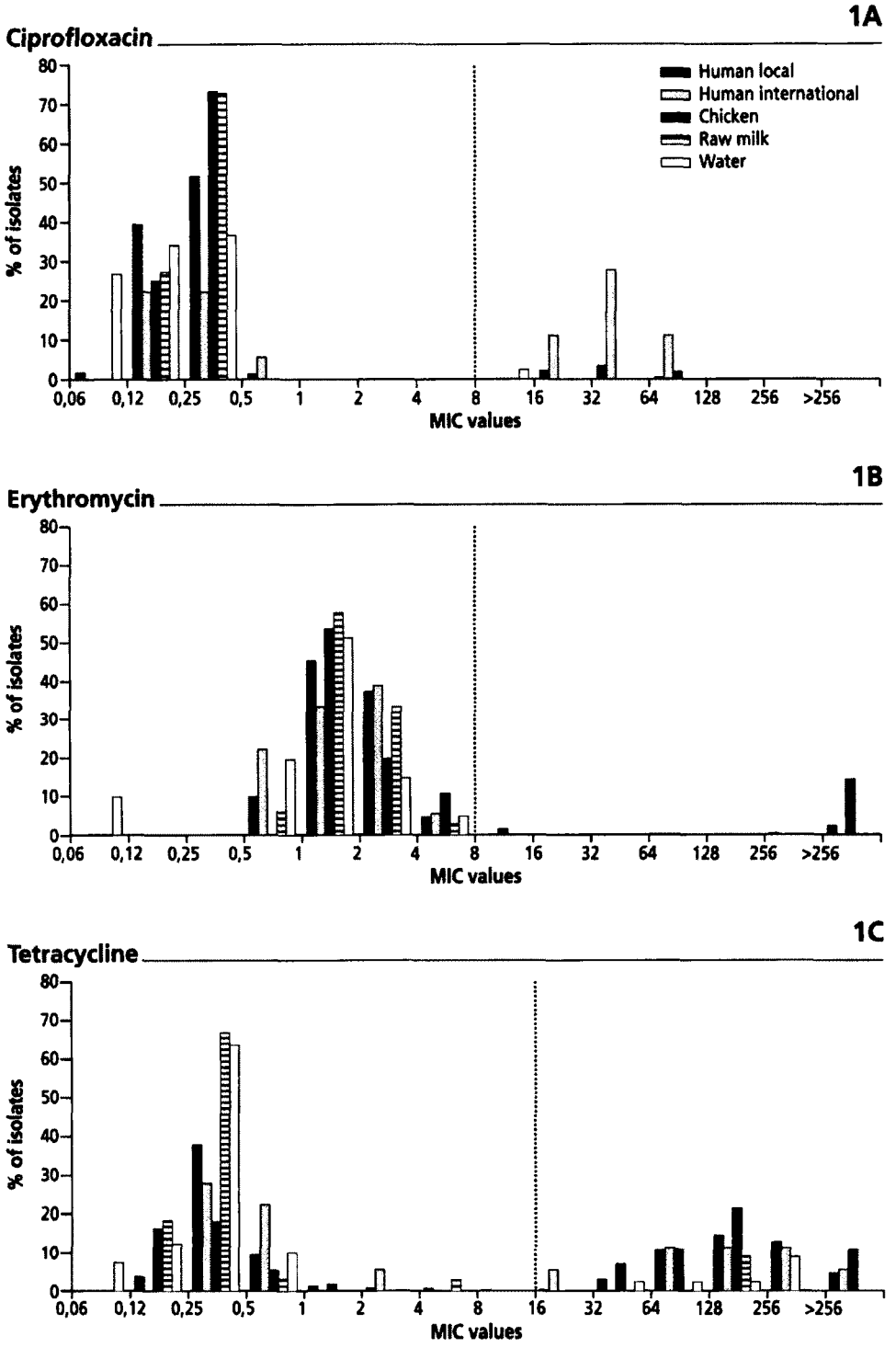


FIGURE 1. MIC histograms of three antimicrobials for 384 *C. jejuni* isolates. The vertical lines represent the resistance breakpoint for each antimicrobial.

Resistance to ciprofloxacin was significantly more frequent in human isolates acquired abroad than in those acquired locally (50% versus 5.9%; $p<0.001$); ciprofloxacin resistance was almost absent in water, chicken, and raw milk isolates (Table 1). In contrast, resistance to erythromycin was significantly more common in chicken than in locally-acquired human isolates (16% versus 3.0%, respectively; $p<0.001$); no erythromycin resistance was found among water, raw milk and human isolates acquired abroad. Resistance to tetracycline was significantly more common in chicken and human isolates acquired locally (58.9% and 45.8%, respectively) than in raw milk and water isolates (9.1% and 7.3%, respectively, $p<0.001$). Tetracycline resistance was also observed in 44.4% of human isolates acquired abroad.

TABLE 1. Antimicrobial resistance of 384 *C. jejuni* isolates.

	Water (n = 41)	Human acquired locally (n = 236)	Human acquired abroad (n = 18)	Chicken (n = 56)	Raw milk (n = 33)
Ciprofloxacin					
% resistant isolates (#)	2.4% (1)	5.9% (14)	50.0% (9)	1.8% (1)	0% (0)
MIC range (mg/liter)	0.06–8	0.06–64	0.12–64	0.12–64	0.12–0.25
MIC_{50}[a] (mg/liter)	0.12	0.25	0.5	0.25	0.25
MIC_{90}[b] (mg/liter)	0.25	0.25	32	0.25	0.25
Erythromycin					
% resistant isolates (#)	0% (0)	3.0% (7)	0% (0)	16.0% (9)	0% (0)
MIC range (mg/liter)	0.06–4	0.5–>256	0.5–4	1–>256	0.5–1
MIC_{50} (mg/liter)	1	1	1	1	1
MIC_{90} (mg/liter)	2	2	2	>256	2
Tetracycline					
% resistant isolates (#)	7.3% (3)	45.3% (107)	44.4% (8)	58.9% (33)	9.1% (3)
MIC range (mg/liter)	0.06–64	0.12–>256	0.25–>256	0.12–>256	0.12–128
MIC_{50} (mg/liter)	0.25	0.5	0.5	64	0.25
MIC_{90} (mg/liter)	0.5	256	256	256	4

[a] MIC for 50% of strains tested.
[b] MIC for 90% of strains tested.

Between 2000-2001 and 2002-2003, the ciprofloxacin resistance rate remained stable among chicken (2.7% versus 0%) and human isolates acquired locally (5.0% versus 6.1%). Erythromycin resistance rates went from 10.8% in 2000-2001 to 26.3% in 2003 among chicken isolates (p=0.27). The prevalence of erythromycin resistance remained stable in human isolates acquired locally (2.8% in 2000-2001 versus 3.0% in 2002-2003). Finally, the rates of tetracycline resistance went from 36.2% in 2000-2001 to 42.4% in 2002-2003 (p=0.18) among human isolates acquired locally, and from 45.9% in 2000-2001 to 84.2% in 2003 among chicken isolates (p=0.57).

Four human and four chicken erythromycin-resistant isolates from our study have been further characterized *(20)*, and all showed an A→G transition at position 2059 of the 23S rRNA gene, except for one human isolate which exhibited an A→G transition at position 2058.

A subset of isolates from the present study, 149 locally-acquired human isolates and 37 chicken isolates, were typed by PFGE in a previous study, in which we found that 46% of chicken isolates had genotypes similar to those of 22% human isolates *(43)*. Among the 60 human and 17 chicken tetracycline-resistant isolates analyzed, 42 isolates were distributed among 21 clusters (Dice coefficient $\geq$ 90%) containing 70 isolates. Of the 4 human and the 4 chicken erythromycin-resistant isolates tested, 5 isolates were distributed among 3 clusters containing 14 isolates. Finally, among the 7 ciprofloxacin-resistant isolates tested (one from chicken and 6 from humans), 2 isolates belonged to 2 clusters containing a total of 7 isolates. The remaining isolates had unique PFGE profiles.

DISCUSSION

It is estimated that there are more than 2,000,000 infections, 13,000 hospitalizations and 100 deaths caused by Campylobacter each year in the United States *(41)*, and that 18% of these infections are caused by strains resistant to at least one antimicrobial agent *(5)*. NARMS (National Antimicrobial Resistance Monitoring System) has documented an increased proportion of human infections with ciprofloxacin-resistant *C. jejuni* between 1989 and 2003 *(48)*: no isolates resistant to ciprofloxacin were identified in 1989-1990, 12% were resistant in 1997, 21% in 2002, and 17% in 2003. Resistance to tetracycline was already 42% in 1989-1990 and has slightly decreased to 38% in 2003. Resistance to erythromycin has remained low at 3% or less.

In Québec, the resistance rate to ciprofloxacin for *C. jejuni* human clinical isolates was 0% in 1985-1986, 3.5% in 1992-1993, and 12.7% in 1995-1997 *(17)*. During the same period, resistance to tetracycline went from 19.1% in 1985-1986, 40.7% in 1992-1993 and 55.7% in 1995-1997, and no erythromycin-resistant isolates were reported. Another study of 23 human *C. jejuni* isolates collected in 1998-1999 in Québec showed resistance rates of 9% for ciprofloxacin, 9% for erythromycin and 39% for tetracycline *(24)*; of note, no distinction was made between locally- and internationally-acquired isolates in these two studies. Then, between 1999 and 2001, Gaudreau and Gilbert reported that 9%, 6% and 58% of 109 locally-acquired *C. jejuni* human clinical isolates were resistant to ciprofloxacin, erythromycin, and tetracycline, respectively *(16)*. In our study, we report lower rates of resistance to ciprofloxacin (5.9%), erythromycin (3.0%) and tetracycline (45.3%), suggesting that from 2000 to 2003 in Québec, antimicrobial resistance has remained stable among locally-acquired *C. jejuni* human clinical isolates, and might even have decreased.

In our study, 50% of human isolates acquired abroad were resistant to ciprofloxacin; this should be taken into account when empirically treating returning travellers. Several other studies have reported high ciprofloxacin resistance rate among *C. jejuni* isolates acquired internationally *(6, 12, 16, 27, 33, 53, 60)* among patients returning from Southern Asia (84.6%) and Southern Europe (65.1%), and more particularly from Thailand (81.3%), India (71.1%), and Spain (62.5%) *(12, 31)*. Macrolides are the antimicrobials of choice in this setting, but high erythromycin resistance rates have also been reported among human *Campylobacter* spp. isolates from India (15.8%) and Spain (10.0%) *(31)*.

Fluoroquinolone-resistant organisms have been reported to be associated with more severe disease, including diarrhoea of 2 to 5 days longer than that of persons with ciprofloxacin-susceptible infection, even in the absence of ciprofloxacin treatment and independently of foreign travel *(12, 25, 44, 50, 59, 61)*. These results suggest that the virulence of resistant organisms may differ from that of susceptible strains. This hypothesis is further supported by the recent finding of enhanced *in vivo* fitness of fluoroquinolone-resistant *C. jejuni* in chickens, in the absence of antibiotic selection pressure *(38)*.

Some investigators suggest that resistance in *C. jejuni* and *C. coli* is driven by use of antibiotics for treating human infections rather than by veterinary use. Although induction of macrolide resistance during treatment has been reported infrequently *(15)*, induction of fluoroquinolone resistance during treatment has been reported in as many as 28% of patients infected with a susceptible strain *(11, 47)*. However, the treated patient is unlikely to be a source of quinolone-resistant campylobacter for other people, because person-to-person transmission of campylobacter is not considered epidemiologically important *(12)*.

On the other hand, the emergence of quinolone-resistant campylobacter isolated from humans and chickens seems to have coincided with the introduction of quinolones in veterinary medicine in the US and in different European countries *(10, 25, 54-56, 59, 63, 64)* and their absence in countries like Australia where they are not licensed *(62)*. It has been described that treatment with enrofloxacin of broiler chickens infected with quinolone-susceptible *C. jejuni* does not eradicate the organism but rather rapidly selects for quinolone resistance in *C. jejuni (23, 32, 39, 40)*.

In Canada, licensing of the veterinary use of fluoroquinolones in 1987 was withdrawn in 1997 *(11)*. However, erythromycin, chlortetracycline and oxytetracycline are registered for use as growth promoters in broilers in Canada and in disease prevention and therapy in many breeds such as swine, sheep, turkey and cattle *(30)*; little data regarding the frequency and average duration of use of these drugs are available. Our study showed almost no fluoroquinolone resistance among chicken (1.8%) and raw milk (0%) isolates collected from 2000 to 2003 in Québec. A prior study of 180 chicken boilers *C. jejuni* isolates collected between 1998 and 1999 in Québec also showed resistance rates of 1% for ciprofloxacin, 7% for erythromycin and 66% for tetracycline *(24)*. The high resistance rate to erythromycin and tetracycline, and the low resistance rate to ciprofloxacin among chicken isolates may reflect the veterinary use of these particular antimicrobial agents. Although the differences were not statistically significant, the trend for an increased resistance rate for erythromycin and tetracycline among chicken isolates is worrisome and should be monitored closely. On the other hand, the significantly lower erythromycin resistance among human isolates from domestically-acquired infections in our study may reflect the fact that there are several other important sources for human infection in addition to chicken *(1)*.

Additional data from Alberta showed no erythromycin resistance among 101 chicken *C. jejuni* isolates collected during 2001 *(20)*. In Ontario, about 3% of 332 chicken *C. jejuni* isolates from 1998 to 2001 were resistant to erythromycin *(35)*. In contrast, Health Canada reported a 22.3% erythromycin resistance rate among 94 retail chicken *Campylobacter* spp. isolates from Québec Province, compared with 9.0% in 78 isolates from Ontario (p=0.02) *(29)*; however, rates were not calculated for *C. jejuni* isolates specifically. These data suggest that erythromycin resistance among chicken isolates might be more prevalent in Québec than in other provinces in Canada. This hypothesis should be verified by further studies. However, in order to monitor trends in antimicrobial resistance among campylobacter, isolates should be differentiated to the species level, especially if any conclusions regarding zoonotic spread of resistance are to be made.

We are concerned about the high erythromycin resistance rate observed among our chicken isolates, for medical reasons. Since poultry is suspected to be the primary source of *C. jejuni* infections for humans, erythromycin resistance in chicken isolates might eventually translate into an increase of the *C. jejuni* erythromycin resistance rate in humans. The finding of a *C. jejuni* chicken isolate resistant to all three drugs used for treatment in humans is also worrisome. This finding suggests that cases of campylobacteriosis acquired from undercooked or mishandled retail chicken may not respond to empirical therapy. Therefore, the use of *in vitro* susceptibility testing of Campylobacter may take on greater importance in ensuring rapid and appropriate management of patients in food-borne campylobacteriosis *(19)*. The possibility that resistant bacteria or resistance genes may be transferred from animals to humans should also be studied very closely.

To our knowledge, only one prior study has evaluated antimicrobial resistance of *C. jejuni* isolates from water samples, and only 9 isolates were tested *(37)*: among these 9 isolates, one was resistant to tetracycline and none was resistant to ciprofloxacin or erythromycin. Few other studies have evaluated *C. jejuni* isolates from cattle, with resistance rates ranging from 5.1-25.0% for fluoroquinolones, 2.9-8.3% for erythromycin, and 0-39% for tetracycline *(1, 4, 51)*.

We provide the first study of antimicrobial resistance for *C. jejuni* isolates collected from humans, retail chickens, raw milk and environmental water in Canada, and the largest study of water isolates. However, our study has some limitations. It was difficult to test the hypothesis and interpret data on sources of transmission of antimicrobial resistant isolates when using isolates collected in different locations and time frame. Detailed background information such as the history of antibiotic usage in the dairy barns and poultry farms was not available for the *C. jejuni* isolates from milk and chicken in this study.

Finally, when adding the antimicrobial resistance data available to the PFGE results on a subset of human and chicken isolates, it was difficult to draw any additional conclusion on the putative source of antimicrobial resistance other than the distribution of antimicrobial resistant isolates is very heterogeneous. PFGE is not suitable for determining the population genetics of bacterial species, which is better studied by methods like multilocus sequence typing (MLST) *(8)*. A MLST study of these isolates is currently underway, and should help better understand the transmission of antimicrobial resistance among these isolates. Nevertheless, additional studies are needed to monitor trends in antimicrobial resistance among food, environment and human *C. jejuni* isolates, as well as antibiotic use in animals. MLST combined with geographical and time distribution analyses of isolates from different

sources collected from a well-defined region would provide new insights on the epidemiology of this infection.

ACKNOWLEDGMENTS

The study was funded by the Centre de Recherche Clinique du Centre Hospitalier Universitaire de Sherbrooke. We thank Gaétan Lamontagne and Marie Nadeau from the MAPAQ for providing the raw milk isolates. We also thank the Canadian Infectious Disease Society (CIDS) for the Safe Drinking Water/CFID Studentship Award (S.L.).

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. Allos B. M. 2001. *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin. Infect. Dis.* 32: 1201-1206.
3. Altekruse S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow. 1999. *Campylobacter jejuni*--an emerging foodborne pathogen. *Emerg. Infect. Dis.* 5: 28-35.
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. Barza M., and K. Travers. 2002. Excess infections due to antimicrobial resistance: the "Attributable Fraction". *Clin. Infect. Dis.* 34 Suppl 3: S126-130.
6. Cardinale E., J. A. Dromigny, F. Tall, M. Ndiaye, M. Konte, and J. D. Perrier-Gros-Claude. 2003. Fluoroquinolone susceptibility of Campylobacter strains, Senegal. *Emerg. Infect. Dis.* 9: 1479-1481.
7. Clinical and Laboratory Standard Institute (CLSI). 2006. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline. CLSI document M45-A. Clinical and Laboratory Standard Institute, Wayne, PA.
8. Clinical and Laboratory Standard Institute (CLSI). 2006. Molecular methods for bacterial strain typing; proposed guideline. CLSI document MM11-P. Clinical and Laboratory Standard Institute, Wayne, PA.
9. Denis M., C. Soumet, K, Rivoal, G. Ermel, D. Blivet, G. Salvat, and P. Colin. 1999. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Lett. Appl. Microbiol.* 29: 406-410.
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 isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *J. Antimicrob. Chemother.* 27: 199-208.
11. Engberg J., F. M. Aarestrup, D. E. Taylor, P. Gerner-Smidt, and I. Nachamkin. 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. *Emerg. Infect. Dis.* 7: 24-34.
12. Engberg J., J, Neimann, E. M. Nielsen, F. M. Aerestrup, and V. Fussing. 2004. Quinolone-resistant Campylobacter infections: risk factors and clinical consequences. *Emerg. Infect. Dis.* 10: 1056-1063.
13. 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.
14. Fermer C., and E. O. Engvall. 1999. Specific PCR identification and differentiation of the thermophilic campylobacters, *Campylobacter jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*. *J. Clin. Microbiol.* 37: 3370-3373.

15. Funke G., R. Baumann, J. L. Penner, and M. Altwegg. 1994. Development of resistance to macrolide antibiotics in an AIDS patient treated with clarithromycin for *Campylobacter jejuni* diarrhea. *Eur. J. Clin. Microbiol. Infect. Dis.* 13: 612-615.
16. Gaudreau C., and H. Gilbert. 2003. Antimicrobial resistance of *Campylobacter jejuni* subsp. *jejuni* strains isolated from humans in 1998 to 2001 in Montreal, Canada. *Antimicrob. Agents Chemother.* 47: 2027-2029.
17. Gaudreau C., and H. Gilbert. 1998. Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. *Antimicrob. Agents Chemother.* 42: 2106-2108.
18. Gaudreau C., and H. Gilbert. 1997. Comparison of disc diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. *J. Antimicrob. Chemother.* 39: 707-712.
19. Ge B., D. G. White, P. F. McDermott, W. Girard, S. Zhao, S. Hubert, and J. Meng. 2003. Antimicrobial-resistant campylobacter species from retail raw meats. *Appl. Environ. Microbiol.* 69: 3005-3007.
20. Gibreel A., V. N. Kos, M. Keelan, C. A. Trieber, S. Levesque, S. Michaud, and D. E. Taylor. 2005. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*: molecular mechanism and stability of the resistance phenotype. *Antimicrob. Agents Chemother.* 49: 2753-2759.
21. Gibreel A., and D. E. Taylor. 2006. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J. Antimicrob. Chemother.* 58: 243-255.
22. Gibreel A., D. M. Tracz, L. Nonaka, T. M. Ngo, S. R. Connell, and D. E. Taylor. 2004. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to *tet*(O)-mediated tetracycline resistance. *Antimicrob. Agents Chemother.* 48: 3442-3450.
23. Griggs D. J., M. M. Johnson, J. A. Frost, T. Humphrey, F. Jorgensen, and L. J. Piddock. 2005. Incidence and mechanism of ciprofloxacin resistance in *Campylobacter* spp. isolated from commercial poultry flocks in the United Kingdom before, during, and after fluoroquinolone treatment. *Antimicrob. Agents Chemother.* 49: 699-707.
24. Guevremont E., E. Nadeau, M. Sirois, and S. Quessy. 2006. Antimicrobial susceptibilities of thermophilic Campylobacter from humans, swine, and chicken broilers. *Can. J. Vet. Res.* 70: 81-86.
25. 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 F. J. Angulo. 2004. Antimicrobial resistance among Campylobacter strains, United States, 1997-2001. *Emerg. Infect. Dis.* 10: 1102-1109.
26. Hakanen A., P. Huovinen, P. Kotilainen, A. Siitonen, and H. Jousimies-Somer. 2002. Quality control strains used in susceptibility testing of *Campylobacter* spp. *J. Clin. Microbiol.* 40: 2705-2706.
27. Hakanen A., H. Jousimies-Somer, A. Siitonen, P. Huovinen, and P. Kotilainen. 2003. Fluoroquinolone resistance in *Campylobacter jejuni* isolates in travelers returning to Finland: association of ciprofloxacin resistance to travel destination. *Emerg. Infect. Dis.* 9: 267-270.
28. Harnett N., S. McLeod, Y. A. Yong, C. Hewitt, M. Vearncombe, and C. Krishnan. 1995. Quinolone resistance in clinical strains of *Campylobacter jejuni* and *Campylobacter coli*. *J. Antimicrob. Chemother.* 36: 269-270.

29. Health Canada. 2003. Canadian Integrated Program for Antimicrobial Resistance Surveillance. p. 41-42, 92.
30. Health Canada. 2002. Report of the Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health. p. 62-79.
31. Helms M., J. Simonsen, K. E. Olsen, and K. Molbak. 2005. Adverse health events associated with antimicrobial drug resistance in Campylobacter species: a registry-based cohort study. *J. Infect. Dis.* 191: 1050-1055.
32. Humphrey T. J., F. Jorgensen, J. A. Frost, H. Wadda, G. Domingue, N. C. Elviss, D. J. Griggs, and L. J. Piddock. 2005. Prevalence and subtypes of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. *Antimicrob. Agents Chemother.* 49: 690-698.
33. Kassenborg H. D., K. E. Smith, D. J. Vugia, T. Rabatsky-Ehr, M. R. Bates, M. A. Carter, N. B. Dumas, M. P. Cassidy, N. Marano, R. V. Tauxe, and F. J. Angulo. 2004. Fluoroquinolone-resistant Campylobacter infections: eating poultry outside of the home and foreign travel are risk factors. *Clin. Infect. Dis.* 38 Suppl 3: S279-284.
34. Kos V. N., M. Keelan, and D. E. Taylor. 2006. Antimicrobial susceptibilities of *Campylobacter jejuni* isolates from poultry from Alberta, Canada. *Antimicrob. Agents Chemother.* 50: 778-780.
35. Larkin C., C. Van Donkersgoed, A. Mahdi, P. Johnson P, B. McNab, and J. Odumeru. 2006. Antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from hog, beef, and chicken carcass samples from provincially inspected abattoirs in Ontario. *J. Food Prot.* 69: 22-26.
36. Lévesque S., J. Martel, E. Frost, and S. Michaud. 2005. Prevalence of Campylobacter in river and tributary water in the Eastern Townships, Québec, Canada. In: 13th International Workshop on Campylobacter, Helicobacter and Related Organisms, September 5, 2005, Gold Coast Queensland, Australia.
37. Lindmark H., B. Harbom, L. Thebo, L. Andersson, G. Hedin, B. Osterman, T. Lindberg, Y. Andersson, A. Westoo, and E. Olsson Engvall. 2004. Genetic characterization and antibiotic resistance of *Campylobacter jejuni* isolated from meats, water, and humans in Sweden. *J. Clin. Microbiol.* 42: 700-706.
38. Luo N., S. Pereira, O. Sahin, J. Lin, S. Huang, L. Michel, and Q. Zhang. 2005. Enhanced in vivo fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. *Proc. Natl. Acad. Sci. U. S. A* .102: 541-546.
39. Luo N., O. Sahin, J. Lin, L. O. Michel, and Q. Zhang. 2003. *In vivo* selection of Campylobacter isolates with high levels of fluoroquinolone resistance associated with *gyr*A mutations and the function of the CmeABC efflux pump. *Antimicrob. Agents Chemother.* 47: 390-394.
40. McDermott P. F., S. M. Bodeis, L. L. English, D. G. White, R. D. Walker, S. Zhao, S. Simjee, and D. D. Wagner. 2002. Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. *J. Infect. Dis.* 185: 837-840.
41. Mead P.S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5: 607-625.
42. Michaud S., S. Ménard, R. D. Arbeit. 2004. Campylobacteriosis, Eastern Townships, Quebec. *Emerg. Infect. Dis.* 10: 1844-1847.

43. Michaud S., S. Ménard, and R. D. Arbeit. 2005. Role of real-time molecular typing in the surveillance of campylobacter enteritis and comparison between PFGE profiles of chicken and human isolates. *J. Clin. Microbiol.* 43: 1105-1111.
44. Molbak K. 2005. Human health consequences of antimicrobial drug-resistant Salmonella and other foodborne pathogens. *Clin. Infect. Dis.* 41: 1613-1620.
45. Nachamkin I. 2003. *Campylobacter* and *Arcobacter*. In: Murray P. R., E. J. Baron, M. A. Pfaller, J. H. Jorgensen, and R. H. Yolken (eds), Manual of Clinical Microbiology. 8th ed. Washington, D.C.: American Society for Microbiology, pp. 902-914.
46. Nachamkin I., B. M. Allos, and T. W. Ho. 1998. Campylobacter species and Guillain-Barre syndrome. *Clin. Microbiol. Rev.* 11: 555-567.
47. Nachamkin I., J. Engberg, and F. Moller Aarestrup. 2000. Diagnosis and antimicrobial susceptibility of Campylobacter species. In: Nachamkin I., and M. J. Blaser (eds), Campylobacter. Washington, D.C.: American Society of Microbiology, pp. 45-66.
48. National Antimicrobial Resistance Monitoring System (NARMS). 2006. NARMS 2003 Annual Report. Atlanta, GA.
49. National Committee for Clinical Laboratory Standards. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Vol. 23. Wayne, PA.
50. Nelson J. M., K. E. Smith, D. J. Vugia, T. Rabatsky-Ehr, S. D. Segler, H. D. Kassenborg, S. M. Zansky, K. Joyce, N. Marano, R. M. Hoekstra, and F. J. Angulo. 2004. Prolonged diarrhea due to ciprofloxacin-resistant campylobacter infection. *J. Infect. Dis.* 190: 1150-1157.
51. 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.
52. Piddock L. J., V. Ricci, K. Stanley, and K. Jones. 2000. Activity of antibiotics used in human medicine for *Campylobacter jejuni* isolated from farm animals and their environment in Lancashire, UK. *J. Antimicrob. Chemother.* 46: 303-306.
53. Rautelin H., A. Vierikko, M. L. Hanninen, and M. Vaara. 2003. Antimicrobial susceptibilities of campylobacter strains isolated from finnish subjects infected domestically or from those infected abroad. *Antimicrob. Agents Chemother.* 47: 102-105.
54. Reina J., N. Borrell, and A. Serra. 1992. Emergence of resistance to erythromycin and fluoroquinolones in thermotolerant Campylobacter strains isolated from feces 1987-1991. *Eur. J. Clin. Microbiol. Infect. Dis.* 11: 1163-1166.
55. Saenz Y., M. Zarazaga, M. Lantero, M. J. Gastanares, F. Baquero, and C. Torres. 2000. Antibiotic resistance in campylobacter strains isolated from animals, foods, and humans in Spain in 1997-1998. *Antimicrob. Agents Chemother.* 44: 267-271.
56. Sanchez R., V. Fernandez-Baca, M. D. Diaz, P. Munoz, M. Rodriguez-Creixems, and E. Bouza. 1994. Evolution of susceptibilities of *Campylobacter* spp. to quinolones and macrolides. *Antimicrob. Agents Chemother.* 38: 1879-1882.

57. Sato K., P. C. Bartlett, J. B. Kaneene, and F. P. Downes. 2004. Comparison of prevalence and antimicrobial susceptibilities of *Campylobacter* spp. isolates from organic and conventional dairy herds in Wisconsin. *Appl. Environ. Microbiol.* 70: 1442-1447.

58. Slater E. R., and R. J. Owen. 1997. Restriction fragment length polymorphism analysis shows that the hippuricase gene of *Campylobacter jejuni* is highly conserved. *Lett. Appl. Microbiol.* 25: 274-278.

59. Smith K. E., J. M. Besser, C. W. Hedberg, F. T. Leano, J. B. Bender, J. H. Wicklund, B. P. Johnson, K. A. Moore, and M. T. Osterholm. 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. *N. Engl. J. Med.* 340: 1525-1532.

60. The Campylobacter Sentinel Surveillance Scheme Collaborators. 2002. Ciprofloxacin resistance in *Campylobacter jejuni*: case-case analysis as a tool for elucidating risks at home and abroad. *J. Antimicrob. Chemother.* 50: 561-568.

61. Travers K., and M. Barza. 2002. Morbidity of infections caused by antimicrobial-resistant bacteria. *Clin. Infect. Dis.* 34 Suppl 3: S131-134.

62. Unicomb L. E., J. Ferguson, R. J. Stafford, R. Ashbolt, M. D. Kirk, N. G. Becker, M. S. Patel, G. L. Gilbert, M. Valcanis, and L. Mickan. 2006. Low-level fluoroquinolone resistance among *Campylobacter jejuni* isolates in Australia. *Clin. Infect. Dis.* 42: 1368-1374.

63. Van Looveren M., G. Daube, L. De Zutter, J. M. Dumont, C. Lammens, M. Wijdooghe, P. Vandamme, M. Jouret, M. Cornelis, and H. Goossens. 2001. Antimicrobial susceptibilities of Campylobacter strains isolated from food animals in Belgium. *J. Antimicrob. Chemother.* 48: 235-240.

64. Velazquez J. B., A. Jimenez, B. Chomon, and T. G. Villa. 1995. Incidence and transmission of antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J. Antimicrob. Chemother.* 35: 173-178.

CHAPITRE 2

AVANT-PROPOS DE L'ARTICLE

Titre

Multilocus sequence typing of *Campylobacter jejuni* isolates from humans, chickens, raw milk and environmental water in Quebec

Auteurs

Simon Lévesque, Eric Frost, Robert D. Arbeit et Sophie Michaud.

État de l'article à ce jour

Publié dans la revue Journal of Clinical Microbiology, Vol. 46, No. 10, 2008, Pages 3404–11.

Le formulaire « Autorisation d'intégration d'un article écrit en collaboration à un mémoire ou une thèse » a été signé par chaque co-auteure ou co-auteur conformément à la « Directive relative au dépôt des essais, des mémoires et des thèses » de l'Université de Sherbrooke.

RÉSUMÉ DE L'ARTICLE

Le typage moléculaire des isolats bactériens est essentiel pour déchiffrer l'épidémiologie des infections à *Campylobacter jejuni*. Nous avons utilisé deux différentes méthodes (MLST et *flaA* SVR) sur 289 isolats (163 isolats humains, 56 isolats de poulet, 34 isolats de lait cru et 36 isolats d'eau), obtenus au Québec sur une période de trois ans. Nous avons également inclus dans l'étude l'analyse d'un sous-groupe de 131 isolats fait par EGCP. Nous avons obtenu par MLST 96 STs et 20 CCs, incluant 49 STs (73 isolats, 25%) et 39 allèles nouvellement identifiés et jamais documentés dans la base de données internationale. La fréquence des nouveaux STs fut significativement plus élevée parmi les isolats d'eau comparativement aux autres sources (18/36 [50%] versus 55/235 [22%], respectivement ; p<0,001). Neuf des dix plus prévalent CCs comprenaient des isolats humains et des isolats d'au moins une autre source et cinq comprenaient exclusivement ou majoritairement des isolats humains et de poulet. Cependant, les isolats d'eau et de lait cru étaient prédominants parmi les autres CCs associés aux humains, suggérant que les infections sporadiques à *C. jejuni* chez l'humain peuvent fréquemment provenir d'autres sources que le poulet. Les trois systèmes de typage eurent un bon taux de discrimination, avec un index de discrimination >0,9. Parmi les 131 isolats analysés par EGCP, chacun des 20 génotypes représentés par ≥2 isolats correspondait à un seul CC. Par contre, parmi les 14 génotypes par *flaA* SVR les plus prévalents (5 à 27 isolats chacun), huit (57%) comprenaient des isolats retrouvés dans plusieurs CCs. Les raisons de ces résultats discordants ne sont cependant pas claires. La résistance aux antibiotiques fut

aléatoirement distribuée parmi les CCs et semble être plus reliée à l'origine de l'isolat plutôt qu'à son génotype. Même si le MLST est une technique fastidieuse et dispendieuse, elle demeure la seule méthode de génotypage du *C. jejuni* capable de déchiffrer les relations épidémiologiques entre les isolats.

Contribution de l'étudiant

J'ai contribué totalement à l'obtention et à l'analyse de tous les résultats décrits dans l'article, sauf pour les résultats de PFGE. J'ai également écrit entièrement la première ébauche du manuscrit et participé à la correction avant publication.

MULTILOCUS SEQUENCE TYPING OF *CAMPYLOBACTER JEJUNI* ISOLATES FROM HUMANS, CHICKENS, RAW MILK AND ENVIRONMENTAL WATER IN QUEBEC

Simon Lévesque[1], Eric Frost [1], Robert D. Arbeit [2], Sophie Michaud [1].

1. Department of Microbiology and Infectious Diseases, Faculté de Médecine de l'Université de Sherbrooke, Québec, Canada.

2. Infectious Diseases Section, Tufts University School of Medicine, Boston, MA.

Corresponding author: Sophie Michaud, M.D., M.P.H., C.S.P.Q., F.R.C.P.C.
Department of Microbiology and Infectious Diseases
Faculté de Médecine de l'Université de Sherbrooke
3001, 12e avenue Nord
Sherbrooke, Québec J1H 5N4
Phone: (819) 564-5321
Fax: (819) 564-5392
E-mail: Sophie.Michaud@USherbrooke.ca

Financial support: Fonds de la Recherche en Santé du Québec.

Running head: MLST of *C. jejuni*

Key words: *Campylobacter jejuni*, multilocus sequence typing, epidemiology, water, chicken, human, milk, antimicrobial resistance

Word count (text): 4140

ABSTRACT

Molecular strain typing is essential for deciphering the epidemiology of *Campylobacter jejuni* infections. We applied two different methods – MLST, *flaA* SVR – to 289 isolates (163 human, 56 chicken, 34 raw milk, 36 environmental water) collected in Québec over three years; in addition, the analysis included the PFGE typing results of a subset of 131 isolates previously studied. MLST defined 96 sequence types (STs) and 20 clonal complexes (CCs), including 49 STs (73 isolates, 25%) and 39 alleles not previously documented in an international database. The frequency of new STs was significantly higher among water isolates than from other sources (18/36 [50%] vs. 55/253 [22%], respectively; $p<0.001$). Nine of the ten most prevalent CCs included isolates from humans and at least one other source; five comprised exclusively or mostly human and chicken isolates. However, water and milk were the predominant non-human sources among the remaining CCs, suggesting sporadic *C. jejuni* infections in humans may frequently arise from sources other than chicken. All three typing systems were discriminatory (index >0.9). Among 131 isolates analyzed by PFGE, each of the 20 types represented by ≥2 isolates corresponded to a single CC. In contrast, among the 14 most prevalent *flaA* SVR types (5 to 27 isolates each), eight (57%) included isolates that represented multiple different CCs. The basis for these discordant results was uncertain. Antimicrobial resistance was randomly distributed among CCs and appeared to be more closely related to the source of an isolate than its genotype. Although MLST is labor-intensive and expensive, it remains the single best method for genotyping *C. jejuni* and deciphering epidemiologic relationships among isolates.

Abstract word count: 265

INTRODUCTION

Campylobacter jejuni is the leading reported cause of bacterial gastroenteritis in developed countries (3). It is also the leading notifiable enteric food- and waterborne disease in Canada, with 11 543 reported cases in 2002 (http://dsol-smed.phac-aspc.gc.ca). In Quebec, nearly 3000 cases of diarrheal illness are attributed annually to Campylobacter enteritis, more than the combined total caused by *Salmonella* and *Shigella* species, *E. coli* O157:H7 and *Yersinia enterocolitica* (1).

The organisms colonize a range of hosts, including domestic animals and wild birds, and fecal shedding readily contaminates ground water (3). While outbreaks are well documented, most clinical cases are isolated, sporadic infections for which the source is rarely apparent. Consumption of contaminated food, especially poultry has been considered the most prevalent source (2); however, recent studies implicate environmental water and unpasteurized milk as potentially important (28).

Given the limitations of conventional clinical epidemiological approaches (22, 28, 30), recent investigations and surveillance studies have emphasized molecular strain typing methods. Pulse-field gel electrophoresis (PFGE), a highly discriminatory technique that has been effectively applied to other enteric pathogens (42), can successfully confirm *C. jejuni* outbreaks suspected by epidemiological surveillance (15), but has proven only modestly successful in linking small clusters and sporadic cases to particular sources (17, 29).

MLST (multi-locus sequence typing) has emerged as the state-of-the-art method for resolving bacterial population genetics (9, 24). An MLST system for *C. jejuni* has recently been developed (10) and indicates that the species is genetically

diverse, with a weakly clonal population structure, marked by frequent intra- and interspecies horizontal genetic exchange (10, 11, 25). Some MLST-defined lineages of *C. jejuni* have been linked to a restricted geographical area (12) or to particular ecological niches, such as bathing beaches (10), wild birds (6), chickens, pigs, bovines or sheep (25). Although MLST is highly reproducible, portable, and easy to interpret, it is complex and expensive to perform.

A more practical, but still robust, strain typing alternative to MLST is needed. To date, flagellin is the only virulence gene of *C. jejuni* that has proven to be sufficiently diverse to be informative. A simple and effective typing system was developed based on primers directed at highly conserved regions of the *fla* locus which provided a PCR product that could be analyzed for restriction fragment length polymorphism (RFLP) (11, 34). However, previous studies revealed that the flagellin genes can undergo recombination, which limits the stability of the locus for strain typing (16).

flaA, the gene encoding the primary structural flagellin protein of *C. jejuni*, has a short variable repeat (SVR) region of 150 base pairs (27). Analysis of the SVR sequences provided reproducible and discriminatory strain typing results comparable to conventional *fla*-typing, but required appreciably less effort.

We applied both MLST and *flaA* SVR typing to a collection of 289 *C. jejuni* isolates cultured from humans, chickens, raw milk and environmental water in Québec over a 3-year period; this report describes the congruence among the genotypic relationships defined by these two DNA-based strain typing methods and considers their relative utility for epidemiologic and surveillance studies of *C. jejuni*. In addition, the analysis includes the PFGE typing results of a subset of 131 isolates previously

studied (29, 30) as well as antimicrobial resistance profiles to ciprofloxacin, erythromycin and tetracycline.

MATERIALS AND METHODS

Isolates. Only *C. jejuni* isolates were included in this study. Isolates were obtained as previously described (21) from human stools (n=163), fresh whole retail chicken (n=56), raw milk (n=34), and environmental water (n=36). The human isolates originated from fecal samples of patients with diarrhea submitted to the Eastern Townships hospital microbiology laboratories between 1998 and 2003 (8 in 1998-1999, 153 in 2000-2001, 32 in 2002, and 69 in 2003). For subjects with recurrent infections, only the first *C. jejuni* isolate was included in the study. Human isolates were considered to be acquired internationally if the subject had traveled abroad during the entire 10-day period before the onset of symptoms. Isolates for which this information was not available were excluded from the study. Overall, 139 isolates were acquired locally and 24 internationally, including nine from Europe (France, 5; Spain, 2; and United Kingdom and Eastern Europe, one each), ten from Central and South America (Peru, 3; Haiti, 2; and Argentina, Bolivia, Dominican Republic, Mexico, and Mexico or Argentina, one each), plus one each from Chad, Tunisia and Indonesia. For two isolates the country of origin was uncertain.

The water isolates were obtained from river and tributary water samples collected in the Eastern Townships between May 13 and August 12, 2003 (23). In brief, each sample represented about 500 ml of water collected in a sterile Nalgene bottle that was transported on ice, held at 4°C and tested within 24 h. The water was filtered through a sterile 0.45 μm membrane filter; some samples required pre-filtration with a 1.5μm membrane. The filters were transferred into a Whirl-Pak bag containing 100 ml of Park-Sanders enrichment broth with 0.5 ml of Supplement A (0.2% vancomycin and 0.2% trimethoprim lactate) and 5 ml of Supplement B (0.064%

sodium cefoperazone in brucella broth), incubated for 4 h at 37°C in a microaerobic atmosphere, and then at 42°C for an additional 44 h. One ml of the suspension was transferred into a second Park-Sanders broth (10 ml) and incubated at 42°C for 24 h in a microaerobic atmosphere. Then, 100 µl of the final enrichment broth was plated on Karmali agar and incubated in a microaerobic atmosphere at 42°C for 48 h. Plates that were negative for Campylobacter at that time were re-incubated for an additional 24 h. Isolates were identified to the species level by routine phenotyping methods (32) and by two PCR methods (7, 14). Isolates which had a hippurate-negative phenotype but had a hippurate gene detected by PCR were identified as *C. jejuni* (38).

The chicken isolates were cultured from fresh whole retail chickens purchased in grocery stores in the Eastern Townships (37 isolates in 2000-2001 and 19 in 2003) (28, 29). The raw milk isolates were cultured in Québec province in 2000-2001 and provided by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ).

Culture of isolates and preparation of genomic DNA. All *C. jejuni* isolates were grown on 5% (vol/vol) defibrinated sheep blood TSA (Oxoid inc., Nepean, On) in a microaerobic atmosphere at 42°C for 24-48 h. Genomic DNA extraction was made by transferring one single colony into 25 µl of NaOH 0.5 N. After 5 minutes, 25 µl of Tris 1M pH 8.0 and 450 µl of sterile distilled water were added. DNA extracts were stored at -20°C.

Multilocus sequence typing. MLST was carried out as previously described by Dingle et al. (10) with modified amplification conditions (denaturation at 94°C [30 sec], annealing at 50°C (*uncA*), 55°C (*aspA*, *gltA*, *glyA*, *pgm* and *tkt*) or 60°C (*glnA*) [30 sec], and extension at 72 °C [1 min]). For some isolates, primers of the extended MLST system were used (31). PCR products were visualized on 1% (w/vol) agarose

gel electrophoresis. Sequencing reactions were made in collaboration with Genome Quebec (http://www.genomequebec.mcgill.ca). Sequences were compared and analyzed with BioNumerics version 3.5 (Applied Maths). Allele numbers, sequence types and clonal complexes were assigned by submitting DNA sequence to the *C. jejuni* MLST database website (http://pubmlst.org/campylobacter).

***flaA* SVR sequencing.** A fragment of 641 bp of *flaA* gene containing SVR was amplified using the Consensus Forward Primer for *flaA* (5'-ATG GGA TTT CGT ATT AAC AC) (43) and FLA625 RU (5'-CAA GTC CTG TTC CAA CTG AAG) (27). These primers were also used for sequencing. A fragment of 348 bp (corresponding to nucleotides 266 to 613 of the *flaA* gene of the *C. jejuni* ATCC 33560 strain) was used as a reference for comparing the different alleles. Allele numbers were determined from the existing *flaA* SVR database (http://hercules.medawar.ox.ac.uk/flaA/).

PFGE fingerprint analysis. PFGE typing results of *Kpn*I digests of a subset of 95 human and 36 chicken isolates were determined in previous studies (29, 30).

Antimicrobial susceptibility testing. Agar dilution antimicrobial susceptibility tests were performed with ciprofloxacin, erythromycin and tetracycline by agar dilution according to Clinical and Laboratory Standards Institute (CLSI) (33), as described previously (21). Antimicrobial susceptibility results were not available for two locally-acquired and six internationally-acquired human isolates because the stored isolates were not viable.

Phylogenetic analyses. Linkage analysis was performed by calculating the index of association (I_A) using the algorithm of Maynard-Smith (40) included in the START2 package, version 0.5.13 (19). If there is complete linkage equilibrium, i.e., a random association between alleles of different loci indicating a freely recombining

population, then I_A = 0. If there is linkage disequilibrium, indicating a clonal population structure in which recombination has been rare or absent, then I_A is significantly different from 0 (40). The ratio of nonsynonymous to synonymous substitutions (d_N/d_S) and the UPGMA tree were also calculated with the START2 package program. We determined the founding genotype for each clonal complex using eBURST (13), by querying the *C. jejuni* MLST database website and cross-checking with the UPGMA tree.

Statistical analysis. Proportions were compared with Statistix for Windows version 7.1 (Analytical Software, Tallahassee, Fl.), using Chi-square and Fisher's exact two-tailed tests and a significance level of 5%. The discrimination index (DI) was determined using the method of Hunter and Gaston (18).

RESULTS

Population Structure. A total of 96 STs were identified among the 289 isolates analyzed (Table 1). Seventy-nine STs, representing 264 (91.3%) isolates, were assigned to 20 previously described CCs. The remaining 25 isolates were distributed among 17 STs which could not be assigned to any of the known lineages; these include 14 (39%) of the water isolates. The 10 most prevalent CCs (representing all CCs with 5 or more isolates) comprised 239 (83%) of all isolates. Within each CC a single ST accounted for 30% to 100% of the isolates representing that CC; collectively, these dominant ("modal") STs accounted for 141 (49%) isolates (Table 1). The UPGMA tree was consistent with the isolate distribution resolved by eBURST (data not shown).

Table 1. Distribution of 289 *C. jejuni* isolates among Clonal Complexes and Sequence Types. CC, Clonal Complex; ST, Sequence Type; Modal ST, Sequence Type within the Clonal Complex represented by the largest number of isolates.

Clonal Complex	No. Isolates	No. ST	Modal ST	No. (%) Isolates of CC in modal ST
21	76	13	21	44 (58%)
42	17	3	42	11 (65%)
45	53	9	45	35 (66%)
48	5	4	48	2 (40%)
49	7	4	467	3 (43%)
61	15	3	61	13 (87%)
353	32	17	353	10 (31%)
508	10	1	132	10 (100%)
607	13	4	1212	6 (46%)
1275	11	4	637	7 (64%)
Misc	25	17	na	na
Unclassified	25	17	na	na
Total	289	96	10 (10%)	141 (49%)

Allelic diversity. A total of 194 alleles were identified across all seven loci, ranging from 24 alleles at *aspA* to 38 at *pgm* (Table 2). Overall, 39 (20%) of the alleles were previously unreported (last database query, December 2007); the frequency of

new alleles at a locus ranged from 12% (*aspA* and *glnA*) to 28% (*tkt* and *uncA*). The proportion of variable sites per allele ranged from 6.5% for *glnA* to 18.6% for *uncA*. The I_A for the complete data set was 3.519; when only one example of each ST was included, I_A was 1.6779. Both values are significantly different from 0 and consistent with a predominantly clonal population with a moderate degree of horizontal recombination (40). The ratio of nonsynonymous to synonymous substitutions (d_N/d_S) varied across the seven loci, ranging from 0.0125 for *uncA* to 0.0812 for *glyA*.

TABLE 2. Allelic diversity among 289 *C. jejuni* isolates.

Locus	Fragment size (bp)	No. of alleles	No. (%) of new alleles[a]	No. of variable sites[b]	% of variable sites[b]	d_N/d_S[b]
aspA	477	24	3 (12%)	72	15.1	0.0431
glnA	477	26	3 (12%)	31	6.5	0.0370
gltA	402	26	6 (23%)	36	9.0	0.0555
glyA	507	23	5 (22%)	58	11.4	0.0812
tkt	459	32	9 (28%)	56	12.2	0.0597
pgm	498	38	6 (16%)	73	14.7	0.0449
uncA	489	25	7 (28%)	91 (41)	18.6 (8.4)	0.0125 (0.0176)

[a] Number of new alleles identified in this study and submitted to the international MLST database.
[b] Figures in parenthesis exclude *uncA* allele 17, which may have come from a different *Campylobacter* species.

Previously unreported sequence types. Overall, 49 (51%) STs representing 73 (25%) isolates were previously unreported (Table 3; these have been submitted to the international database). Twenty-eight (57%) of the new STs resulted from new allele sequences and the remainder from new combinations of previously described alleles (Table 3). Of note, ST-1227, represented by one water isolate, had new allele sequences detected in all seven genes. Most (38/49; 78%) of the new STs were represented by a single isolate. Among the four sources studied, water had the highest proportion of isolates representing new STs (18/36; 50%) compared with 22%

(36/163) of human, 26% (15/56) of chicken, 12% (4/34) of raw milk ($p<0.001$. Chi square test). Thirty-five (71%) of the new STs could be assigned to known CCs (Table 2).

TABLE 3. New Sequence Types (ST) observed in this study.

Clonal Complex	New ST	No. of isolates	Source[a]	Allele[b]						
				aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*
21	1208	1	H	2	1	1	3	2	1	**103**
21	1209	2	H	2	1	**137**	3	2	1	5
21	1214	1	C	2	1	1	53	2	1	5
21	1459	1	H	2	1	1	2	2	1	5
21	1461	1	H	2	7	12	3	2	1	5
22	1686	1	H	118	3	6	4	3	3	17
45	1215	4	C (2), H (2)	4	7	10	4	121	7	1
45	1217	1	C	4	7	**139**	4	1	7	1
45	1222	4	RM	4	7	**140**	4	1	7	1
45	1228	1	W	4	7	10	4	1	7	**102**
45	1685	1	H	4	7	10	4	121	7	17
48	1460	1	H	6	4	5	2	11	1	5
49	1220	1	C	3	1	5	2	11	11	6
52	1235	1	H	9	17	2	10	**227**	3	6
52	2393	1	H	**181**	17	2	10	11	3	6
61	1244	1	H	1	1	2	2	**225**	3	17
61	1684	1	H	2	4	2	2	6	**181**	5
179	1207	2	H	12	6	**137**	**176**	40	32	3
353	1210	3	H	7	17	5	2	**224**	3	6
353	1211	1	H	7	17	5	2	10	3	3
353	1218	1	C	7	17	12	2	11	3	6
353	1232	1	H	7	17	5	10	11	3	6
353	1233	1	H	7	17	5	10	10	**177**	6
353	1462	1	H	7	4	5	2	11	3	**106**
353	1480	2	H	7	17	5	10	2	**177**	6
353	2394	1	H	**182**	17	5	2	10	3	6
353	2395	1	H	7	4	5	2	11	3	6
443	2396	1	H	24	2	2	15	10	3	12
460	1213	1	C	7	30	2	2	89	59	6
607	1212	6	C (4), H (2)	8	2	5	53	11	3	**105**
607	1216	1	C	9	2	5	53	11	3	1
692	991	1	W	37	52	57	26	107	29	23
1275	1223	2	W	27	33	22	49	43	9	31
1275	1225	1	W	27	33	22	49	43	7	31
1275	1231	1	W	27	2	22	104	43	86	31
UA	1206	1	H	2	59	**136**	105	126	25	23
UA	1219	3	C (2), H (1)	7	17	5	10	11	61	1
UA	1221	1	C	2	84	5	10	119	**178**	26
UA	1224	2	W	18	22	22	97	115	**175**	47

Clonal Complex	New ST	No. of isolates	Source[a]	Allele[b]						
				aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*
UA	1226	5	W	1	6	61	**176**	40	**180**	1
UA	1227	1	W	**122**	**163**	**135**	**175**	**219**	**176**	**101**
UA	1229	1	W	97	121	97	125	174	152	**100**
UA	1230	1	W	1	6	61	4	40	**180**	1
UA	1234	1	H	28	34	27	33	**223**	**182**	33
UA	1236	1	W	94	117	97	125	174	147	**100**
UA	1237	1	W	18	21	22	104	134	101	60
UA	1456	1	H	2	59	4	**187**	131	24	57
UA	1457	1	H	2	**165**	73	**147**	**220**	**190**	**104**
UA	1458	1	H	63	**164**	**141**	**188**	27	**179**	18

UA, unassigned; C, chicken; H, human; RM, raw milk; W, water.

[a] Number in parentheses is number of isolates from each source when more than one source was represented. All isolates were from Canada except as follows: Peru: ST 1233, 1480, 2396; Bolivia: ST 2395; Bolivia or Argentina (indeterminate): ST 2394; Haiti: ST 1234, 1458; Indonesia: ST 2393.

[b] New alleles identified in this study are in boldface.

Association between clonal complexes and source of isolation. Isolates from all four sources were represented only in the two largest CCs (CCs 21 and 45) (Figure 1). Nine of the 10 most prevalent CCs included isolates from humans and at least one other source. Five comprised exclusively or mostly human and chicken isolates; among the remaining CCs, water and milk were the predominant non-human sources. CC 1275 comprised exclusively water isolates, accounting for 31% (11/36) of all isolates from that source. Among the 24 isolates acquired outside of Canada, 21 were distributed among seven CCs; the remaining isolates were not associated with any existing CC. Eight international isolates were found in CC 353, representing 25% of isolates of this CC; an additional six isolates were found in CC 21.

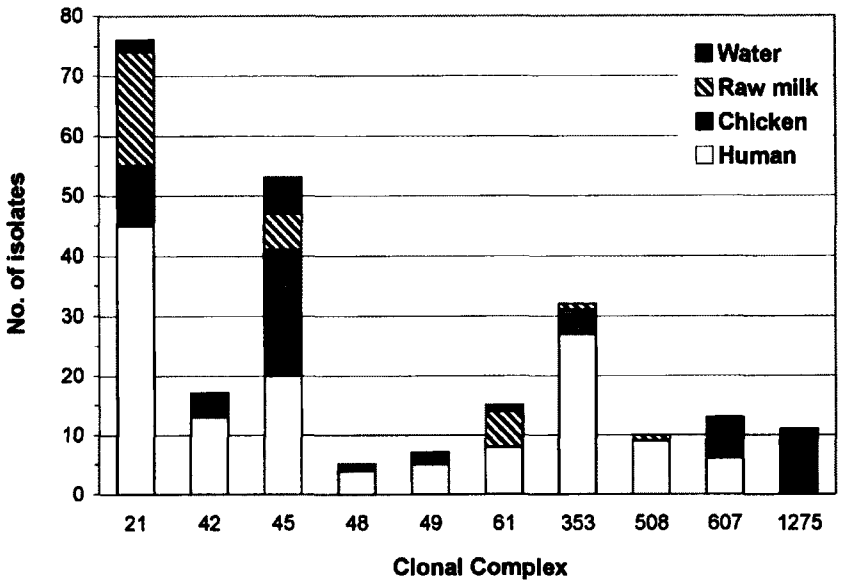


FIGURE 1. Distribution of sources for *C. jejuni* isolates representing the ten most prevalent clonal complexes detected.

Distribution of antibiotic resistance by isolate source. The most common antimicrobial susceptibility pattern detected was susceptibility to all three antibiotics tested (Figure 2). This was observed in 150 (53%) of the 281 isolates evaluated, but was significantly less prevalent among human and chicken isolates (43% and 39%, respectively) compared to raw milk and water isolates (88% and 89%, respectively; $p<0.0001$, Chi square). The most common resistance observed was to tetracycline (41%); only 21 (7.5%) isolates were resistant to ciprofloxacin and only 16 (5.7%) to erythromycin. Erythromycin resistance was most prevalent among isolates from chickens (9/56, 16%); whereas ciprofloxacin resistance was most prevalent among those from humans (19/155, 12%). Of note, resistance to ciprofloxacin was significantly more frequent among the human isolates acquired abroad compared with

those acquired locally (9/18 versus 10/127, respectively; $p < 0.0001$, Fisher's exact test). Tetracycline resistance was also observed in 44% of human isolates acquired abroad. There was no apparent association between antibiotic resistance and CC or ST (data not shown).

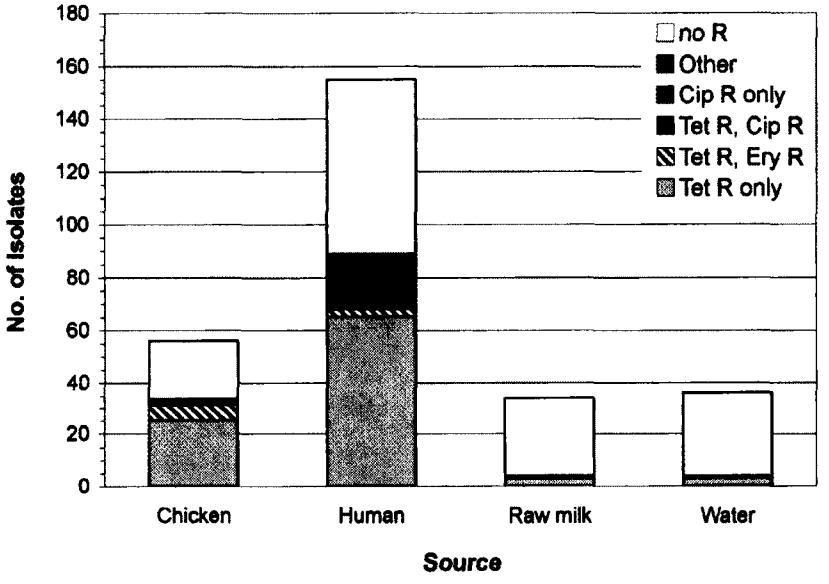


FIGURE 2. Distribution of antibiotic susceptibility patterns for *C. jejuni* isolates by source. R, resistant; Tet, tetracycline; Cip, ciprofloxacin; Ery, erythromycin.

Association between *flaA* SVR type and CC. A total of 91 *flaA* SVR types were identified among 289 isolates. There were 47 (52%) *flaA* SVR types that corresponded to unique isolates. The 14 (15%) most prevalent *flaA* SVR types included at least 5 isolates each, for a total of 156 (54%) isolates (Table 4). These included six *flaA* SVR types each of which was associated with only a single CC. Within each of the remaining eight most prevalent *flaA* SVR types, there was a single most common (modal) CC which represented all but 1 to 3 isolates (Table 4).

Similarly, 23 of the 30 *flaA* types represented by 2 to 4 isolates were associated with a single CC. Thus, among all 242 isolates with *flaA* types represented by more than a single isolate, *flaA* SVR type was predictive of CC for 215 (89%). There was no apparent association between *flaA* SVR type and either antibiotic resistance or source (data not shown).<

TABLE 4. Relationship between *flaA* SVR types and Clonal Complexes.

flaA SVR Type	No. Isolates	Modal Clonal Complex	No. (%) of Isolates in modal CC	Nonconforming CCs (no. of isolates)
36	27	21	25 (93%)	48 (1), 607 (1)
49	15	21	13 (87%)	42 (2)
361	5	21	4 (80%)	48 (1)
792	5	21	5 (100%)	-
359	10	42	10 (100%)	-
21	18	45	16 (89%)	443 (2)
2	16	45	15 (94%)	1275 (1)
8	11	45	11 (100%)	-
42	14	61	13 (93%)	45 (1)
34	9	353	6 (67%)	48 (1), 460 (2)
45	6	353	6 (100%)	-
14	5	353	3 (60%)	607 (2)
172	10	508	10 (100%)	-
621	5	1275	5 (100%)	-
Misc[a]	86	na	73 (85%)	na
Total	242[b]		215 (89%)	

[a] *flaA* types represented by 4, 3 or 2 isolates (8, 10 and 12 types, respectively). For *flaA* types with two isolates of different CC, both isolates were considered non-conforming.
[b] There were 47 isolates with unique *flaA* types.

Associations between PFGE types and CC. PFGE analysis of *Kpn*I restriction digests was performed for 131 isolates. A total of 87 PFGE types were identified; 67 (77%) of these were represented by a single isolate. The remaining 20 types comprised 2 to 6 isolates each; each of these PFGE types was associated with a single CC (Table 5). Thus, among the 64 isolates with PFGE types represented by more than a single isolate, the PFGE profile was 100% predictive of CC.

TABLE 5. Relationship among PFGE types, Clonal Complexes and STs for 131 isolates of *C. jejuni*.

PFGE Type[a]	No. isolates	Clonal Complex	STs represented[b]	*flaA* types represented[b]
3	2	21	806	41
5	6	21	50 (5) 1461	36 (5), 265 (1)
14	6	21	21 (5), 262	49 (5), 37 (1)
15	2	21	21	49
16	2	21	262	54
17	3	21	21	361
18	4	21	21	36
Unique	13	21	21 (5), 50 (4), others (4)	36 (5), 49 (3), 265 (2), others (3)
9	2	42	459	359
10	5	42	42	359 (3), 607 (2)
68	2	42	604	359
Unique	4	42	42 (3), 604	49, 359, 440, 607
1	5	45	45 (2), 1215 (3)	2 (4), 778 (1)
2	2	45	45, 1215	2
19	3	45	45	21
20	2	45	45	21
Unique	14	45	45 (10), 583, 766, 1217, 1685	2 (3), 8 (3), 21 (3), 22 (2), 42, 5, 70
13	3	61	61	42
Unique	1	61	1684	122
11	2	179	1207	779
8	2	257	929	16 (1), 873 (1)
Unique	1	257	929	16
6	2	353	353 (1), 405 (1)	780
Unique	13	353	353 (3), others (10)	45 (3), 780, others (9)
7	6	508	132	172
Unique	2	508	132	172
4	3	607	924 (2), 1216 (1)	790
Unique	2	607	607, 1212	756, 781
Other	17	na	na	na

[a] PFGE types are shown for all 22 types represented by 2 or more isolates. "Unique" indicates PFGE types represented by single isolates within the Clonal Complex. "Other" indicates PFGE types represented by single isolates within the remaining Clonal Complexes.

[b] The ST and *flaA* types represented within the PFGE type; numbers in parentheses indicate the number of isolates. Numbers are omitted where either a single ST or *flaA* type was observed among isolates of a given PFGE type or where the ST or *flaA* type was represented by only a single isolate. "Others" indicate STs or *flaA* types within the CC associated only with unique PFGE types.

Relationships among PFGE type, *flaA* SVR type and ST. The relationships among the three typing systems are depicted graphically in Figure 3. For three CCs, all PFGE types represented by two or more isolates are shown, as well as the ST and *flaA* types associated with all of those isolates. There were no simple linear relationships among the typing systems. Individual ST could be associated with multiple PFGE types (e.g., ST-21 and ST-262 in CC 21); conversely, a single PFGE type could comprise isolates with different ST (e.g. PFGE types 1, 2 and 19 in CC 45). Similarly complex relationships were observed for *flaA* types. The discriminatory power of the typing methods was similar across the 289 isolates studied; the DI was 0.97 for *flaA* SVR, 0.95 for MLST (ST) and 0.88 for MLST (CC), respectively. Among the 131 isolates for which PFGE typing results were available, the DI was 0.99 for PFGE, 0.96 for *flaA* SVR, 0.95 for MLST (ST), and 0.85 for MLST (CC).

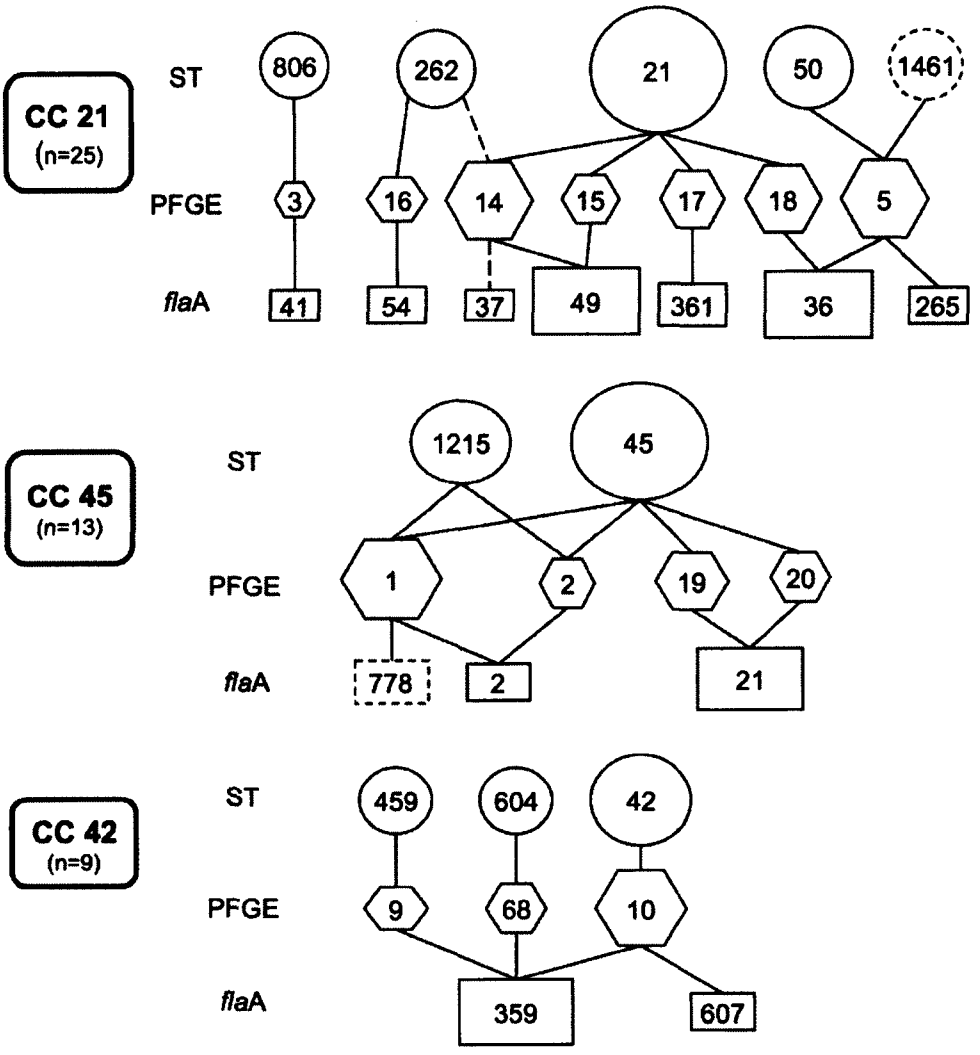


FIGURE 3. Relationships among genotypes of *C. jejuni* as defined by ST (circle), *flaA* SVR (rectangle) and PFGE (hexagon) typing. For each of the three clonal complexes indicated, all PFGE types represented by two or more isolates within the clonal complex are shown as well as the ST and *flaA* SVR types associated with those isolates. Dashed borders indicate that the enclosed types were represented by only a single isolate within the entire series of 289 isolates. The size of figures is roughly proportional to the number of isolates represented; see Table 5 for precise numbers.

DISCUSSION

Each of the molecular methods considered here – MLST, *flaA* SVR and PFGE – has been previously used for typing *C. jejuni* isolates, either in outbreak investigation settings (4, 37), on strain collections from various sources and geographical areas (36) or in human population-based studies (11, 12). The distinctive features of this study were the application of all three molecular techniques to a collection of isolates from diverse sources, including humans, whole-retail chickens, raw milk and environmental water from the same geographical region with the addition of antimicrobial resistance testing as a clinically important phenotype.

The Eastern Townships comprise 102 municipalities (80% rural) in an area of about 10,000 km^2 with approximately 300,000 inhabitants. All of the chicken and water isolates analyzed in this study originated from the Eastern Townships as did 83% of the human isolates; the raw milk isolates were cultured elsewhere in the Québec Province. Among the 289 isolates, MLST analysis at seven loci identified 194 alleles, which in turn defined 96 STs; almost 10% of the isolates could not be assigned to known lineages. Thus, even though the great majority of our isolates originated from a relatively small area, the genetic heterogeneity was comparable to that observed in the Campylobacter MLST database. These findings suggest that the high diversity of the Campylobacter population observed on an international level is also reflected among the genotypes from a relatively small, regional microcosm.

Consistent with this, we observed 39 (20%) new alleles and 49 (51%) new STs. This genotypic diversity was distributed among isolates from all four sources examined. Isolates from humans were found in all but one of the CCs represented by more than one isolate, suggesting that potentially pathogenic strains are not restricted to specific lineages. Five of the most prevalent CCs (42, 48, 49, 353 and 607),

representing about 25% of all isolates, comprised exclusively or predominantly human and chicken isolates. However, water and raw milk were the predominant non-human sources among the remaining CCs, suggesting that sporadic *C. jejuni* infections in humans may frequently arise from sources other than chicken. Population-based studies, such as that of Sopwith et al. (41), are needed to better define the role of the additional sources and the influence of seasonal and regional factors (28). We have recently initiated a 3-year study across the Eastern Townships to correlate the genotypes of Campylobacter isolates from clinical and other sources with geographic and temporal factors.

Excluding the human isolates, some CCs appeared to be preferentially associated with particular ecologic niches, although others represented all the sources examined. These observations support the hypothesis that some genotypes are associated with specific hosts, as suggested by the international *C. jejuni* MLST database as well as other studies (6, 9, 10, 25, 26).

In this context the water isolates raised especially interesting questions. Most of the unassigned STs were composed exclusively of water isolates, which also accounted for a disproportionate number of new alleles. We speculate that this reflects the exceptional diversity of environmental isolates; an alternative explanation is the relatively limited number of Canadian environmental isolates previously available in the international MLST database. CC 1275 in our series was composed exclusively of water isolates and in the database includes isolates from wild birds (61%) as well as environmental water (13%) and bathing beach sand (10%). Similarly, CCs 177 and 179 include isolates from both the environment (sand) and wild bird (6, 10, 25). Beaches typically have high concentrations of wild birds, particularly seagulls, as did the areas where we obtained environmental water isolates. These observations raise

two hypotheses. First, some environmental isolates might be preferentially adapted to colonize the intestines of wild birds and, given the opportunity, are capable of causing human disease. We are continuing to collect seagull and environmental water isolates from the same Eastern Townships area to explore this possibility. A second hypothesis is that some lineages represented among the environmental isolates may be largely non-pathogenic; this might be further investigated by virulence factor studies.

Several molecular strain typing systems have been described for *C. jejuni*; however, there have been relatively few comparative studies and the most effective typing method for particular questions remains unresolved. In this study of a large collection of *C. jejuni* isolates from diverse sources, MLST and *flaA* SVR demonstrated comparable discriminatory power. PFGE profiles were available for only ~ 45% of the isolates. Within this subset, the discriminatory indexes calculated for *flaA* SVR and MLST were similar to that for the total study collection, while that for PFGE was higher. However, each of these molecular typing systems has particular strengths and limitations as well as significant operational differences. The method of choice may vary depending on the specific question at hand.

MLST is now the established standard for analyzing population genetics. MLST provides objective, sequence-based data that is portable and readily analyzed and has an established, unified nomenclature that greatly facilitates integrating different studies (12). However, MLST, which examines seven loci, requires appreciable effort, time and expense. Further, in this series, the two most prevalent types (ST-21 and ST-45) represented over 25% of the isolates, which may significantly limit resolving specific epidemiologic relationships. For this reason, it would be interesting to generate other clonal complexes with the subgroup founding genotypes identified with eBURST. When we analyzed all the isolates to date included

in CC 21 with eBURST, we identified 5 subgroup founding genotypes (ST-19, ST-50, ST-53, ST-104 and ST-262) which met the criteria for forming new clonal complexes. First, each complex had an abundant central genotype and second, each central genotype had many single-, double-, and triple-locus variants (9). Creation of these new clonal complexes would probably help to understand specific epidemiologic relationships in bigger clonal complexes.

Although the discriminatory power of *flaA* SVR, which is also based on nucleotide sequencing, was comparable to MLST, the isolates were more evenly distributed among the different types. Such diversity is potentially more useful for distinguishing among epidemiologically unrelated isolates (4, 9). However, among *flaA* SVR types represented by two or more isolates, 10% of the isolates were discordant with respect to CC, suggesting that *flaA* SVR does not correlate consistently with chromosomal genotype. Other studies have also suggested that *flaA* SVR alone is poorly suited for investigating the molecular epidemiology of *C. jejuni* (8, 9, 11, 12, 35).

PFGE remains the most practical method for focused clinical molecular epidemiology studies, including the identification and tracking of outbreak strains. The variation among PFGE profiles reflects not only nucleotide sequence changes affecting the target restriction sites, but also chromosomal insertions, deletions and rearrangements. Consequently, PFGE typically has a higher "clock speed" and greater discriminatory power (5). On the other hand, the high diversity in the PFGE types within each CC may make the method less effective in long-term longitudinal studies of the epidemiology of Campylobacter (36). The major limitations of PFGE are the subjectivity of visual interpretations of the patterns, the frequent need to confirm

computer-detected matches and the difficulties of sharing typing results among different laboratories.

Linkage analysis of these isolates indicated a moderately clonal structure, consistent with prior studies of *C. jejuni* (40). However, the relationships among the genotypes defined by the three typing systems were complex and non-linear (Figure 3). At this time, we cannot resolve whether this reflects rapid divergence of the sequences contributing to the different typing results or relatively frequent horizontal recombination events, previously noted among *C. jejuni*. Also, as discussed before (39), we cannot detect departure from clonality in our isolate population using the I_A value.

Antimicrobial resistance was randomly distributed among CCs and appeared to be more closely related to the source of the isolate than to its genotype. Resistance to erythromycin and ciprofloxacin were more frequent among isolates from chickens and from patients with recent international travel, respectively. These results suggest that the emergence of resistance among *C. jejuni* reflects antimicrobial selection pressure on isolates from different lineages rather than the diffusion of a unique clone. In their studies of *C. jejuni* chicken isolates in Senegal, Kinana et al. similarly observed that quinolone resistance was variable within STs (20). Of interest, they also noted the highest rate of quinolone resistance in CC 353. Our findings have also important implications on the use of antimicrobial in food animals; because campylobacter may be transferred from animals to humans via food or water, the emergence of multidrug resistance to fluoroquinolones and macrolides in campylobacter strains from the food chain raises concerns that the treatment of human infections will be compromised (21).

The strongest observation from these studies is the rich genetic diversity of *C. jejuni*. This suggests that the sources for sporadic clinical infections are also likely to be diverse and that robust molecular strain typing tools will be required to decipher these relationships. *flaA* SVR typing, which involves sequencing only a single locus, would facilitate large-scale studies. However, the discordant results observed by us and others, although infrequent, remain unexplained and potentially problematic. At this time, MLST appears to be the single most effective tool for molecular strain typing of *C. jejuni* and uniquely suitable for extended, collaborative investigations.

ACKNOWLEDGMENTS

The study was supported by an award from the Fonds de Recherche en Santé du Québec (S.M.) and by the Safe Drinking Water/CFID Studentship Award of the Canadian Infectious Disease Society (S.L.). The Campylobacter Multilocus Sequence Typing website (http://campylobacter.mlst.net) used in the analysis was developed by Man-Suen Chan and sited at the University of Oxford.

We thank Kate Dingle from Oxford University for technical advice and helpful discussion on the MLST method; and Gaétan Lamontagne and Marie Nadeau of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) for providing the raw milk isolates.

REFERENCES

1. L'entérite à *Campylobacter* au Québec: état de situation et propositions d'action: Comité provincial sur l'entérite à Campylobacter au Québec, 1999.
2. **Allos BM.** 2001. *Campylobacter jejuni* infections: update on emerging issues and trends. Clin. Infect. Dis. **32:** 1201-1206.
3. **Altekruse S. F., N.J. Stern, P. I. Fields, and D. L. Swerdlow.** 1999. *Campylobacter jejuni*--an emerging foodborne pathogen. Emerg. Infect. Dis. **5**:28-35.
4. **Clark C. G., L. Bryden, W. R. Cuff, P. L. Johnson, F. Jamieson, B. Ciebin, and G. Wang.** 2005. Use of the oxford multilocus sequence typing protocol and sequencing of the flagellin short variable region to characterize isolates from a large outbreak of waterborne *Campylobacter* sp. strains in Walkerton, Ontario, Canada. J. Clin. Microbiol. **43**:2080-2091.
5. CLSI 2007. Molecular methods for bacterial strain typing; approved guideline.MM11-A, vol. 27, Clinical and Laboratory Standard Institute, Wayne, PA.
6. **Colles F. M., K. Jones, R. M. Harding, and M. C. Maiden.** 2003. Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. Appl. Environ. Microbiol. **69**:7409-7413.
7. **Denis M., C. Soumet, K. Rivoal, G. Ermel, D. Blivet, G. Salvat, and P. Colin.** 1999. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Lett. Appl. Microbiol. **29**:406-410.
8. **Dingle K. E., F. M. Colles, D. Falush, and M. C. Maiden.** 2005. Sequence typing and comparison of population biology of *Campylobacter coli* and *Campylobacter jejuni*. J. Clin. Microbiol. **43**:340-347.
9. **Dingle K. E., F. M. Colles, R. Ure, J. A. Wagenaar, B. Duim, F. J. Bolton, A. J. Fox, D. R. Wareing, and M. C. Maiden.** 2002. Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. Emerg. Infect. Dis. **8**:949-955.
10. **Dingle K. E., F. M. Colles, D. R. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J. Bootsma, R. J. Willems, R. Urwin, and M. C. Maiden.** 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J. Clin. Microbiol. **39**:14-23.
11. **Djordjevic S. P., L. E. Unicomb, P. J. Adamson, L. Mickan, and R. Rios.** 2007. Clonal complexes of *Campylobacter jejuni* identified by multilocus sequence typing are reliably predicted by restriction fragment length polymorphism analyses of the *flaA* gene. J. Clin. Microbiol. **45**:102-108.
12. **Duim B., P. C. Godschalk, N. van den Braak, K. E. Dingle, J. R. Dijkstra, E. Leyde, J. van der Plas, F. M. Colles, H. P. Endtz, J. A. Wagenaar, M. C. Maiden, and A. van Belkum.** 2003. Molecular evidence for dissemination of unique *Campylobacter jejuni* clones in Curacao, Netherlands Antilles. J. Clin. Microbiol. **41**:5593-5597.
13. **Feil E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. **186:** 1518-1530.
14. **Fermer C., and E. O. Engvall.** 1999. Specific PCR identification and differentiation of the thermophilic campylobacters, *Campylobacter jejuni, C. coli, C. lari*, and *C. upsaliensis*. J. Clin. Microbiol. **37**:3370-3373.

15. **Fitzgerald C., L. O. Helsel, M. A. Nicholson, S. J. Olsen, D. L. Swerdlow, R. Flahart, J. Sexton, and P. I. Fields.** 2001. Evaluation of methods for subtyping *Campylobacter jejuni* during an outbreak involving a food handler. J. Clin. Microbiol. **39**:2386-2390.
16. **Harrington C. S., F. M. Thomson-Carter, and P. E. Carter.** 1997. Evidence for recombination in the flagellin locus of *Campylobacter jejuni*: implications for the flagellin gene typing scheme. J. Clin. Microbiol. **35**:2386-2392.
17. **Hedberg C. W., K. E. Smith, J. M. Besser, D. J. Boxrud, T. W. Hennessy, J. B. Bender, F. A. Anderson, and M. T. Osterholm.** 2001. Limitations of pulsed-field gel electrophoresis for the routine surveillance of Campylobacter infections. J. Infect. Dis. **184**:242-244.
18. **Hunter P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26**:2465-2466.
19. **Jolley K. A., E. J. Feil, M. S. Chan, and M. C. Maiden.** 2001. Sequence type analysis and recombinational tests (START). Bioinformatics **17**:1230-1231.
20. **Kinana A. D., E. Cardinale, F. Tall, I. Bahsoun, J. M. Sire, B. Garin, S. Breurec, C. S. Boye, and J. D. Perrier-Gros-Claude.** 2006. Genetic diversity and quinolone resistance in *Campylobacter jejuni* isolates from poultry in Senegal. Appl. Environ. Microbiol. **72**:3309-3313.
21. **Lévesque S., E. Frost, and S. Michaud.** 2007. Comparison of antimicrobial resistance of *Campylobacter jejuni* isolated from humans, chickens, raw milk and environmental water in Québec. J. Food Protection **70**:729-735.
22. **Lévesque S., K. St-Pierre, E. Frost, and S. Michaud.** Determination of the optimal culture conditions for detecting *Campylobacter* spp. in environmental water. 2005. Abstr. 13th International Workshop on Campylobacter, Helicobacter and Related Organisms, abstr. A49.
23. **Lévesque S., J. Martel, E. Frost, and S. Michaud.** Prevalence of Campylobacter in river and tributary water in the Eastern Townships, Québec, Canada. 2005. Abstr. 13th International Workshop on Campylobacter, Helicobacter and Related Organisms, abstr. A52.
24. **Maiden M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA. **95**:3140-3145.
25. **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.
26. **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.
27. **Meinersmann R. J., L. O. Helsel, P. I. Fields, and K. L. Hiett.** 1997. Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. J. Clin. Microbiol. **35**:2810-2814.
28. **Michaud S., S. Ménard, and R. D. Arbeit.** 2004. Campylobacteriosis, Eastern Townships, Quebec. Emerg. Infect. Dis. **10**:1844-1847.

29. **Michaud S., S. Ménard, and R. D. Arbeit.** 2005. Role of real-time molecular typing in the surveillance of campylobacter enteritis and comparison between PFGE profiles of chicken and human isolates. J. Clin. Microbiol. **43:**1105-1111.
30. **Michaud S., S. Ménard, C. Gaudreau, and R. D. Arbeit.** 2001. Comparison of *Sma*I-defined genotypes of *Campylobacter jejuni* examined by *Kpn*I: A population-based study. J. Med. Microbiol. **50:**1075-1081.
31. **Miller W. G., S. L. On, G. Wang, S. Fontanoz, A. J. Lastovica, and R. E. Mandrell.** 2005. Extended multilocus sequence typing system for *Campylobacter coli*, *C. lari*, *C. upsaliensis*, and *C. helveticus*. J. Clin. Microbiol. **43:**2315-2329.
32. **Nachamkin I.** 2003. *Campylobacter* and *Arcobacter*, p. 902-914. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, J. H. Jorgensen, and R. H. Yolken (ed.), Manual of clinical microbiology. 8th ed. ASM Press, Washington, DC.
33. **NCCLS.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-A3, vol. 23, National Committee for Clinical Laboratory Standards, Wayne, PA.
34. **Petersen L., and D. G. Newell.** 2001. The ability of *fla*-typing schemes to discriminate between strains of *Campylobacter jejuni*. J. Appl. Microbiol. **91:** 217-224.
35. **Price E. P., V. Thiruvenkataswamy, L. Mickan, L. Unicomb, R. E. Rios, F. Huygens, and P. M. Giffard.** 2006. Genotyping of *Campylobacter jejuni* using seven single-nucleotide polymorphisms in combination with *flaA* short variable region sequencing. J. Med. Microbiol. **55:**1061-1070.
36. **Sails A. D., B. Swaminathan, and P. I. Fields.** 2003. Clonal complexes of *Campylobacter jejuni* identified by multilocus sequence typing correlate with strain associations identified by multilocus enzyme electrophoresis. J. Clin. Microbiol. **41**: 4058-4067.
37. **Sails A. D., B. Swaminathan, and P. I. Fields.** 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.
38. **Slater E. R., and R. J. Owen.** 1997. Restriction fragment length polymorphism analysis shows that the hippuricase gene of *Campylobacter jejuni* is highly conserved. Lett. Appl. Microbiol. **25:** 274-278.
39. **Smith J. M.** 1999. The detection and measurement of recombination from sequence data. Genetics. **153:** 1021-1027.
40. **Smith J. M., N. H. Smith, M. O'Rourke, and B. G. Spratt.** 1993. How clonal are bacteria? Proc. Natl. Acad. Sci. USA. **90:**4384-4388.
41. **Sopwith W., M. Ashton, J. A. Frost, K. Tocque, S. O'Brien, M. Regan, and Q. Syed.** 2003. Enhanced surveillance of campylobacter infection in the north west of England 1997-1999. J. Infect. **46:**35-45.
42. **Swaminathan B., T. J. Barrett, and the CDC PulseNet Task Force Force.** 2000. A national molecular subtyping network for food-borne bacterial disease surveillance in the United States, p. 529-535. *In* I. Nachamkin and M. C. Blaser (ed.), Campylobacter. 2nd ed., ASM Press, Washington, DC.
43. **Wassenaar T. M., and D. G. Newell.** 2000. Genotyping of *Campylobacter* spp. Appl. Environ. Microbiol. **66:**1-9.

CHAPITRE 3

AVANT-PROPOS DE L'ARTICLE

Titre

High-resolution melting system to perform Multilocus sequence typing of *Campylobacter jejuni*

Auteurs

Simon Lévesque, Sophie Michaud, Robert D. Arbeit et Eric Frost.

État de l'article à ce jour

Publié dans la revue PLOS One 2011 **6**:e16167.

Le formulaire « Autorisation d'intégration d'un article écrit en collaboration à un mémoire ou une thèse » a été signé par chaque co-auteure ou co-auteur conformément à la « Directive relative au dépôt des essais, des mémoires et des thèses » de l'Université de Sherbrooke.

RÉSUMÉ DE L'ARTICLE

Le Multi-locus sequence typing (MLST) est devenu la méthode de référence pour l'étude de l'évolution génétique des populations bactériennes, mais cette méthode demeure dispendieuse et fastidieuse. Dans cette étude, nous avons évalué le potentiel du High-resolution melting (HRM) pour l'identification d'allèles connues par MLST pour le *Campylobacter jejuni*, dans le but de réduire le coût et le temps technique de la méthode MLST. Chaque allèle du système MLST classique fut amplifié en deux ou trois sous fragments, qui furent par la suite analysés par HRM. Le système HRM fut validé en utilisant 47 isolats de *C. jejuni* déjà caractérisés par le système MLST classique. Ces isolats ont été choisis pour leur provenance variée, tels que l'environnement, les animaux ou encore l'humain, et ils incluent les six *sequence type* (ST) les plus prévalents et les allèles les plus fréquents. Le système HRM fut utilisé pour la validation d'un groupe additionnel de 84 isolats de *C. jejuni* provenant du poulet. Pour ces derniers isolats, 92 % des allèles furent déterminés en 35 heures de travail et le coût fut de 20 $ pour le HRM par rapport à 100 $ pour la méthode MLST classique basée sur le séquençage. Le HRM a le potentiel de complémenter les méthodes d'analyses basées sur du séquençage pour l'étude des mutations ponctuelles et de faciliter une vaste gamme d'études basées sur des méthodes génotypiques.

Contribution de l'étudiant

J'ai participé au design de l'étude et des expériences. J'ai contribué totalement à l'obtention et à l'analyse de tous les résultats décrits dans l'article. J'ai également écrit entièrement la première ébauche du manuscrit et participé à la correction avant la publication.

HIGH-RESOLUTION MELTING SYSTEM TO PERFORM MULTILOCUS SEQUENCE TYPING OF *CAMPYLOBACTER JEJUNI*

Simon Lévesque[1], Sophie Michaud [1], Robert D. Arbeit[2], Eric H. Frost [1]

1. Department of Microbiology and Infectious Diseases, Faculté de Médecineet des sciences de la santé de l'Université de Sherbrooke, Québec, Canada.

2. Infectious Diseases Section, Tufts University School of Medicine, Boston, MA.

Corresponding author: Eric Frost, Ph.D.
Department of Microbiology and Infectious Diseases
Faculté de Médecine de l'Université de Sherbrooke
3001, 12e avenue Nord
Sherbrooke, Québec J1H 5N4
Phone: (819) 564-5321
Fax: (819) 564-5392
E-mail: Eric.Frost@USherbrooke.ca

Financial support: The Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Centre de Recherche Clinique Étienne Le-Bel.

Running head: Genotyping of *Campylobacter jejuni*

Key words: *Campylobacter jejuni*, High-resolution melting, Multilocus sequence typing, molecular epidemiology

Word count (text): 2508

ABSTRACT

Multi-locus sequence typing (MLST) has emerged as the state-of-the-art method for resolving bacterial population genetics but it is expensive and time consuming. We evaluated the potential of high resolution melting (HRM) to identify known MLST alleles of *Campylobacter jejuni* at reduced cost and time. Each MLST locus was amplified in two or three sub fragments, which were analyzed by HRM. The approach was investigated using 47 *C. jejuni* isolates, previously characterized by classical MLST, representing isolates from diverse environmental, animal and clinical sources and including the six most prevalent sequence types (ST) and the most frequent alleles. HRM was then applied to a validation set of 84 additional *C. jejuni* isolates from chickens; 92% of the alleles were resolved in 35 hours of laboratory time and the cost of reagents per isolate was $20 compared with $100 for sequence-based typing. HRM has the potential to complement sequence-based methods for resolving SNPs and to facilitate a wide range of genotyping studies.

Abstract word count: 159

INTRODUCTION

Campylobacter jejuni is the leading reported cause of bacterial gastroenteritis in developed countries (1). The organisms colonize a range of hosts, including domestic animals and wild birds, and fecal shedding readily contaminates ground water (1). While outbreaks are well documented, most clinical cases represent isolated, sporadic infections for which the source is rarely apparent. Consumption of contaminated food, especially poultry has been considered the most prevalent source (2); however, recent studies implicate environmental water and unpasteurized milk as potentially important (3).

Multi-locus sequence typing (MLST), a genotyping system based on single-nucleotide polymorphisms (SNPs) of housekeeping genes, has emerged as the state-of-the-art method for resolving bacterial population genetics (4, 5). A recently developed MLST system for *C. jejuni*(6) indicates that the species is genetically diverse, with a weakly clonal population structure, marked by frequent intra- and interspecies horizontal genetic exchange (6, 7, 8). Some MLST-defined lineages of *C. jejuni* have been linked to a restricted geographical area (9) or to particular ecological niches, such as bathing beaches (7), water (10), wild birds (11), chickens, pigs, bovines or sheep (12). Although MLST is highly reproducible, portable, and easy to interpret, it is complex and expensive to perform.

The development of fluorescent DNA binding dyes with improved saturation properties has allowed a more precise assessment of sequence variation based on the analysis of DNA melting curves. This technique, referred to as high resolution melting (HRM), can distinguish single base variation and then has the potential to identify SNPs without the burden of sequencing (13, 14). After PCR amplification, amplicons are subjected to melting curves with a fluorescence monitoring of a saturating dye that

does not inhibit PCR. This approach provides a simple, closed-tube, semi-automated and cost-effective method for detecting base substitutions and small insertions or deletions (15). Merchant-Patel et al. (16) recently reported the application of HRM for typing the flagellin-encoding *flaA* gene of *Campylobacter jejuni*; their results demonstrated that the method is both accurate and easy to implement.

In this study, we describe the novel application of an HRM protocol optimized to perform MLST of *C. jejuni* isolates. Our goal was to resolve the *C. jejuni* sequence types as defined in the existing MLST database (http://pubmlst.org/campylobacter) at substantially lower cost than the conventional sequence-based method.

RESULTS

For all 47 isolates, successful amplifications were achieved across the 17 sub fragments spanning the seven MLST loci. Table 1 lists all SNPs included in this study. The SNP position in the fragment did not have a strong effect on the T_m separation, even if the SNP was near the amplification primer. Excluding *uncA*, about 90% of SNPs were transition mutations (T to C or C to T, A to G or G to A), but inversion mutations (G to C or C to G and A to T or T to A) were also readily detected.

TABLE 1.SNPs in locus fragments.

Allele	SNPs position (5' to 3') in locus fragments[ab]										
	9	45	84	174	279	342	414	476			
aspA-1	T	G	G	G	C	C	T	C			
aspA-2	T	G	A	A	T	C	C	T			
aspA-4	C	A	G	G	C	T	T	C			
aspA-7	T	G	A	A	T	C	T	T			
aspA-8	T	G	G	G	T	C	C	T			
	12	33	45	108	112	132	202	267	369	384	465
glnA-1	G	A	A	A	C	A	A	C	C	T	A
glnA-2	G	A	A	G	C	A	A	C	C	T	A
glnA-4	G	G	A	G	T	A	G	T	T	C	G
glnA-7	A	A	A	G	C	T	A	C	T	T	A
glnA-17	G	G	G	G	T	A	A	C	C	T	A

	12	39	200	201	207	294	320	348	396
gltA-1	A	C	T	G	C	C	G	A	A
gltA-2	G	T	T	G	T	C	A	A	A
gltA-3	A	T	C	G	C	T	A	G	A
gltA-5	A	C	T	G	C	C	A	A	A
gltA-10	A	T	T	C	C	T	A	A	G

	3	42	51	57	114	120	129	136	138	198	208	213	237	259	264	267	285	286	303	309	312	320	504
glyA-2	T	C	C	T	T	A	C	C	T	C	G	C	A	A	C	A	A	C	T	T	G	C	C
glyA-3	C	T	T	C	T	A	C	C	T	T	A	T	A	G	T	G	A	T	C	T	A	C	T
glyA-4	T	C	C	T	C	G	G	T	A	T	A	T	A	G	C	G	G	C	C	C	A	T	C
glyA-53	T	T	T	C	T	A	C	C	T	T	A	T	G	G	T	G	A	T	C	T	A	C	T

	33	41	45	81	150	162	165	168	171	216	219	219	249	267	291	316	324	342	348	372	405	408	435	453	471	494
pgm-1	A	C	T	A	A	A	A	T	A	A	C	C	A	C	G	T	C	C	G	T	T	T	T	C	C	C
pgm-2	G	T	C	G	G	G	T	A	G	G	T	T	G	T	T	C	C	T	A	C	T	C	T	T	T	T
pgm-5	A	C	T	G	A	A	A	T	A	A	C	C	A	T	G	T	T	T	G	T	T	T	C	C	C	C
pgm-6	A	C	T	G	G	G	C	A	G	A	C	C	A	T	T	C	C	T	A	C	C	C	T	T	T	C
pgm-10	A	C	T	G	A	A	G	T	A	A	C	C	A	T	G	T	C	T	G	T	T	T	T	C	C	C
pgm-11	G	T	C	G	G	G	C	A	G	G	T	T	G	T	T	C	C	T	A	C	T	C	T	T	T	T

	12	21	72	117	138	141	162	174	189	297	330	435
tkt-1	C	C	T	C	C	T	A	A	C	C	T	C
tkt-3	C	C	T	C	C	T	A	A	C	C	C	C
tkt-7	T	T	A	C	A	C	A	G	T	T	C	T
tkt-9	T	T	A	T	C	T	G	G	T	T	C	T

	3	189	234	375
uncA-1	T	C	G	C
uncA-3	C	C	G	T
uncA-5	C	T	G	C
uncA-6	C	C	G	C
uncA-17[b]				
uncA-105	T	C	A	C

[a] The numbering starts at the first nucleotide of each comparison fragment for each locus on the *C. jejuni* MLST database website. Numbers not underlined are in the left fragment, numbers with intermittent underlining are in the middle fragments and numbers with solid underling are in the right fragment.
[b] Left fragment: 12 SNPs; middle fragment: 29 SNPs; right fragment: 28 SNPs

For each sub fragment, the expected 3 to 6 alleles were resolved by HRM as distinct difference plots (Figure 2). The reproducibility of the system was confirmed at multiple levels. The same DNA extracts were run in duplicate or triplicate wells of the same plate and in replicate wells across different runs. In addition, gene fragments representing the same MLST allele were amplified from DNA extracts of at least 6 different *C. jejuni* isolates. The HRM curves for the same DNA preparations or for the same sequences (SNPs) amplified from different isolates were readily grouped together; conversely, curves for different alleles could be consistently resolved.

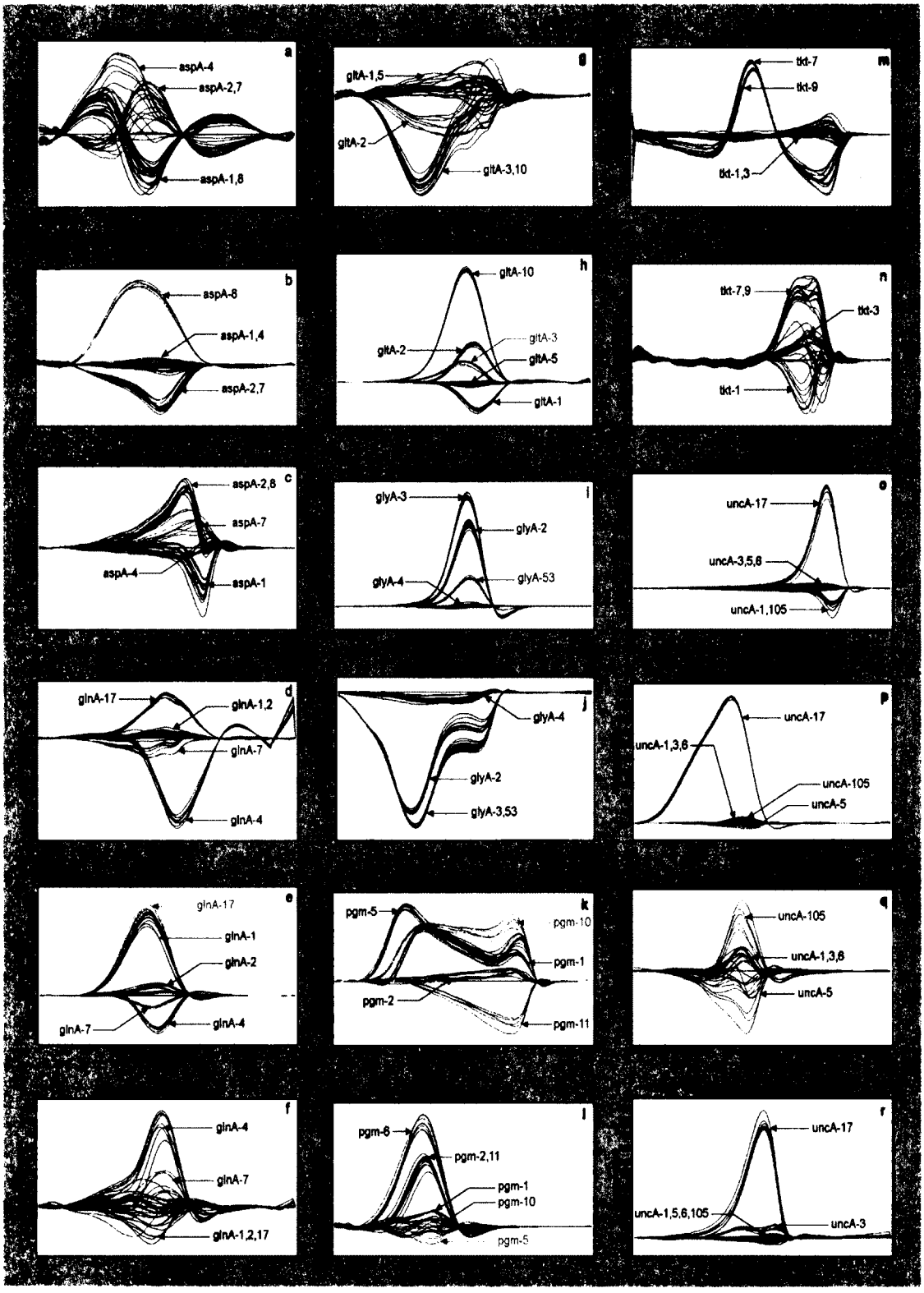


FIGURE 2.Difference plots for the normalized and temperature shifted melting curves for all locus fragments. **a:** asp left. **b:** asp middle. **c:** asp right. **d:**gln left. **e:**gln middle. **f:**gln right. **g:**glt left. **h:**glt right. **i:**gly left. **j:**gly right. **k:**pgm left. **l:**pgm right. **m:**tkt left. **n:**tkt right. **o:**unc left. **p:**unc middle.**q:**unc middle without allele unc-17. **r:**unc right. Arrows link allele numbers with corresponding same color curves.

Since each MLST locus was divided into two or three sub fragments for the HRM analysis, it was necessary to consider the HRM profiles for all of the sub fragments together in order to assign an MLST allele. For example, *aspA* was represented by five alleles and the locus was analyzed in three fragments (Table 2). The left fragment (199 bp) contained three SNPs; however, within this sub fragment, there were only three unique sequences –alleles *aspA*-1 and -8 were identical as were *aspA*-2 and -7. The middle fragment (197 bp) contained two SNPs generating three unique sequences; but in this region, *aspA*-1 and -4 were identical, as were *aspA*-2 and -7. Finally, the right fragment (247 bp) included 3 SNPs generating 4 unique sequences, with *aspA*-2 and -8 being identical. Within each sub fragment, the unique sequences had distinct HRM profiles (Figure 2A, 2B, and 2C). The combination of profiles across the three sub fragments resolved the five different alleles.

TABLE 2. Oligonucleotide primers used in the study.

Locus	Locus fragment	Forward (5' to 3')		Reverse (5' to 3')		Amplicon size (bp)
aspA	asp left	ASP HRM F3	GTG AAT TTA AAA CTT TTG CCG TA	ASP HRM R6	TCG ATC AAA TCC TCA GCC ACA GTA	199
	asp middle	ASP HRM F5	TAA GAG AAG TGA CAG GTT TTG AAT	ASP HRM R7	GGA AGA TTA ATC TCA TTA AGA CCA CAT T	187
	asp right	ASP HRM F7	GAC TTA AGA CTT TTA AGT AGT GGT CC	ASP HRM R4	GCA TTA CAA CAG AAT TAA ATA AGC TAT ATG C	247
glnA	gln left	GLN HRM F6	AAC CTG ATG CTC AAA GTG C	GLN HRM R5	CAT TTT TCA TAC ATT TGT CCT TTG	106
	gln middle	GLN HRM F7	CTA TCA TAG TAT TTT GTG ATG TGT ATG	GLN HRM R4	CTA AAG AAT CAA TTG GCT GAA CTG G	318
	gln right	GLN HRM F4	CTG GAC ACA GGC CAA GAA ACA AAG GTG	GLN HRM R2	GAG CTA CCA TTT TTA CAA CAT ATT TAT AAA TTT G	231
gltA	glt left	GLT HRM F1	CGC GTC TTG AAG CAT TTC GTT AT	GLT HRM R1	CCA CTA TAG TAG GGA TTT TAG CTA C	225
	glt right	GLT HRM F2	GAA TAT ATG GAA ATG GCA GCT AG	GLT HRM R2	GCA TGA GTT GAA CCC ACA GC	272
glyA	gly left	GLY HRM F3	GAT AAA ATT TTA GGA ATG GAT TTA AGT CAT G	GLY HRM R1	CAC AAC AAG ACC TGC AAT ATG	288
	gly right	GLY HRM F2	GCC TAT CTT TTT GCT GAT ATA GCA	GLY HRM R2	AAA ACA TTA GCT AAA ACT TGA GC	317
pgm	pgm left	PGM HRM F1	GAA GTT ATA GTA ATG AGT GAT AAA CCT AAT G	PGM HRM R1	TTT AAA GCA CCA TTA CTC ATT ATA GT	275
	pgm right	PGM HRM F4	GGT AAA TTA CAA TCA AGT GTT GTG GC	PGM HRM R3	CTT TTT TTT CTG CAA TTT TAA G	328
tkt	tkt left	TKT HRM F1	CAT GCA AGT GCT TTG CTT TAT AGT	TKT HRM R1	CCC ATC TCC GCA AAG ACA A	261
	tkt right	TKT HRM F2	GCT AGG CAG TGA TTT AAT CGA TCA	TKT HRM R2	GAT GAT AAG ACA AGG TTT TGT GGA	304
uncA	unc left	UNC HRM F6	GGT GCT ATG GAA TAT ACT ATT GTT G	UNC HRM R3	GAC ATT TCG CGA TAA GCT ACA GC	176
	unc middle	UNC HRM F7	GTT TAT GAT GAT TTG AGC AAG C	UNC HRM R4	GTT GGA ATA TAA GCA GAA ACA TCT CC	221
	unc right	UNC HRM F8	GGT GCT GGT TCT TTG ACG GCA TTG	UNC HRM R2	GTG CAA AAG CTT GAA GCT CTC TA	265

The *uncA* alleles in the demonstration set included *uncA*-17, which is derived from *C. coli* (6) and differs from the other *uncA* alleles by multiple SNPs, representing both transition and substitution mutations. Consequently, the HRM profile for each sub fragment of *uncA*-17 was highly divergent from the profiles for the other *uncA* alleles, with appreciably higher values for the relative signal difference (y axis, Figure 2O, P and R). For the middle sub fragment the other alleles were particularly difficult to resolve when *uncA-17* was present (Figure 2P), but readily distinguished when *uncA-17* was excluded (Figure 2Q).

To evaluate the relative efficiency and cost of performing MLST by HRM compared with conventional direct sequencing, we analyzed a confirmation set of 84 additional *C. jejuni* isolates from chickens. Using HRM, we resolved 92% of the MLST alleles. Moreover, the analysis required only 35 hours of laboratory time and reagents cost only $20 (Canadian) per isolate compared with $100 for sequence-based typing (data not shown).

DISCUSSION

MLST has emerged as the state-of-the-art method for studying bacterial population genetics. The MLST system for *C. jejuni* has been used in population studies of isolates from different geographical areas (17), from human and non-human sources (7, 9), as well as in molecular epidemiologic analyses of outbreaks (18, 19). However performing MLST remains laborious and expensive. We have shown here that HRM can complement full MLST characterization of *C. jejuni* by identifying the most common alleles more rapidly and at lower cost.

HRM can resolve the SNPs that define the different alleles in the MLST system because two DNA amplicons that differ at even a single nucleotide will have different melting profiles. For the demonstration set of 47 diverse isolates, HRM resolved all 35 predicted alleles among the seven MLST genes. The differences in melting profiles among alleles varied with the number and type of SNPs as well as the gene fragment being amplified. For example, the profiles for *unc*-17, an allele which is known to come from *C. coli* (6, 8, 10, 11, 20), showed very strong differences in relative fluorescence signal and very sharp groupings (Figure 2O, P and Q). However, sub fragments where the alleles differed by only a single SNP often showed readily distinguished melting profiles (e.g., *aspA*-2, 7 and *aspA*-1, 8 in Figure 2A and *aspA*-7 and *aspA*-1 in Figure 2C). Even in instances where the relative fluorescence signal differences were quite small (0.8 – 3.0) and, consequently, the profiles less tightly clustered (Figure 2A, C and F), reliable interpretation was possible based on the differences in the overall profiles considered across the range of temperatures.

This strategy for typing *C. jejuni* isolates has many important advantages, but the single greatest benefit is the reduction in the total time and cost required. HRM requires neither agarose gel analysis, sequencing, nor sequence analysis. We estimate

that the per isolate cost to perform MLST using HRM is 20-30% that of sequencing. This is achieved without compromising the portability of MLST since the existing nomenclature can be used. As of March 2010, the *C. jejuni* MLST database contained more than 4300 alleles among almost 9000 isolates. The 47 isolates in our study were drawn from the six major clonal complexes and included alleles whose frequency in the current database ranged from 40% and 68% (*pgm* and *uncA*, respectively). We were able to resolve most of these alleles using a single reference isolate for each of the six major sequences types. Distinguishing all known alleles might require additional reference isolates. However, an advantage of this HRM system is that any sequenced allele can be used as the reference profile. Our experience with the 84 *C. jejuni* isolates from chickens demonstrated that the system is particularly efficient when analyzing ecological niches with relatively few ST variations. Analyzing isolates from niches with more variation, novel niches, or from several niches simultaneously would be less efficient as it would require additional reference strains or sequencing more samples, but would still be less expensive than sequencing of all genes.

Obviously, an HRM system cannot replace sequence-based MLST. If a previously unidentified melting profile is encountered, it is necessary to revert to sequencing; however, once identified, the new profile can be used for reference in subsequent HRM analyses. If the sequence proves to be a new allele, it can be submitted to the database.

At a technical level, HRM can be performed using cyclers that accept 384-well plates, permitting high-throughput studies. Because HRM compares amplicons from independent PCR reactions, it is essential to standardize the quantity of DNA used in order to minimize reaction-to-reaction variability. We observed that variation in DNA

quantity or quality could shift amplification curves; such offsets have been previously observed to compromise the HRM groupings (21).

HRM can be readily applied to a wide range of genetic analyses that involve detection of a single SNP or a signature allele representing a specific set of SNPs. Examples in microbiology include studies requiring the identification of particular clonal complexes, sequence types or individual mutations. By selecting the locus amplified and the reference standard for the HRM system based on the objective of the study, this approach can be applied to questions in pathogenesis, ecology, epidemiology and antibiotic resistance. As just one example, the NAP1/027 epidemic strain of *C. difficile* belongs to MLST type 35 (24). Identifying a signature allelic profile could serve as a rapid shortcut for preliminary strain detection (22), minimizing the challenges and effort associated with PFGE or sequencing. Analogous situations arise in numerous studies across all levels of biology, from resistance mutations in viruses to human alleles associated with clinical disease

In summary, we have demonstrated that by analyzing multiple loci concurrently HRM technology can resolve the SNPs that are the basis of MLST. In our studies of >120 *C. jejuni* isolates from diverse geographical sources and representing diverse genotypes, the HRM results were consistent with sequencing and thus could be expressed using the existing MLST nomenclature, but were obtained with greater speed, less effort and at lower cost. HRM has the potential to complement classical sequence-based methods and facilitate a wide range of genotyping studies.

MATERIALS AND METHODS

Isolates.

Table 3 lists the source, MLST alleles, sequence type and clonal complex of 47 *C. jejuni* isolates used in this study; all have been previously reported [10] and analyzed by the standard MLST protocol [7]. Isolates were selected to represent diverse sources and to include the six most prevalent sequence types (ST) and most frequent alleles for each locus.

TABLE 3.*C. jejuni* isolates used in the study.

Isolate	Source	aspA	glnA	gltA	glyA	pgm	tkt	uncA	ST[a]	CC[b]
001A-0058	Human	2	1	1	3	2	1	5		
001A-0078	Human	2	1	1	3	2	1	5		
001B-0003	Chicken	2	1	1	3	2	1	5		
001B-0035	Chicken	2	1	1	3	2	1	5		
001B-0046	Chicken	2	1	1	3	2	1	5	21	21
006A-0001	Raw milk	2	1	1	3	2	1	5		
006A-0004	Raw milk	2	1	1	3	2	1	5		
007A-0018	Water	2	1	1	3	2	1	5		
007A-0031	Water	2	1	1	3	2	1	5		
001A-0059	Human	4	7	10	4	1	7	1		
001A-0060	Human	4	7	10	4	1	7	1		
001B-0010	Chicken	4	7	10	4	1	7	1		
001B-0011	Chicken	4	7	10	4	1	7	1	45	45
001B-0024	Chicken	4	7	10	4	1	7	1		
007A-0023	Water	4	7	10	4	1	7	1		
007A-0030	Water	4	7	10	4	1	7	1		
007A-0032	Water	4	7	10	4	1	7	1		
001A-0005	Human	7	17	5	2	10	3	6		
001A-0016	Human	7	17	5	2	10	3	6		
001A-0085	Human	7	17	5	2	10	3	6		
001A-0259	Human	7	17	5	2	10	3	6	353	353
001A-0263	Human	7	17	5	2	10	3	6		
001A-0273	Human	7	17	5	2	10	3	6		
001A-0274	Human	7	17	5	2	10	3	6		
001B-0008	Chicken	7	17	5	2	10	3	6		
001A-0162	Human	1	4	2	2	6	3	17		
001A-0163	Human	1	4	2	2	6	3	17		
001A-0166	Human	1	4	2	2	6	3	17		
001A-0238	Human	1	4	2	2	6	3	17	61	61
006A-0014	Raw milk	1	4	2	2	6	3	17		
006A-0020	Raw milk	1	4	2	2	6	3	17		
006A-0026	Raw milk	1	4	2	2	6	3	17		
006A-0028	Raw milk	1	4	2	2	6	3	17		
001A-0064	Human	1	2	3	4	5	9	3		
001A-0084	Human	1	2	3	4	5	9	3		
001A-0088	Human	1	2	3	4	5	9	3		
001A-0168	Human	1	2	3	4	5	9	3	42	42
001B-0009	Chicken	1	2	3	4	5	9	3		
001B-0012	Chicken	1	2	3	4	5	9	3		
001B-0052	Chicken	1	2	3	4	5	9	3		
006A-0053	Raw milk	1	2	3	4	5	9	3		
001A-0287	Human	8	2	5	53	11	3	105		
001A-0289	Human	8	2	5	53	11	3	105		
001B-0029	Chicken	8	2	5	53	11	3	105	1212	1212
001B-0055	Chicken	8	2	5	53	11	3	105		
001B-0056	Chicken	8	2	5	53	11	3	105		
001B-0057	Chicken	8	2	5	53	11	3	105		

[a] ST; sequence type

[b] CC; clonal complex

DNA extraction.

All *C. jejuni* isolates were grown on 5% (vol/vol) defibrinated sheep blood TSA (Oxoid Inc., Nepean, On) in a micro aerobic atmosphere at 42°C for 24-48 h. Isolated colonies were used to inoculate Mueller-Hinton broth (Oxoid Inc., Nepean, On), grown to 0.5 McFarland standard density, 0.5 ml of the broth transferred to a microfuge tube, centrifuged at 13000 rpm for 10 minutes and the supernatant discarded. Genomic DNA was extracted from the pellet by adding 10 µl of NaOH 0.5 N. After 5 minutes, 10 µl of Tris 1M pH 8.0 and 980 µl of sterile distilled water were added. DNA extracts were stored at -20°C. DNA concentration was measured using a NanoVue spectrophotometer (GE Healthcare Life Science, Piscataway, NJ, USA).

Primer design.

The fragments for the seven loci in the MLST system (402 to 507 bp) are longer than the maximum that can be efficiently analyzed by HRM (100 to 300bp) [13]. Consequently, for each locus two or three sub fragments were analyzed to provide adequate resolution of the known alleles. Oligonucleotide primers used are listed in Table 2. In the majority of cases, the 3' end (for forward primers of left locus fragments) and the 5' end (for reverse primers of right locus fragments) were the last nucleotides before/after the comparison fragment for each locus on the *C. jejuni* MLST database website. In four cases (GLN HRM F7, TKT HRM F1, TKT HRM R2, UNC HRM F6) the primer was upstream or downstream from the comparison fragment by -8, -4, +4 and -6 nucleotides, respectively. One primer (GLY HRM F3) included the first nucleotide of the comparison fragment. Internal primers overlapped each other to cover the entire sequence. Primers were synthesized by Integrated DNA Technologies (Coralville, Iowa, USA) and used without further purification.

PCR and HRM analysis.

Real-time PCR cycling was performed in a 96-well plate on a LightCycler® 480 II real-time PCR system (Roche). Each plate must contain at least two reference isolates for each allele that would be identified on the plate together with the unknown samples. The reaction was performed in a 15 µl PCR mix containing 1X LightCycler® 480 High Resolution Melting Master Kit (Roche), 3.5 mM $MgCl_2$, 0.5 µM of each primer and between 10 and 20 ng of DNA. The amplification protocol consisted of a first denaturation step at 95°C [5 min], 45 cycles of denaturation at 95°C [10 s], annealing at 55°C [30 s], and extension at 72°C [30 s]. The HRM step consisted of a first denaturation step at 95°C [1 min], followed by a renaturation step at 40°C [1 min]. Melting curves were generated by ramping from 70°C to 95°C at 0.02°C/sec, 25 acquisitions/°C.

During amplification, fluorescence data were normalized and then plotted using the automated grouping functionality provided by the LightCycler® 480 II Gene Scanning Software version 1.5.0.39 and by manual editing. Figure 1A shows the compilation of curves representing successful amplification of the left fragment of *gly* for 96 isolates. All curves reached a similar plateau height and, as per manufacture's recommendations, the mean cycle number at which fluorescence exceeded background (referred to as the crossing point or cycle threshold) was <30 with a range of less than 7 across all samples. Reactions that did not meet these criteria were discarded and the fragment amplified again in a subsequent run. The software automatically analyzed the raw melting curve data and set the pre-melt (initial fluorescence) and post-melt (final fluorescence) signals of all samples to uniform values (Figure 1B); occasionally, manual adjustments were made to optimize group separation. Next, the software shifted the normalized curves along the temperature axis to equalize the point at which

the dsDNA in each sample becomes completely denatured (temperature shift, Figure 1C). For each locus, the default of 5 was used as the threshold value in the temperature shift step. In the final step each shifted, normalized curve is plotted (difference plot, Figure 2I) as the difference relative to an arbitrarily chosen reference curve among the samples analyzed on the plate, usually one of the known reference isolates. The software groups together similar curves according to an adjustable sensitivity value. In these displays (Figure 2A to 2R) the differences between melting curve profiles for different alleles are readily appreciated. Curves not grouped with one of the reference isolates would have to be run subsequently with other reference isolates containing the allele or sequenced. If the reference isolates were not grouped together correctly, the run would be repeated.

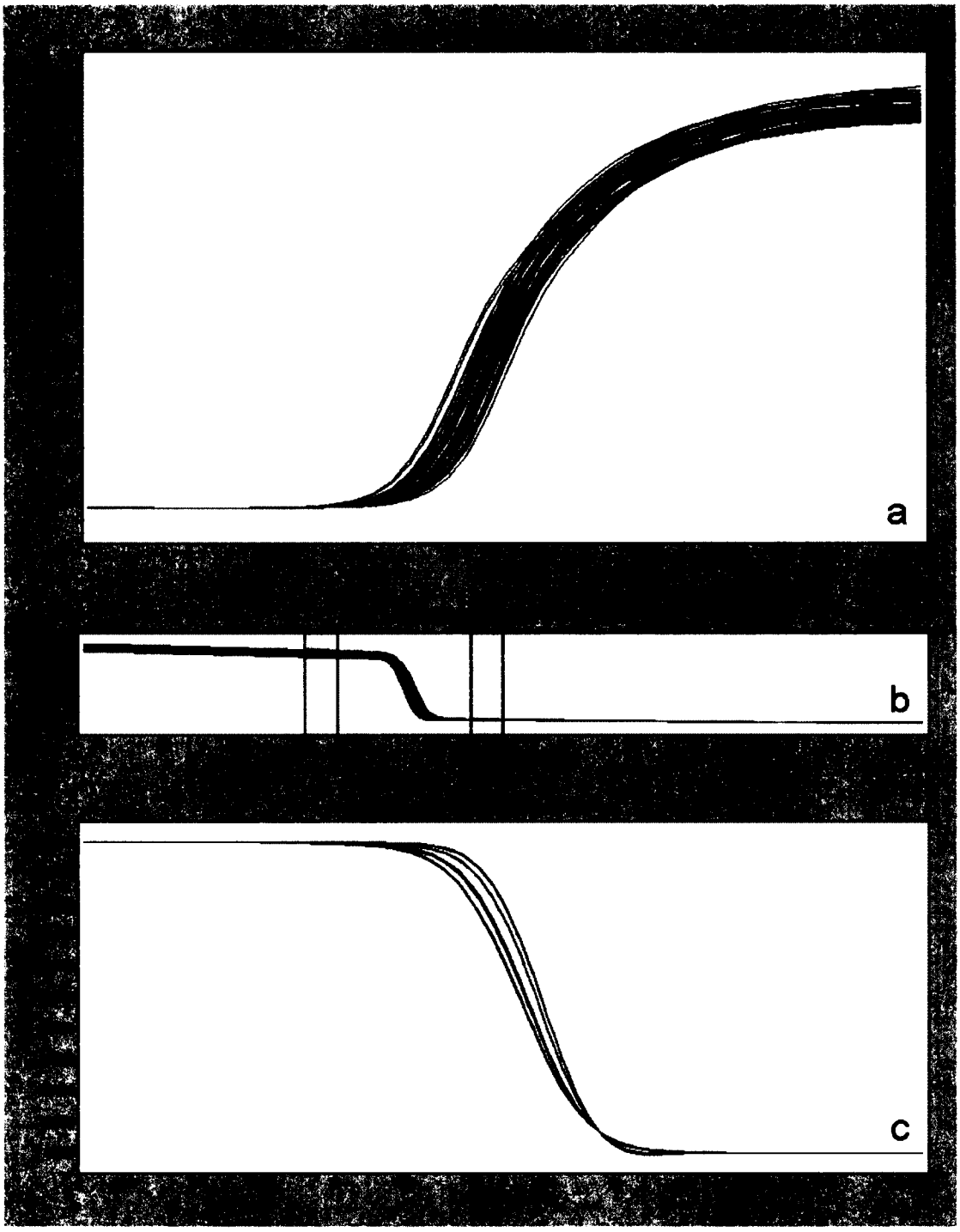


FIGURE 1. Data preparation for HRM. **a:** Amplification curves for *tkt* right fragment for 96 isolates. **b:** Normalization of raw melting curve data. Green box: pre-melt (initial fluorescence). Blue box: post-melt (final fluorescence). **c:** Normalized and shifted melting curves.

ACKNOWLEDGMENTS

This study was funded by the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada and the Centre de Recherche Clinique Étienne Le-Bel du Centre Hospitalier Universitaire de Sherbrooke. The Campylobacter Multilocus Sequence Typing website (http://campylobacter.mlst.net) used in the analysis was developed by Man-Suen Chan and sited at the University of Oxford. We thank the other members of the CampyloGIS project (Goze Bénié, Ferdinand Bonn, Olivier Thomas, Sylvain Quessy, François Milord, Suzanne Ménard, Jacques Désilets, Michel Pomerleau, André Lavoie, Rémi Desbiens, Djoan Bonfils and Karen St-Pierre). We thank Frederic Chano from Roche Diagnostics and Sylvie Deslandes for technical support.

AUTHORS CONTRIBUTIONS

S.L. and E.F. designed research; S.L. performed research; S.L. and E.F. analyzed data; S.L., S.M., R.D.A and E.F. wrote the manuscript.

REFERENCES

1. Altekruse SF, Stern NJ, Fields PI, Swerdlow DL (1999) *Campylobacter jejuni*-an emerging foodborne pathogen. Emerg Infect Dis 5: 28-35.
2. Allos BM (2001) *Campylobacter jejuni* infections: update on emerging issues and trends. Clin Infect Dis 32: 1201-6.
3. Michaud S, Menard S, Arbeit RD (2004) Campylobacteriosis, Eastern Townships, Quebec. Emerg Infect Dis 10: 1844-7.
4. Dingle KE, Colles FM, Ure R, Wagenaar JA, Duim B, et al. (2002) Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. Emerg Infect Dis 8: 949-55.
5. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, et al. (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A 95: 3140-5.
6. Dingle KE, Colles FM, Wareing DR, Ure R, Fox J, et al. (2001) Multilocus sequence typing system for *Campylobacter jejuni*. J Clin Microbiol 39: 14-23.
7. Djordjevic SP, Unicomb LE, Adamson PJ, Mickan L, Rios R (2007) Clonal complexes of *Campylobacter jejuni* identified by multilocus sequence typing are reliably predicted by restriction fragment length polymorphism analyses of the flaA gene. J Clin Microbiol 45: 102-8.
8. Schouls LM, Reulen S, Duim B, Wagenaar JA, Willems RJ, et al. (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.
9. Duim B, Godschalk PC, van den Braak N, Dingle KE, Dijkstra JR, et al. (2003) Molecular evidence for dissemination of unique *Campylobacter jejuni* clones in Curacao, Netherlands Antilles. J Clin Microbiol 41: 5593-7.
10. Levesque S, Frost E, Arbeit RD, 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: 3404-11.
11. Colles FM, Jones K, Harding RM, Maiden MC (2003). Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. Appl Environ Microbiol 69: 7409-7413.
12. Manning G, Dowson CG, Bagnall MC, Ahmed IH, West M, et al. (2003) Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl Environ Microbiol 69: 6370-9.
13. Taylor CF (2009) Mutation scanning using high-resolution melting. Biochem Soc Trans 37: 433-7.
14. Erali M, Voelkerding KV, Wittwer CT (2008) High resolution melting applications for clinical laboratory medicine. Exp Mol Pathol 85: 50-8.
15. Millat G, Chanavat V, Julia S, Crehalet H, Bouvagnet P, et al. (2009) Validation of high-resolution DNA melting analysis for mutation scanning of the LMNA gene. Clin Biochem 42: 892-8.
16. Merchant-Patel S, Blackall PJ, Templeton J, Prise EP, Tong SY, et al. (2010) *Campylobacter jejuni* and *Campylobacter coli* genotyping by high-resolution melting analysis of a flaA fragment. Appl Environ Microbiol 76: 493-9.

17. Sails AD, Swaminathan B, Fields PI (2003) Clonal complexes of *Campylobacter jejuni* identified by multilocus sequence typing correlate with strain associations identified by multilocus enzyme electrophoresis. J Clin Microbiol 41: 4058-67.
18. Clark CG, Bryden L, Cuff WR, Johnson PL, Jamieson F, et al. (2005) Use of the oxford multilocus sequence typing protocol and sequencing of the flagellin short variable region to characterize isolates from a large outbreak of waterborne *Campylobacter sp.* strains in Walkerton, Ontario, Canada. J Clin Microbiol 43:2080-91.
19. Sails AD, Swaminathan B, Fields PI (2003) Utility of multilocus sequence typing as an epidemiological tool for investigation of outbreaks of gastroenteritis caused by *Campylobacter jejuni*. J Clin Microbiol 41: 4733-9.
20. Miller WG, On SL, Wang G, Fontanoz S, Lastovica AJ, et al. (2005) Extended multilocus sequence typing system for *Campylobacter coli*, *C. lari*, *C. upsaliensis*, and *C. helveticus*. J Clin Microbiol 43: 2315-29.
21. Roche diagnostics GmbH (2008) High resolution melting: optimisation strategies. LightCycler® 480 Real-Time PCR system, Technical note No.1. Available: https://www.roche-applied-science.com/sis/rtpcr/htc/htc_docs/LC480_Technical%20Note%201_HRM.pdf Accessed 14 August 2010.
22. Best EL, Fox AJ, Frost JA, Bolton FJ (2005) Real-time single-nucleotide polymorphism profiling using Taqman technology for rapid recognition of *Campylobacter jejuni* clonal complexes. J Med Microbiol 54: 919-25.
23. Best EL, Fox AJ, Frost JA, Bolton FJ (2004) Identification of *Campylobacter jejuni* multilocus sequence type ST-21 clonal complex by single-nucleotide polymorphism analysis. J Clin Microbiol 42: 2836-9.
24. Killgore G, Thompson A, Johnson S, Brazier J, Kuijper E. et al. (2008) Comparison of seven techniques for typing international epidemic strains of *Clostridium difficile*: restriction endonuclease analysis, pulsed-field gel electrophoresis, PCR-ribotyping, multilocus sequence typing, multilocus variable-number tandem-repeat analysis, amplified fragment length polymorphism, and surface layer protein A gene sequence typing. J Clin Microbiol 46: 431-7.

CHAPITRE 4

AVANT-PROPOS DE L'ARTICLE

Titre

Use of amplified-fragment length polymorphism to study the ecology of *Campylobacter jejuni* in environmental water and to predict multilocus sequence typing clonal complexes

Auteurs

Simon Lévesque, Karen St-Pierre, Eric Frost, Robert D. Arbeit et Sophie Michaud.

État de l'article à ce jour

Publié dans la revue Applied and Environmental Microbiology, Vol. 78, No. 7, 2012, Pages 2470–73.

Le formulaire « Autorisation d'intégration d'un article écrit en collaboration à un mémoire ou une thèse » a été signé par chaque co-auteure ou co-auteur conformément à la « Directive relative au dépôt des essais, des mémoires et des thèses » de l'Université de Sherbrooke.

RÉSUMÉ DE L'ARTICLE

Le Campylobacter est la source la plus commune de diarrhée bactérienne à travers le monde. Ce genre bactérien colonise une grande variété d'hôtes, allant des animaux domestiques aux oiseaux sauvages. La grande quantité de microorganismes excrétés dans leurs matières fécales permet au Campylobacter de contaminer l'eau environnementale, incluant les ruisseaux, les rivières et les lacs. Cette étude avait pour but de déterminer la variabilité génétique du Campylobacter dans l'eau environnementale et d'évaluer la capacité de l'AFLP à prédire les complexes clonaux du MLST. Nous avons utilisé l'AFLP comme première méthode d'analyse moléculaire. Nous avons par la suite utilisé les résultats générés par l'AFLP pour sélectionner un sous-groupe d'isolats que nous avons analysés par MLST. Nous avons comparé la corrélation des relations génétiques pour les résultats obtenus avec les deux méthodes. Les 920 isolats de *Campylobacter jejuni* analysés étaient similaires à 64,05 % et 101 différents types identifiés par AFLP étaient regroupés parmi 71 groupes; indiquant un haut niveau de diversité génétique. Nous avons analysé 266 des 920 isolats de *C. jejuni* par MLST. Un total de 137 STs fut identifié. Les STs les plus fréquents furent les ST-45, ST-3889 et ST-637. Cent dix-neuf (119) STs furent associés avec un ou deux isolats seulement. Globalement, 95 STs furent rapportés pour la première fois. Le profil AFLP d'un isolat prédit faiblement son ST ou CC d'appartenance déterminé par MLST. Même si l'AFLP est une méthode de typage moléculaire rapide et relativement peu coûteuse, parmi cette collection d'isolats d'eau très diversifiée, les regroupements d'AFLP ne corrèlent pas avec les CC déterminés

par MLST. Ceci suggère que l'AFLP n'est pas adéquat pour déchiffrer les relations génétiques les populations de Campylobacter, ce qui pourrait être problématique pour de plus larges analyses épidémiologiques.

Contribution de l'étudiant

J'ai participé au design de l'étude et des expériences. J'ai contribué totalement à l'obtention et à l'analyse de tous les résultats décrits dans l'article. J'ai également écrit entièrement la première ébauche du manuscrit et participé à la correction avant la soumission.

USE OF AMPLIFIED-FRAGMENT LENGTH POLYMORPHISM TO STUDY THE ECOLOGY OF *CAMPYLOBACTER JEJUNI* IN ENVIRONMENTAL WATER AND TO PREDICT MULTI-LOCUS SEQUENCE TYPING CLONAL COMPLEXES

Simon Lévesque[1], Karen St-Pierre[1], Eric Frost[1], Robert D. Arbeit[2], Sophie Michaud[1*]

1. Department of Microbiology and Infectious Diseases, Faculté de Médecine de l'Université de Sherbrooke, Québec, Canada.

2. Infectious Diseases Section, Tufts University School of Medicine, Boston, MA.

Corresponding author: Sophie Michaud, MD, MPH, CSPQ, FRCPC
Department of Microbiology and Infectious Diseases
Faculté de Médecine de l'Université de Sherbrooke
3001, 12e avenue Nord
Sherbrooke, Québec J1H 5N4
Phone: (819) 564-5321
Fax: (819) 564-5392
E-mail: Sophie.Michaud@USherbrooke.ca

Financial support: The Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Centre de Recherche Clinique Étienne Le-Bel.

Running head: Ecology of *C. jejuni.* in environmental water using AFLP and MLST

Key words: *Campylobacter jejuni*, environmental water, AFLP, MSLT, molecular epidemiology.

Word count (text): 1608

ABSTRACT

We determined the genetic variability among water isolates of *Campylobacter jejuni* using AFLP and MLST. Across a highly diverse collection of isolates, AFLP clusters did not correlate with MLST clonal complexes, suggesting that ALFP is not reliable for deciphering population genetic relationships and may be problematic for larger epidemiologic analyses.

Word count: 50

Campylobacter species are the most common cause of bacterial diarrhea in humans worldwide (1). The genus colonizes a wide variety of hosts, from domestic animals to wild birds, and the extensive burden of excreted organisms provides ample opportunity for *Campylobacter spp.* to contaminate environmental water (1, 3). The contamination of drinking water by *Campylobacter* has resulted in a number of well-characterised outbreaks (7, 12, 14-16, 21, 28), highlighting the potential of any water source to act as a transmission route for *Campylobacter* infection (5).

A number of methods have been developed to investigate genetic diversity among *Campylobacter* in order to trace sources of infection (33). An MLST system for *C. jejuni* has been developed and indicates that the species is genetically diverse, with a weakly clonal population structure, marked by frequent intra- and interspecies horizontal genetic exchange (25, 26). Although MLST is highly reproducible, portable, and easy to interpret, it is complex and expensive to perform. AFLP genotyping is a rapid, universally applicable typing method based on PCR and capillary electrophoresis, which has been reported to have high discriminatory power (24).

In this study, we used AFLP to determine the genetic variability among water isolates both across season and sampling site (10, 29). A subset of the isolates was typed by MLST to compare the the genetic relationships obtained by the two typing methods.

C. jejuni isolates (n = 950) were obtained from environmental water in a previous study (31). For isolates previously identified as *Campylobacter spp.*, species identification was performed using a protocol adapted from the real-time PCR system of Chaban et al. (6) (see supplemental material). AFLP analysis was performed using a

protocol adapted from the AFLP microbial fingerprint protocol of Applied Biosystems (2) and from Duim et al. (11) (see supplemental material) using *C. jejuni* strain ATCC 33560 to control for band pattern reproducibility. Banding patterns were calculated using the Dice coefficient and were expressed as percentage similarities. For cluster analysis of AFLP banding patterns, the unweighted-pair-group method using average linkages (UPGMA) was used (32).

MLST was performed using high-resolution melting (HRM) (23) on a subset of the collection chosen to represent the different sites and seasons as well as AFLP results. Alleles unresolved by HRM were resolved by sequencing (9, 22). For some isolates, primers of the extended MLST system (26) or new primers designed for this study (see Table S2 in the supplemental material) were used. Minimum spanning trees were constructed within BioNumerics, using the allelic data set. Novel sequence types (ST) not assigned to a clonal complex (CC) within the *C. jejuni* MLST database were examined with eBURST3 (13, 30). The association between AFLP patterns and CC was examined using the Wallace coefficient within BioNumerics using publicly available scripts (http://biomath.itqb.unl.pt/ClusterComp) (4).

Evenly distributed band patterns (50 to 500 bp long) were obtained by AFLP for 920 of 950 *C. jejuni* isolates tested whereas only high molecular weight fragments, unsuitable for AFLP analysis, were obtained for 30 isolates (3%). The 920 *C. jejuni* isolates were similar at a level of 64% and 101 different AFLP types were observed, indicating a high level of genetic diversity comparable to other studies with *C. jejuni* isolates (17-20, 24, 27).

Overall, 96.7% of isolates (890/920) were part of 71 clusters of 2 to 209 isolates (median, 4 isolates); by definition, a cluster represented isolates within a similarity coefficient of 90%. The four largest AFLP clusters contained respectively

209, 145, 98 and 58 isolates each. Cluster analysis revealed three main subgroups containing 780 (subgroup A, 85% of the collection), 44 (subgroup B, 5% of the collection) and 25 (subgroup C, 2.7% of the collection) isolates; where each subgroup represented isolates with a similarity coefficient of 85% (see Figure S1 in the supplemental material). The four largest AFLP clusters belonged to subgroup A.

The diversity of AFLP patterns at each sampling site is shown in Table S3 (see supplemental material). When sampling sites were analysed individually, the similarity coefficient among the isolates varied between 65% and 85%. Comparison of the distribution of the 3 main subgroups among the sampling sites showed that the proportion of genotypes was different from site to site. Isolates from subgroup A were present in most sites (from 65% to 96% of isolates in sites) and exclusively in two sampling sites (H4 and M1).

In order to determine whether the AFLP results correlated with the clonal population structure defined by MLST, we analysed 266 (29%) of the 920 *C. jejuni* isolates by MLST. A total of 137 STs were identified (Table 1) including 68 STs, representing 176 (66%) isolates, that were assigned to 21 previously described CCs. The remaining 90 isolates were distributed among 69 STs which could not be assigned to any of the known lineages, but 36 (52%) were identified as representing two new CCs (CC ST-1224 and CC ST-4102 assigned by us using eBURST3 (see Table S4 in the supplemental material). The most frequent STs identified were ST-45 (36 isolates), ST-3889 (19 isolates) and ST-637 (16 isolates); 119 STs were associated with only one or two isolates. The most prevalent CCs identified were CC ST-45 (58 isolates), CC ST-1275 (30 isolates) and CC ST-179 (29 isolates), which represented 44% of the typed isolates. eBURST3 also identified 7 other groups of 2 to 6 isolates each with no predicted founders.

Table 1. Distribution of 266 *C. jejuni* isolates among clonal complexes and sequence types.

Clonal complex	No. isolates	No. ST	Modal ST	No. (%) isolates of CC in modal ST
21	8	3	21	5 (63%)
45	58	12	45	36 (62%)
61	5	4	4120	2 (40%)
177	7	4	177	3 (43%)
179	29	8	3889	19 (66%)
283	8	1	267	8 (100%)
682	7	3	682	5 (71%)
1034	8	8	na	na
1224	17	10	1224	6 (35%)
1275	30	11	637	16 (53%)
4102	9	5	1226	3 (33%)
Misc	16	14	na	na
Unclassified	64	54	na	na
Total	266	137	10 (7%)	103 (39%)

CC, clonal complex; ST, sequence type; Modal ST, sequence type within the clonal complex represented by the largest number of isolates.

CC ST-1224, is a distinct lineage linked only with water isolates (Figure 1), with alleles infrequently reported worldwide or reported for the first time in this study. CC ST-4102 is also found only in the Eastern Townships, but contains better known alleles. This complex shares three alleles with ST-179. The lack of evidence linking CC ST-1224 with other ecological niches makes it difficult to hypothesize a source. The fact that these CCs are unique to the Eastern Townships could probably link them to native fauna, not seen in other parts of the world (5, 8) or that does not interchange with the same species in other regions because they do not migrate. The fact that 69 % of the STs in this study are new supports this hypothesis. Since ST-4102 shares three alleles with ST-179 and ST-1224 shares one allele with ST-1275, they may, at some stage, have shared a common host, allowing interchange of alleles, as suggested for

unique CCs found in New-Zealand. These complexes could then have evolved locally in the Eastern Townships. Another hypothesis is that isolates from these complexes could be found elsewhere but because very few environmental isolates have been typed in the international database (about 4% of the 12 774 isolates typed, last query 2011-11-17) they have not been observed.

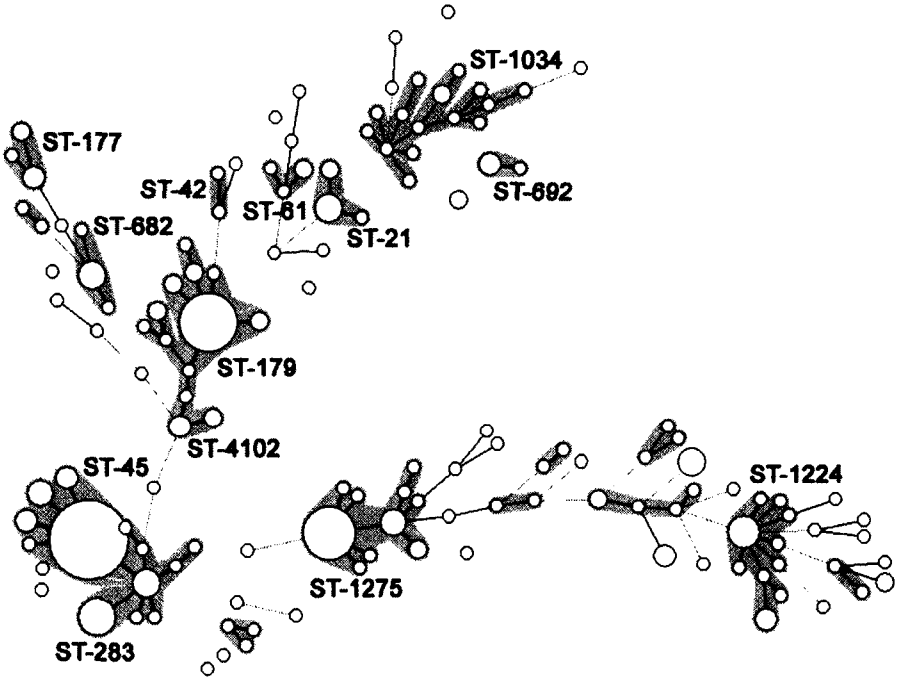


Figure 1. Minimum spanning tree of water isolates based on sequence types. Clusters are identified in grey by the clonal complex number associated with the sequence types in the cluster. Clusters with no clonal complex number were identified by eBURST3 with no predicted founders. Size of the circles represents the number of isolates for each sequence type.

The MLST analysis confirmed the high level of genetic diversity shown by AFLP. The source of the high level of genetic variability among *Campylobacter* isolates from environmental water is not clear. The genotypic variation among isolates from different sampling sites was not associated with simple geographic location (data not shown). *Campylobacter* do not grow efficiently in environmental water, suggesting their presence represents contamination. Although the species is known to

have a weakly clonal population structure with a high level of intraspecies recombination, it seems unlikely that water provides opportunity for such events. This suggests that the high genetic variability may reflect very diverse contamination sources, including birds, wildlife, domestic animals and human waste. Detailed environmental information (e.g., distribution of domestic and wildlife species adjacent to sampling sites) was not available. Analysis of additional collections of isolates will be needed to address this issue.

The 266 isolates analysed by MLST represented 29 AFLP clusters of 2 to 50 isolates each and 38 unique AFLP patterns (Table 2). Within each AFLP cluster a single CC was predominant (Figure S2, S3 and S4 in supplemental material), but the strength of that association varied widely. The MLST heterogeneity among the isolates of AFLP cluster 2 was particularly striking (Figure S3, supplemental material and Table 2). The calculated Wallace coefficient of 0.37 confirmed that the AFLP cluster of an isolate was a poor predictor of its ST or CC. Similar results were observed when AFLP subgroups were analysed by MLST CCs (Table 2).

Table 2. Distribution of clonal complexes for the 266 *C. jejuni* MLST typed among AFLP clusters and subgroups.

				Clonal complexes												
AFLP subgroup (85%)	AFLP cluster (90%)	Number of isolates	Number of isolates typed using MLST	21	45	61	177	179	283	682	1034	1224	1275	4102	Other	UA
	1	209	57	0	40	0	1	1	2	1	0	1	2	1	3	5
	2	145	40	0	4	0	1	1	2	2	3	0	13	1	4	9
A	3	98	36	0	0	0	0	17	0	0	0	2	2	6	0	9
	4	58	13	0	0	0	0	1	1	0	0	5	1	0	0	5
	other	270	78	8	6	3	3	4	2	1	5	9	2	1	7	27
B	all	44	14	0	5	1	0	2	0	1	0	0	2	0	2	1
C	all	25	5	0	0	1	0	0	0	0	0	0	4	0	0	0
other	-	71	23	0	3	0	2	3	1	2	0	0	4	0	0	8
Total	-	920	266	8	58	5	7	29	8	7	8	17	30	9	16	64

Previous studies have concluded that there was good congruence between AFLP and MLST for *Campylobacter* isolates from humans, bovines and chickens (11, 29). Both of those studies represented appreciably smaller collections and emphasized human clinical isolates, which may have restricted diversity based on selection for pathogenesis, compared with the known heterogeneity of isolates in environmental niches (5, 22). Review of the data presented in both those manuscripts indicates that, similar to our results, individual AFLP types were associated with diverse CCs. AFLP, like random amplified polymorphic DNA (RAPD) which preceded it, depends on a certain level of stochastic priming. Using restriction sites helps decrease the randomness somewhat, but since the restriction site alone is not sufficient for reliable priming, there is residual noise. As we look at larger numbers of more diverse isolates, it is increasingly likely that the noise will result in patterns that are similar enough to cluster but in fact, the fragments do not represent the same DNA and so the "match" is not based on actual chromosomal similarity. The poor congruence we observed between the two typing methods suggests caution when applying AFLP to resolve epidemiologic relationships among isolates of *Campylobacter*.

This study showed, by both AFLP and MLST, that *Campylobacter* water isolates collected in the Eastern Townships of Quebec over several years are highly diverse genetically as has been shown elsewhere. Across this highly diverse collection of water isolates, AFLP clusters did not correlate with MLST CCs, suggesting that ALFP is not reliable for deciphering population genetic relationships and may be problematic for larger epidemiologic analyses.

ACKNOWLEDGMENTS

This study was funded by the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada and the Centre de Recherche Clinique Étienne Le-Bel du Centre Hospitalier Universitaire de Sherbrooke. The *Campylobacter* Multilocus Sequence Typing website (http://campylobacter.mlst.net) used in the analysis was developed by Man-Suen Chan and sited at the University of Oxford. We thank the other members of the CampyloGIS project (Goze Bénié, Ferdinand Bonn, Olivier Thomas, Sylvain Quessy, François Milord, Suzanne Ménard, Jacques Désilets, Michel Pomerleau, André Lavoie, Rémi Desbiens, and Djoan Bonfils). We also thank Raphael Sansregret and Maya Zabinski for technical assistance.

REFERENCES

1. **Altekruse, S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow.** 1999. *Campylobacter jejuni*--an emerging foodborne pathogen. Emerg Infect Dis **5**:28-35.
2. **Applied, B.** 2005. Microbial AFLP fingerprint protocol. Applied Biosystems, Foster City, CA, USA.
3. **Blaser, M. J., D. N. Taylor, and R. A. Feldman.** 1983. Epidemiology of *Campylobacter jejuni* infections. Epidemiol Rev **5**:157-76.
4. **Carrico, J. A., C. Silva-Costa, J. Melo-Cristino, F. R. Pinto, H. de Lencastre, J. S. Almeida, and M. Ramirez.** 2006. Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*. J Clin Microbiol **44**:2524-32.
5. **Carter, P. E., S. M. McTavish, H. J. Brooks, D. Campbell, J. M. Collins-Emerson, A. C. Midwinter, and N. P. French.** 2009. Novel clonal complexes with an unknown animal reservoir dominate *Campylobacter jejuni* isolates from river water in New Zealand. Appl Environ Microbiol **75**:6038-46.
6. **Chaban, B., K. M. Musil, C. G. Himsworth, and J. E. Hill.** 2009. Development of cpn60-based real-time quantitative PCR assays for the detection of 14 *Campylobacter* species and application to screening of canine fecal samples. Appl Environ Microbiol **75**:3055-61.
7. **Clark, C. G., L. Price, R. Ahmed, D. L. Woodward, P. L. Melito, F. G. Rodgers, F. Jamieson, B. Ciebin, A. Li, and A. Ellis.** 2003. Characterization of waterborne outbreak-associated *Campylobacter jejuni*, Walkerton, Ontario. Emerg Infect Dis **9**:1232-41.
8. **Devane, M. L., C. Nicol, A. Ball, J. D. Klena, P. Scholes, J. A. Hudson, M. G. Baker, B. J. Gilpin, N. Garrett, and M. G. Savill.** 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. J Appl Microbiol **98**:980-90.
9. **Dingle, K. E., F. M. Colles, D. R. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J. Bootsma, R. J. Willems, R. Urwin, and M. C. Maiden.** 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J Clin Microbiol **39**:14-23.
10. **Duim, B., P. C. Godschalk, N. van den Braak, K. E. Dingle, J. R. Dijkstra, E. Leyde, J. van der Plas, F. M. Colles, H. P. Endtz, J. A. Wagenaar, M. C. Maiden, and A. van Belkum.** 2003. Molecular evidence for dissemination of unique *Campylobacter jejuni* clones in Curacao, Netherlands Antilles. J Clin Microbiol **41**:5593-7.
11. **Duim, B., T. M. Wassenaar, A. Rigter, and J. Wagenaar.** 1999. High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with amplified fragment length polymorphism fingerprinting. Appl Environ Microbiol **65**:2369-75.
12. **Engberg, J., P. Gerner-Smidt, F. Scheutz, E. Moller Nielsen, S. L. On, and K. Molbak.** 1998. Water-borne *Campylobacter jejuni* infection in a Danish town---a 6-week continuous source outbreak. Clin Microbiol Infect **4**:648-656.
13. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol **186**:1518-30.

14. **Frost, J. A., I. A. Gillespie, and S. J. O'Brien.** 2002. Public health implications of *Campylobacter* outbreaks in England and Wales, 1995-9: epidemiological and microbiological investigations. Epidemiol Infect **128:**111-8.
15. **Hanninen, M. L., H. Haajanen, T. Pummi, K. Wermundsen, M. L. Katila, H. Sarkkinen, I. Miettinen, and H. Rautelin.** 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:**1391-6.
16. **Jakopanec, I., K. Borgen, L. Vold, H. Lund, T. Forseth, R. Hannula, and K. Nygard.** 2008. A large waterborne outbreak of campylobacteriosis in Norway: the need to focus on distribution system safety. BMC Infect Dis **8:**128.
17. **Johnsen, G., H. Kruse, and M. Hofshagen.** 2006. Genetic diversity and description of transmission routes for *Campylobacter* on broiler farms by amplified-fragment length polymorphism. J Appl Microbiol **101:**1130-9.
18. **Johnsen, G., H. Kruse, and M. Hofshagen.** 2007. Genotyping of thermotolerant *Campylobacter* from poultry slaughterhouse by amplified fragment length polymorphism. J Appl Microbiol **103:**271-9.
19. **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.
20. **Kudirkiene, E., M. Malakauskas, A. Malakauskas, A. M. Bojesen, and J. E. Olsen.** Demonstration of persistent strains of *Campylobacter jejuni* within broiler farms over a 1-year period in Lithuania. J Appl Microbiol **108:**868-77.
21. **Kuusi, M., J. P. Nuorti, M. L. Hanninen, M. Koskela, V. Jussila, E. Kela, I. Miettinen, and P. Ruutu.** 2005. A large outbreak of campylobacteriosis associated with a municipal water supply in Finland. Epidemiol Infect **133:**593-601.
22. **Levesque, S., E. Frost, R. D. Arbeit, and S. Michaud.** 2008. Multilocus sequence typing of *Campylobacter jejuni* isolates from humans, chickens, raw milk, and environmental water in Quebec, Canada. J Clin Microbiol **46:**3404-11.
23. **Levesque, S., S. Michaud, R. D. Arbeit, and E. H. Frost.** High-resolution melting system to perform multilocus sequence typing of *Campylobacter jejuni.* PLoS One **6:**e16167.
24. **Lindstedt, B. A., E. Heir, T. Vardund, K. K. Melby, and G. Kapperud.** 2000. Comparative fingerprinting analysis of *Campylobacter jejuni* subsp. *jejuni* strains by amplified-fragment length polymorphism genotyping. J Clin Microbiol **38:**3379-87.
25. **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:**6370-9.
26. **Miller, W. G., S. L. On, G. Wang, S. Fontanoz, A. J. Lastovica, and R. E. Mandrell.** 2005. Extended multilocus sequence typing system for *Campylobacter coli, C. lari, C. upsaliensis,* and *C. helveticus.* J Clin Microbiol **43:**2315-29.

27. **Moreno, Y., M. A. Ferrus, A. Vanoostende, M. Hernandez, R. M. Montes, and J. Hernandez.** 2002. Comparison of 23S polymerase chain reaction-restriction fragment length polymorphism and amplified fragment length polymorphism techniques as typing systems for thermophilic campylobacters. FEMS Microbiol Lett **211**:97-103.
28. **Richardson, G., D. R. Thomas, R. M. Smith, L. Nehaul, C. D. Ribeiro, A. G. Brown, and R. L. Salmon.** 2007. A community outbreak of *Campylobacter jejuni* infection from a chlorinated public water supply. Epidemiol Infect **135**:1151-8.
29. **Schouls, L. M., S. Reulen, B. Duim, J. A. Wagenaar, R. J. Willems, K. E. Dingle, F. M. Colles, and J. D. Van Embden.** 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.
30. **Spratt, B. G., W. P. Hanage, B. Li, D. M. Aanensen, and E. J. Feil.** 2004. Displaying the relatedness among isolates of bacterial species -- the eBURST approach. FEMS Microbiol Lett **241**:129-34.
31. **St-Pierre, K., S. Levesque, E. Frost, N. Carrier, R. D. Arbeit, and S. Michaud.** 2009. Thermotolerant coliforms are not a good surrogate for *Campylobacter* spp. in environmental water. Appl Environ Microbiol **75**:6736-44.
32. **Vauterin, L. A., and P. Vauterin.** 1992. Computer-aided objective comparison of electrophoresis patterns for grouping and identification of microorganisms. Eur Microbiol **1**:37-41.
33. **Wassenaar, T. M., and D. G. Newell.** 2000. Genotyping of *Campylobacter* spp. Appl Environ Microbiol **66**:1-9.

SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Species identification. DNA was extracted with the DNeasy Blood & Tissue kit (Qiagen) from 10^8 bacteria grown on blood TSA plates, according to the manufacturer's protocol. DNA concentration was measured using a NanoVue spectrophotometer (GE Healthcare Life Science, Piscataway, NJ, USA). Each reaction mixture consisted of 1X LightCycler® 480 SYBR Green I Master (Roche), 400 nM of the appropriate primers (except for JH0087/JH0088 primers which were used at 200 nM) and 10 ng of DNA in a final volume of 15 µl. All reactions were performed with a LightCycler® 480 II using the following program: 95°C for 10 min, followed by 40 cycles of 10 s at 95°C, 15 s at the appropriate primers temperature and 15 s at 72°C. A final melt at 95°C for 1 min was done prior to a melt curve analysis. Melting curves were generated by ramping from 55°C to 95°C at 0.11°C/sec, 5 acquisitions/°C. Fluorescence data were visualised using normalisation plotting provided by the LightCycler® 480 II Gene Scanning Software version 1.5.0.39. The following control strains were used in each run: *C. jejuni* ATCC 33560, *C. coli* ATCC 49941, *C. lari* LSPQ 3218, *C. upsaliensis* ATCC 49815, *C. fetus* LSPQ 2979 and *C. hyointestinalis* 001A-0061. For isolates which were not identified to the species level, CPN60 sequencing was used.

AFLP analysis. Restriction adapters and PCR primers used in this study are shown in Table S1. The digestion and ligation reactions were performed in the same step in a final volume of 10 µl. The ligation solution (1.25 µM of each restriction site-specific adapter, 50 µM NaCl) was heated 5 min at 95°C then chilled on ice. Five U of *HindIII*,

1 U of *HhaI*, 1 U of T4 DNA ligase, 0.5µg of BSA, and 1X of T4 DNA ligase buffer with ATP were added to the ligation solution. Finally, 0.6 µg of genomic DNA was added and the restriction-ligation mixture was incubated at 37°C for 2 h. The final product was diluted with 190 µl of TE buffer and stored at -20°C. A preselective PCR was carried out in a 15 µl (final volume) mixture containing 5 µl of the restriction-ligation mixture, 1X of PCR Taq buffer, 3.5 mM of $MgCl_2$, 0.2 mM of dNTPs mixture, 0.5 µM of each preselective primer (Table 1) and 1.25 U of Taq DNA polymerase. The reaction conditions were one cycle at 72°C for 2 min, followed by 20 cycles of denaturation at 90°C for 20 s, primer annealing at 56°C for 30 s, and extension at 72°C for 2 min. Five µl of the PCR product were migrated in a 2 % agarose gel, stained with ethidium bromide and visualised on a UV transilluminator. The remaining PCR products were diluted with 185 µl of TE buffer and stored at 4°C. For the final PCR, 5 µl of the diluted preselective PCR products were amplified in a 15 µl (final volume) mixture containing 1X of PCR Taq buffer, 3.5 mM of $MgCl_2$, 0.2 mM of dNTPs mixture, 0.5 µM of Hha primer, 0.3 µM of HindIII FAM primer and 1.25 U of Taq DNA polymerase. The reaction conditions were one cycle at 94°C for 2 min, followed by 10 cycles of denaturation at 94°C for 20 s, primer annealing at 66°C for 30 s (each of the following cycles was one degree lower, until reaching 57°C at the tenth cycle), extension at 72°C for 2 min, followed by 20 cycles of denaturation at 94°C for 20 s, primer annealing at 56°C for 30 s, extension at 72°C for 2 min, and a final elongation step at 60°C for 30 min. PCR products were conserved at 4°C in the dark. The final products were diluted 1:2 in sterile water and the fluorescent fragments were visualized using an ABI 3730XL system with the ABI Genescan 500 LIZ as the internal lane standard, in collaboration with Genome Quebec Innovation Centre at

McGill University. Densitometric curves were processed with BioNumerics version 5.10 software (Applied Maths, Kortrijk, Belgium).

Table S1. Restriction adapters and PCR primers used in this study.

AFLP Step	Primers	Sequence (5' – 3')
Restriction site-specific adapters	Hind3LinkA	GAC TGC GTA CC
	Hind3LinkB	AGC TGG TAC GCA GTC
	HhaLinkA	GAT GAG TCC TGA TCG
	HhaLinkB	ATC AGG ACT CAT C
Preselective PCR primers	HindPre	GAC TGC GTA CCA GCT T
	HhaPre	GAT GAG TCC TGA TCG C
Final PCR primers	HindAPCRFAM	6-FAM-GAC TGC GTA CCA GCT TA
	HhaAPCR	GAT GAG TCC TGA TCG CA

Table S2. New MLST primers used in this study[a].

Locus	Forward (5' – 3')		Reverse (5' – 3')		Amplicon size (bp)
	Primer	Sequence	Primer	Sequence	
aspA	ASP F SL	GTA CCT ATC ACT TTR GGT CGT	ASP R SL	GCT AAA GTA TRC ATT GCT T	575
glnA	GLN F SL	GGA TGG CAR CCT ATA GAA AAA T	GLN R SL	TAA GCT CAT ATG AAC ATG CAT	657
gltA	GLT F SL	AAT TAG ATG TGG TTC ATC TTT	GLT R SL	AGC TCC ACC ATG TGC ATG ACC	557
glyA	GLY F SL	CCA GGT GAT AAA ATT TTA GGA AT	GLY R SL	CCA TCG CTA ACT AGT TTA AAT	599
tkt	TKT F SL	GAT AGA CTT GTA TTT TCA GG	TKT R SL	TAT GAC TGC CTT CAA AGC TCT	586
uncA	UNC F SL	CAA GTK GTW AAA AGA CTW GAA	UNC R SL	CCT CAT CTA AAT CAC TAG CAA	595

a These primers are used for amplification and sequencing.

Table S3. AFLP and MLST evaluation of genetic diversity for individual sampling sites

Sampling site	Number of isolates	Similarity index (%)	Number of clusters[a]	Percentage of isolates in clusters	Number of isolates in the largest cluster	Number of isolates typed by MLST	Most prevalent clonal complex[b]
A1	19	81,04	4	68,4	4	6	UA
A2	27	82,33	5	77,8	12	8	1275
A3	24	78,40	2	58,3	8	6	4102
A4	23	79,85	3	73,9	6	7	179
A5	30	76,51	4	83,3	17	9	45
C1	47	78,34	5	85,1	19	8	179
C2	22	76,60	4	72,7	5	6	NA
C3	39	73,62	6	82,1	15	9	UA
C4	23	83,16	5	87,0	8	7	179
C5	22	82,75	5	68,2	4	6	45, 179
G1	20	72,17	3	60,0	8	9	UA
G2	23	66,41	5	73,9	8	8	UA
G3	30	66,19	6	70,0	7	9	179
H1	40	74,80	3	70,0	15	10	45, 1034
H2	18	81,75	4	77,8	6	4	45
H3	31	79,78	4	83,9	10	9	45, 179
H4	20	83,61	4	80,0	5	5	45
H5	34	69,49	7	82,4	11	8	1224
M1	11	84,59	3	90,9	5	5	45
M2	26	78,38	4	69,2	7	8	21, 692
M3	10	81,37	2	50,0	3	4	45, UA
M4	32	73,74	4	68,8	11	9	45
S1	27	79,96	4	66,7	8	10	UA
S2	39	76,89	7	71,8	8	10	UA
S3	38	80,20	4	73,7	8	11	UA
S4	56	69,43	7	78,6	14	16	NA
S5	15	83,90	3	86,7	6	6	1275
S6	48	70,95	9	79,2	21	10	UA
V1	46	80,91	10	89,1	8	13	NA
V2	19	84,41	4	63,2	4	6	UA
V3	36	65,16	6	83,3	16	14	1275
V4	25	75,44	5	84,0	9	10	NA

a AFLP patterns were clustered with a similarity coefficient of 90%.

[b] UA; unassigned to known clonal complexes, NA; not available since more than two clonal complexes were present in same number.

Table S4. New sequence types (ST) and clonal complexes observed in this study.

Clonal complex[a]	New ST	No. of isolates	Allele[b]						
			aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*
21	NEW	1	NEW	1	1	3	2	1	5
42	**4199**	1	10	7	3	3	5	180	3
42	**4206**	1	1	2	3	3	5	7	3
45	**4094**	1	4	7	10	4	1	7	21
45	**4114**	1	10	7	10	4	42	7	1
45	**4135**	1	4	22	10	4	42	51	1
45	**4136**	1	4	22	10	4	42	7	1
45	**4191**	1	10	7	10	4	42	7	21
45	**4381**	1	4	7	10	4	42	3	1
52	**4127**	1	9	25	2	10	2	3	5
61	**4120**	2	10	4	2	2	6	3	17
61	**4348**	1	7	17	2	2	6	3	5
177	**4141**	2	17	**342**	8	5	8	2	21
177	**4366**	1	35	43	**281**	5	8	46	21
179	**3889**	19	1	6	137	176	40	32	3
179	**4027**	1	12	6	**271**	176	40	32	3
179	**4121**	1	1	6	137	176	40	7	3
179	**4186**	2	1	6	137	176	40	180	3
179	**4189**	2	10	6	137	176	40	32	3
179	**4380**	1	1	6	61	176	40	32	3
460	**4112**	1	24	30	2	2	6	59	5
508	**4194**	1	27	6	22	24	12	28	1
682	**4203**	1	26	2	9	51	8	46	1
692	**4188**	1	37	52	57	**371**	129	29	23
952	**3890**	1	18	22	20	104	205	94	6
952	**4028**	1	18	22	78	98	138	**381**	16
952	**4190**	1	18	22	165	98	116	86	16
952	**4202**	1	18	22	**275**	**351**	122	113	16
952	**4208**	1	18	7	165	98	129	86	16
1034	**4071**	1	2	59	4	27	126	25	23
1034	**4124**	1	2	61	4	100	6	25	23
1034	**4129**	1	2	33	4	105	6	25	23
1034	**4132**	1	2	52	4	48	2	25	23
1034	**4133**	1	2	29	4	48	2	25	23
1034	**4193**	1	2	59	4	48	13	25	23
1034	**4383**	1	2	61	4	64	332	7	3
1224	1224	6	18	22	22	97	115	175	47
1224	**4029**	1	18	22	22	97	115	175	6
1224	**4074**	1	18	22	22	97	115	***387***	47
1224	**4076**	1	18	70	22	97	115	***388***	47
1224	**4104**	1	18	2	78	97	115	175	47
1224	**4105**	1	18	22	22	98	115	175	47
1224	**4130**	3	***242***	***340***	78	97	115	175	47
1224	**4134**	1	9	2	78	97	115	175	47
1224	**4197**	1	18	22	4	97	115	86	47
1224	**4352**	1	18	***353***	22	97	115	86	47
1275	**4096**	1	1	33	22	49	43	82	31

Clonal complex[a]	New ST	No. of isolates	Allele[b]						
			aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*
1275	**4101**	1	76	33	22	49	43	7	31
1275	**4119**	1	10	33	22	6	43	82	31
1275	**4200**	1	10	1	22	49	43	82	31
1275	**4205**	1	76	33	22	49	43	95	31
1275	**4209**	1	18	2	**263**	104	43	**398**	31
1287	**4032**	1	84	106	29	28	74	99	35
4102	1226	3	1	6	61	176	40	180	1
4102	1230	2	1	6	61	4	40	180	1
4102	**2866**	2	***200***	6	61	176	40	180	***224***
4102	**4102**	1	***200***	6	61	176	40	180	3
4102	**4118**	1	***200***	6	61	176	40	7	3
UA	**4030**	1	94	7	90	125	174	147	100
UA	**4031**	1	1	6	61	176	40	32	1
UA	**4033**	2	18	33	22	104	139	175	16
UA	**4073**	1	18	33	72	**364**	116	**382**	6
UA	**4075**	1	18	22	**275**	104	2	94	5
UA	**4093**	5	18	33	31	326	1	86	16
UA	**4095**	1	1	2	**276**	47	40	**369**	1
UA	**4097**	1	64	105	**278**	100	134	**390**	16
UA	**4098**	1	18	33	78	18	139	175	16
UA	**4100**	1	35	43	**281**	5	8	46	1
UA	**4103**	1	161	33	31	104	43	109	**278**
UA	**4113**	1	2	29	4	48	10	25	23
UA	**4115**	1	2	29	4	48	13	24	23
UA	**4116**	1	18	33	72	104	116	86	6
UA	**4117**	1	18	22	**277**	18	139	175	16
UA	**4122**	1	43	**336**	2	**365**	125	25	57
UA	**4123**	1	10	33	22	18	116	7	47
UA	**4125**	1	2	29	84	48	13	25	23
UA	**4126**	1	64	33	31	104	43	258	31
UA	**4128**	1	10	31	8	104	40	134	1
UA	**4131**	1	94	6	90	125	174	147	100
UA	**4137**	1	18	33	72	**364**	116	86	5
UA	**4138**	1	37	4	10	48	58	25	23
UA	**4139**	1	2	4	4	48	2	25	**280**
UA	**4140**	1	18	2	**263**	104	**469**	**398**	31
UA	**4187**	1	2	59	4	48	126	29	23
UA	**4192**	1	18	109	82	18	138	61	16
UA	**4195**	1	1	1	2	176	2	3	5
UA	**4196**	1	18	22	**277**	18	133	175	16
UA	**4198**	1	10	2	95	129	40	134	1
UA	**4201**	1	18	22	22	104	6	94	3
UA	**4204**	2	2	2	73	147	220	190	104
UA	**4207**	1	**253**	6	**282**	**374**	**478**	**392**	**279**
UA	**4349**	1	37	15	75	48	126	25	23
UA	**4350**	1	27	2	22	104	122	86	31
UA	**4351**	1	**254**	70	77	97	115	86	47
UA	**4361**	1	**255**	**339**	**279**	**383**	**479**	**391**	**276**
UA	**4364**	1	1	22	63	**383**	40	7	1
UA	**4365**	1	**243**	**324**	**280**	**370**	**493**	**389**	**277**

Clonal complex[a]	New ST	No. of isolates	Allele[b]						
			aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*
UA	**4367**	1	**244**	**343**	**272**	**366**	**474**	**399**	**289**

[a] UA, unassigned

[b] New alleles, sequence types and clonal complexes identified in this study are in boldface. For the new clonal complexes, alleles found only in the Eastern Townships are in italic.

[c] The new *aspA* allele has a deletion at position 461, so it was not submitted to the MLST database.

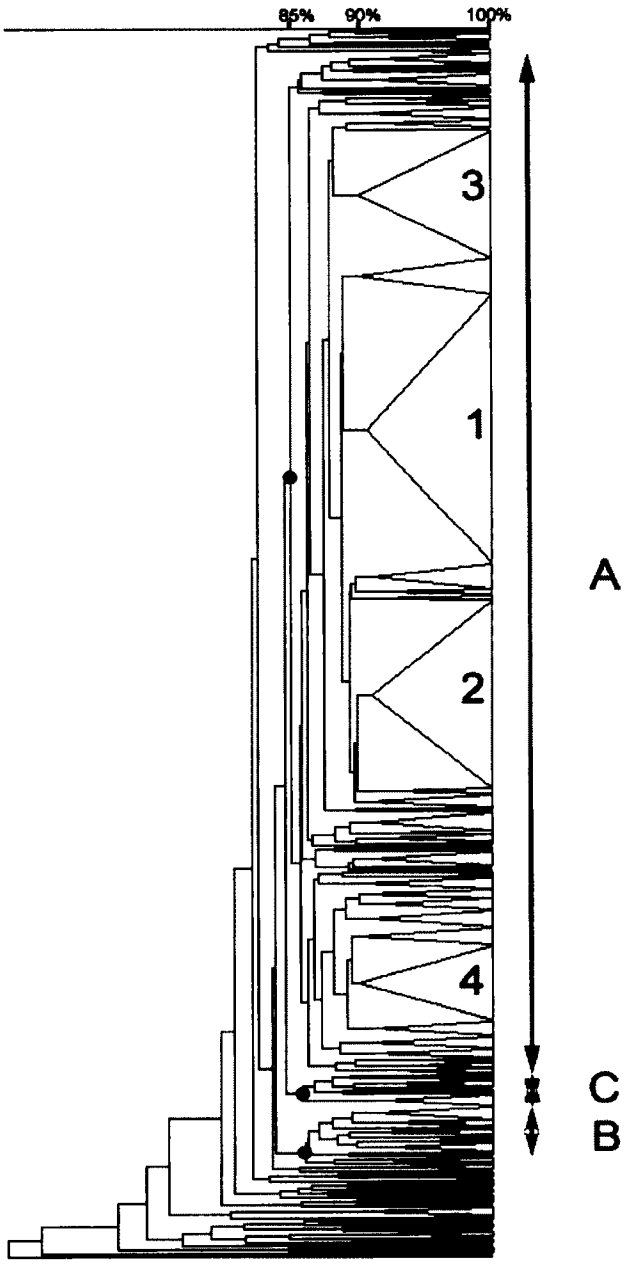


Figure S1. Dendrogram of the cluster analysis of AFLP profiles of 920 *Campylobacter jejuni* isolates. Clonally significant groups of greater than two strains formed at or above the 90 % similarity level are triangulated. Numbers of the four main clusters are indicated in the triangle. Subgroups at 85% of similarity are indicated with arrows and identified with letters. Junctions of subgroups are identified by black dots.

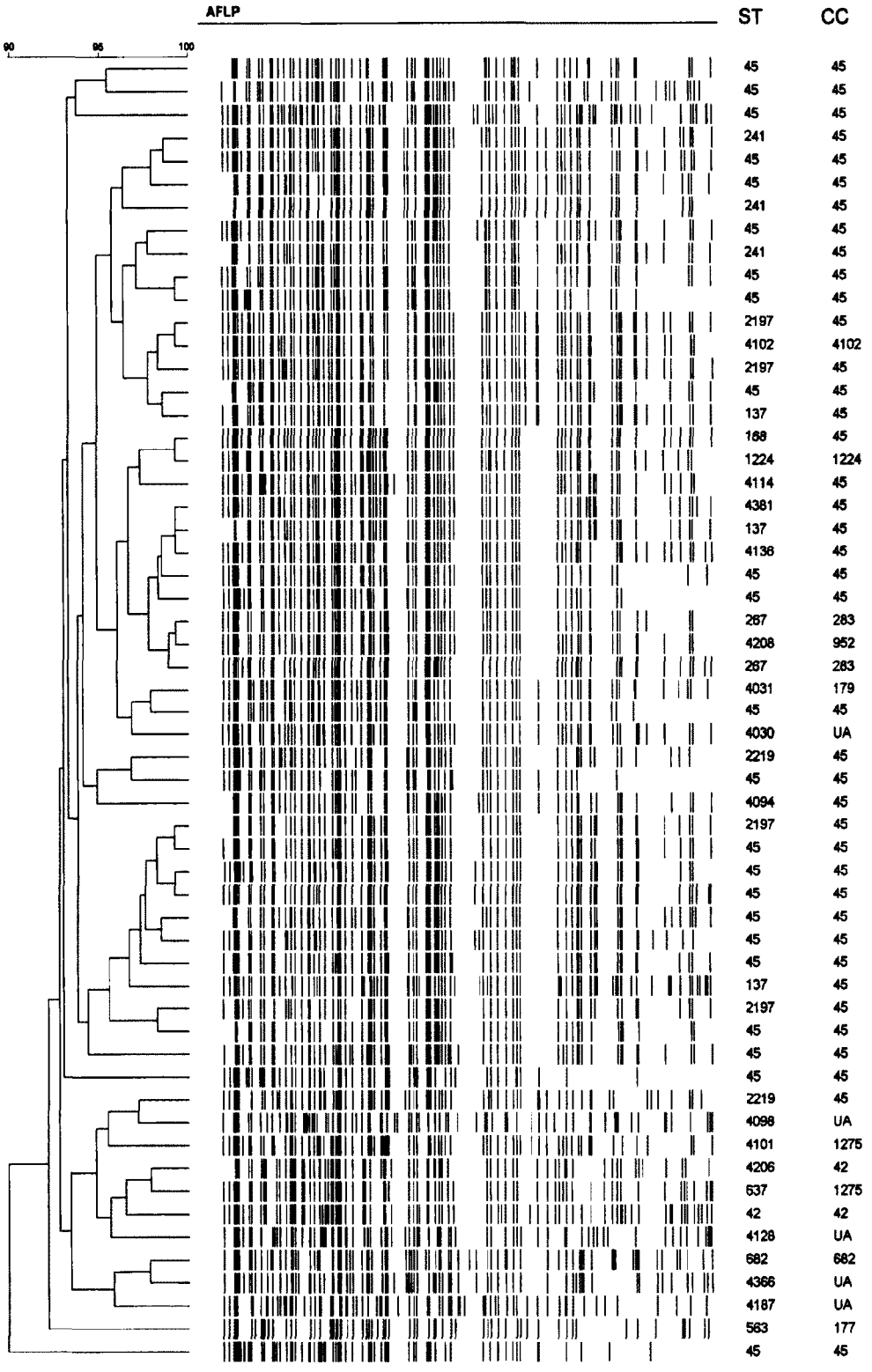


Figure S2. Distribution of sequence types (ST) and clonal complexes (CC) for members of the largest AFLP cluster (57 isolates at 90% of similarity) among the 266 isolates typed by MLST. UA means unassigned ST to a known CC.

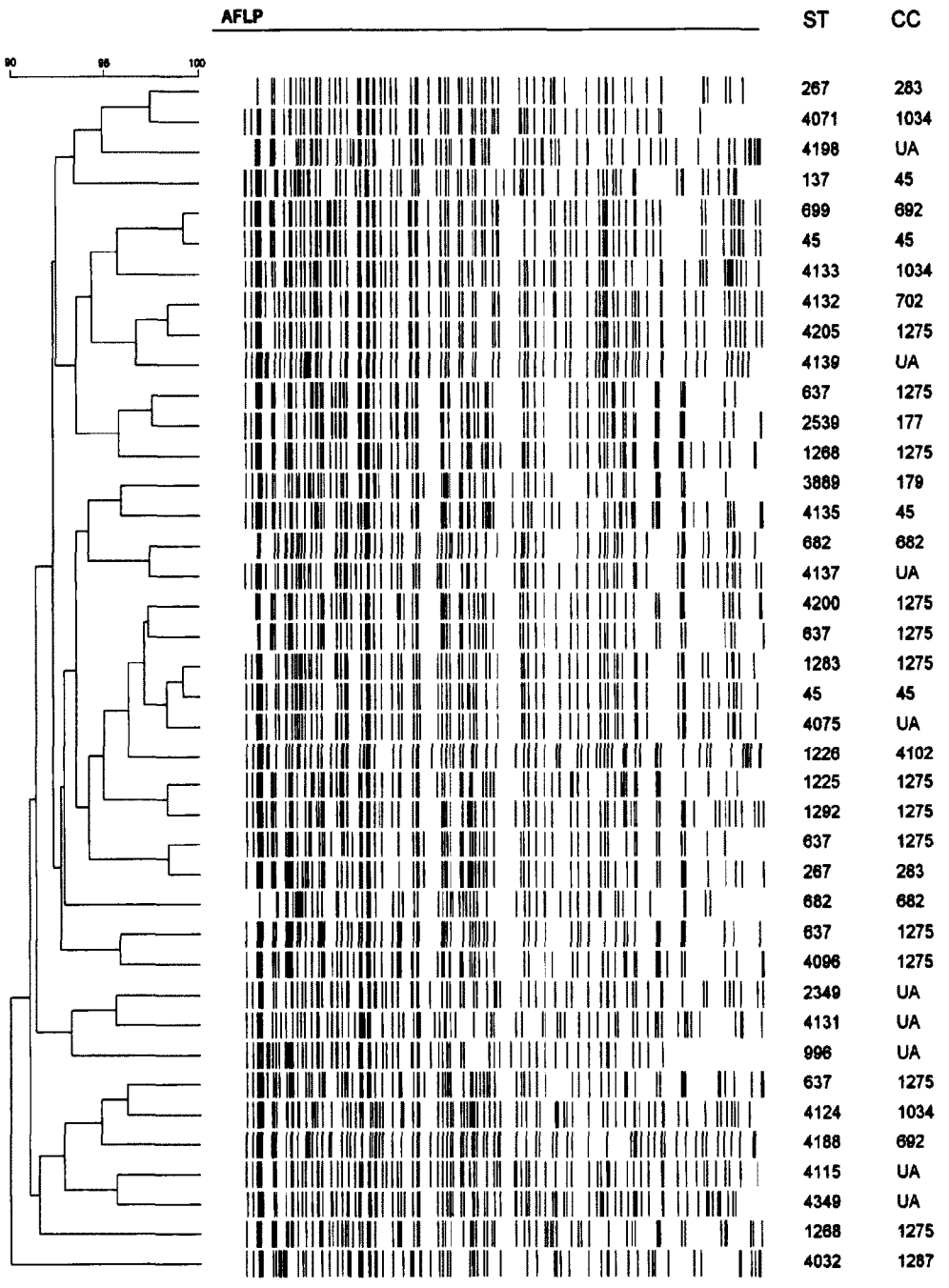


Figure S3. Distribution of sequence types (ST) and clonal complexes (CC) for members of the second largest AFLP cluster (40 isolates at 90% of similarity) among the 266 isolates typed by MLST. UA means unassigned ST to a known CC.

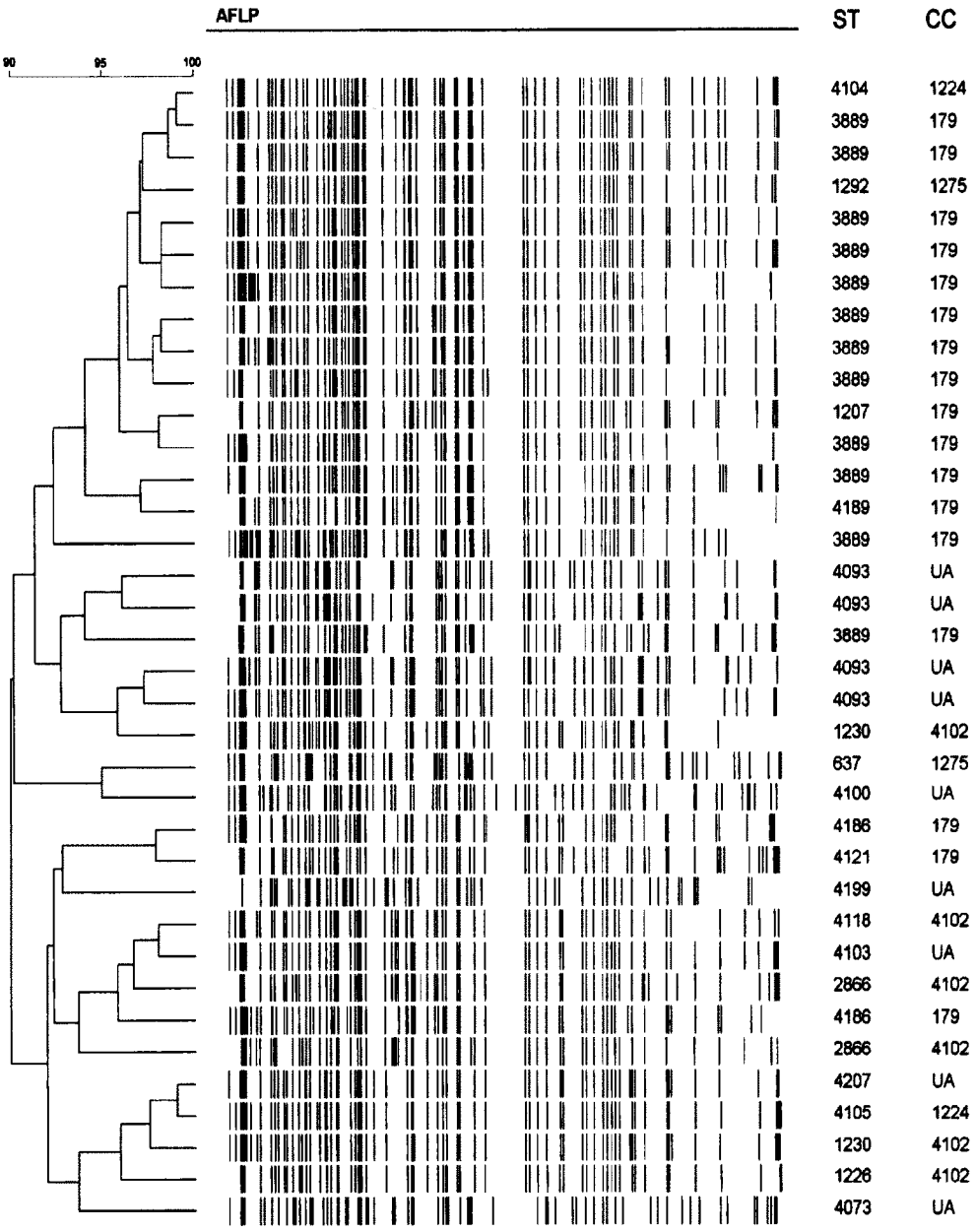


Figure S4. Distribution of sequence types (ST) and clonal complexes (CC) for members of the third largest AFLP cluster (36 isolates at 90% of similarity) among the 266 isolates typed by MLST. UA means unassigned ST to a known CC.

CHAPITRE 5

AVANT-PROPOS DE L'ARTICLE

Titre

A case-case comparison of campylobacteriosis in urban versus rural areas: molecular data to validate risk factors and to attribute sources of infection

Auteurs

Simon Lévesque, Eric Fournier, Nathalie Carrier, Eric Frost, Robert D. Arbeit, Sophie Michaud

État de l'article à ce jour

Soumis pour publication à la revue PLOS ONE le 2 avril 2013.

Le formulaire « Autorisation d'intégration d'un article écrit en collaboration à un mémoire ou une thèse » a été signé par chaque co-auteure ou co-auteur conformément à la « Directive relative au dépôt des essais, des mémoires et des thèses » de l'Université de Sherbrooke.

RÉSUMÉ DE L'ARTICLE

Le Campylobacter est la cause de diarrhée bactérienne la plus importante à travers le monde. La majorité des cas sont des infections sporadiques dont la source est rarement identifiée. Une étude cas-cas des campylobactérioses acquises en Estrie et une étude de prévalence du Campylobacter dans les réservoirs fréquents de la bactérie a été effectuée. Nous avons utilisé le MLST pour appuyer les facteurs de risque identifiés par l'étude cas-cas et pour déterminer les sources des infections sporadiques chez l'humain. De juillet 2005 à décembre 2007, 352 cas de campylobactériose ont été déclarés. Pour l'ensemble de la période, l'incidence cumulative brute fut de 117,2/100 000 habitants. Après exclusion des cas non acquis en Estrie, 241 cas de campylobactériose ont été inclus dans l'analyse finale pour la comparaison cas-cas. Le risque de campylobactériose fut 1,89 fois plus élevé en milieu rural qu'en milieu urbain. L'incidence fut plus élevée en milieu rural qu'en milieu urbain pour le groupe d'âge des 15-34 ans et pour les adultes de plus de 55 ans. Il n'y avait pas de différence significative pour le groupe d'âge des 0-4 ans. Parmi les 45 expositions évaluées par régression logistique conditionnelle univariée (ajusté pour l'âge et le sexe), les 7 variables suivantes ont été significativement plus fréquentes parmi les cas acquis en milieu rural : vivre sur une ferme, exposition professionnelle en lien avec des animaux, l'eau à la maison provenant d'un puits, vivre près d'une ferme, ne pas avoir désinfecté son puits depuis les 6 derniers mois, boire de l'eau filtrée et avoir consommé du lait cru. Aucune différence significative pour l'ensemble des variables associées au poulet n'a été observée entre les cas acquis en milieu rural et urbain. Un

total de 851 isolats *C. jejuni* (178 d'humain, 257 de poulet, 87 de bovin, 266 d'eau et 63 d'oiseau sauvage) ont été analysés par MLST. Parmi les isolats humains, le taux d'incidence des CC ST-21, CC ST-45 et CC ST-61 fut plus élevé en milieu rural qu'en milieu urbain. La détermination de l'implication de chaque source potentielle de contamination a démontré que les campylobactérioses humaines étaient attribuables à 64,5 % aux poulets, 25,8 % aux bovins, 7,4 % à l'eau et 2,3 % aux oiseaux sauvages. Le nombre de cas de campylobactérioses associé à l'eau a été significativement plus élevé en milieu rural. Les analyses restreintes aux groupes d'âge ont démontré que pour les 15-34 ans, les cas étaient plus fréquemment associés aux bovins en milieu rural qu'en milieu urbain. Parmi les sources potentielles, seulement les bovins ont démontré une variation saisonnière, la majorité des cas étant retrouvés durant l'été et l'automne. Les cas humains associés au poulet ont été distribués également durant l'année. Nous avons démontré par cette étude un gradient urbain-rural de campylobactériose. Le nombre de cas excédentaire en milieu rural semble être causé par l'exposition aux bovins, particulièrement pour le groupe d'âge des 15-34 ans via une exposition professionnelle et la consommation d'eau en provenance d'un puits. L'exposition aux bovins est possiblement responsable de la saisonnalité des campylobactérioses. La consommation de poulet est le facteur de risque principal et notre modèle y associe 65 % des cas, indépendamment du milieu d'acquisition et de l'âge. Grâce à ces résultats, nous croyons que des programmes publics de sensibilisation devraient être mis en place en fonction des principaux facteurs de risque identifiés et que des mesures de prévention ciblées et efficaces pourraient être développées.

Contribution de l'étudiant

J'ai participé au design de l'étude et des expériences. J'ai contribué totalement à l'obtention et à l'analyse de tous les résultats décrits dans l'article. J'ai également écrit entièrement la première ébauche du manuscrit et participé à la correction avant la soumission.

A Case-Case Comparison of Campylobacteriosis in Urban Versus Rural Areas: Molecular Data to Validate Risk Factors and to Attribute Sources of Infection

Simon Lévesque[1], Eric Fournier[2], Nathalie Carrier[3], Eric Frost[1,3], Robert D. Arbeit[4], Sophie Michaud[1]

1. Department of Microbiology and Infectious Diseases, Faculté de Médecine de l'Université de Sherbrooke, Québec, Canada.

2. Laboratoire de santé publique du Québec, Institut national de santé publique du Québec, Sainte-Anne-de-Bellevue, Québec, Canada.

3. Centre de Recherche Clinique Étienne Le-Bel du Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, Québec, Canada

4. Infectious Diseases Section, Tufts University School of Medicine, Boston, MA.

Corresponding author: Sophie Michaud, MD, MPH, CSPQ, FRCPC
Department of Microbiology and Infectious Diseases
Faculté de Médecine de l'Université de Sherbrooke
3001, 12e avenue Nord
Sherbrooke, Québec J1H 5N4
Phone: (819) 564-5321
Fax: (819) 564-5392
E-mail: Sophie.Michaud@USherbrooke.ca

Financial support: The Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of

Canada (NSERC) and the Centre de Recherche Clinique Étienne Le-Bel.

Running head: Campylobacteriosis in urban vs rural areas

Key words: *Campylobacter*, case-case comparison, urban, rural, MLST, risk factor, molecular typing

Word count (text): 5 492

ABSTRACT

A prospective case-case study of domestically-acquired *Campylobacter* enteritis and a prevalence study of *Campylobacter* in common reservoirs were conducted. We used MLST to reinforce the case-case findings and to determine the source of sporadic human campylobacter infections. From July 2005 to December 2007, the risk of campylobacteriosis was 1.89-fold higher in rural area than in the urban zone. Conditional multivariate analysis adjusted for age and sex identified two independent factors associated with rural area: a professional exposure to animals (OR=10.6, p=0.032), and water source at home coming from a well (OR=8.3, p<0.0001). A total of 851 *C. jejuni* isolates (178 human, 257 chicken, 87 bovine, 266 water and 63 wild bird isolates) were typed by MLST. Among human isolates, the incidence rates of CC ST-21, CC ST-45 and CC ST-61 were higher among the rural than the urban isolates. The assignment probability for each putative source showed that human *C. jejuni* isolates were attributed to chicken (64.5%), to bovine (25.8%), to water (7.4%) and to wild birds (2.3%). Among putative sources, only bovine showed a clear seasonal variation, since the majority of the cases were found during summer and fall. Human cases associated with chicken were equally distributed all over the year. The seasonality of the infection, as well as the excess number of cases in rural area seems to be caused by bovine exposition, particularly in the 15-34 years old group, probably due to professional exposition and water consumption from a well.

Word count: 243

INTRODUCTION

Campylobacter enteritis is the leading cause of bacterial gastro-enteritis worldwide with an average of 39 cases per 100,000 inhabitants reported annually over the last decade in Canada (3) causing illness in about 1% of the population of the United States, 13,000 hospitalizations, and over 100 deaths each year (50). *Campylobacter* colonizes a wide range of hosts, including domestic animals and wild birds, and thus a substantial burden of *Campylobacter* is excreted via animal fecal material (2). Poultry, raw milk and untreated water have all been well-documented as sources of *Campylobacter* outbreaks (63). Nevertheless, most clinical cases appear as isolated, sporadic infections for which the source is rarely apparent. Identifying the sources and routes of transmission of campylobacteriosis is essential for developing effective, targeted preventive measures.

An urban-rural gradient for *Campylobacter* infections has been described in several countries (46, 51, 53, 57, 60), although only few studies have tried to identify reasons for such geographical variation. In Ontario, Thompson et al. reported 80 cases/100,000 in urban regions, compared to 350-400/100,000 in some rural areas (60) and in Manitoba, Green et al. reported 13.94 cases/100,000 in urban regions compared to 44.38 cases/100,000 in rural areas (19). It is therefore likely that the population attributable risk of contributing factors varies in rural versus urban settings and that preparing chicken or consuming under-cooked chicken could not alone explain the discrepancy.

Many authors have recognized that the analysis of clinical descriptive data have limitations to identify sources of sporadic campylobacteriosis, partly due to the delay between the onset of symptoms and the epidemiological investigation (25, 35, 36). Multi-locus sequence typing (MLST) is suitable for identifying possible

variations in the epidemiology of campylobacteriosis between populations and for source attribution of campylobacteriosis (8, 53). It has demonstrated the ability to improve linkage between apparently sporadic cases encountered in routine surveillance by assigning isolates to sequence type (ST) complexes (8, 11, 53). Some *Campylobacter* STs are principally associated with a particular ecological niche, as ST-61 in cattle, ST-257 in chicken, ST-177 in wild birds or ST-3704 in bank voles (8, 23, 32, 33, 62). Although there is overlap, the association of different STs with particular host sources (33) makes it possible to quantitatively estimate the source of human infection (41, 49, 63). MLST is therefore a valuable tool in testing the significance of suspected epidemiologic exposures in human campylobacteriosis (53).

We conducted a prospective case-case study of domestically-acquired *Campylobacter* enteritis in the Eastern Townships, Québec, to test the hypothesis that some risk factors – such as chicken exposure (i.e. handling raw chicken and eating undercooked poultry) – are common to both urban and rural areas, accounting for the basal rate of campylobacteriosis, while rural particular risk factors are responsible for the excess of cases in rural areas. We conducted in parallel a prevalence study of *Campylobacter* in common *Campylobacter* reservoirs (retailed fresh whole chickens, environmental water, wild birds and cattle) and used MLST to reinforce the case-case findings and to determine the source of sporadic human campylobacter infections.

MATERIALS AND METHODS

Clinical and epidemiological data. The Eastern Townships comprise seven counties with 89 municipalities totalling 298 685 inhabitants. Each municipality was categorized as a small metropolitan area (50 000-249 999 inhabitants), a small non metropolitan city zone (20 000-49 999 inhabitants), a small town zone (2 500-19 999 inhabitants) or a predominantly rural zone (< 2 500 inhabitants), as defined by Statistics Canada (www.statcan.gc.ca), using population data from the 2006 Canadian Census.

Hospital microbiology laboratories routinely report all campylobacter enteritis cases to the regional Public Health Department. Public health nurses interviewed cases by telephone within 2 weeks of reporting, using a structured questionnaire to capture demographic and clinical data, travel history, food history, water consumption, recreational water activity, and animal exposure during the 10 days before the onset of symptoms. All cases reported between July 1, 2005 and December 31, 2007 were eligible for the study. Cases were excluded if the infection was acquired outside Québec (i.e., travel outside the province during the entire 10-day period before the onset of symptoms), if the home address was outside the Eastern Townships or if they declined to participate. For subjects reported on multiple occasions during the study period, only the first episode of infection was considered. Campylobacteriosis data for Québec province were provided by the Ministère de la Santé et des Services Sociaux du Québec.

Isolation and identification of *Campylobacter* isolates. All of the Eastern Townships hospital microbiology laboratories routinely evaluated human stool specimens for *Campylobacter* by using comparable standard methods for isolation and identification (Karmali or Skirrow media incubated for 72 h at 42°C in a microaerobic atmosphere).

Identification of *Campylobacter* isolates to the species level was done by routine phenotypic methods (43). Hospital laboratories were asked to send us all *Campylobacter* isolates cultured during the study period. When *Campylobacter spp.* was sent, species identification was performed by CPN60 analysis as described before (31). Species information for isolates was taken as reported from the laboratories.

In parallel, a prevalence study of environmental water, retailed fresh whole chicken and animal isolates was performed in the Eastern Townships. River water isolates were collected from July, 2005 to October, 2007 (56) according to a previously described protocol (30). From May 22, 2005 to October 16, 2007, 8 fresh, eviscerated whole chickens were purchased weekly in the different counties (one chicken per store) and cultured for *Campylobacter*; for each county, the number of chickens sampled monthly was proportional to the population. Of note, retail chickens sold in the Eastern Townships are produced by multiple companies based elsewhere in Québec Province. From November 11, 2005 to December 11, 2007, 495 bovine (365 dairy cows and 130 beef cattle) fresh feces samples from 99 farms distributed among the seven counties of the Eastern Townships were analysed. From May 10, 2005 to November 14, 2007, 235 gulls (round-billed gull [*Larus delawarensis*] and great black-backed Gull [*Larus marinus*]) fresh feces from two waste management sites located in Eastern Townships were analysed. In addition, we analysed 352 duck (mallard [*Anas platyrhynchos*]) cloaca swabs, 28 snow goose [*Chen caerulescens*] and 24 Canada goose [*Branta canadensis*] fresh feces collected from May 10, 2005 to November 14, 2007. The culture methods are described in more details in supplementary material.

From water, chicken, bovine and wild bird Karmali agar plates, isolates of *Campylobacter* were identified to the species level by routine phenotypic methods as

described previously (56). Genomic DNA was extracted by transferring a single colony of each isolate of *Campylobacter* into 25 µl of 0.5 N NaOH. After 5 minutes at room temperature, 25 µl of Tris 1M pH 8.0 and 450 µl of sterile distilled water were added. DNA extracts were stored at -20°C. Isolates which had a hippurate-negative phenotype but had a hippurate gene detected by PCR were identified as *C. jejuni* (52). Species identification of *Campylobacter* other than *C. jejuni* was also confirmed by CPN60 gene analysis as described before (31). Isolates which died before complete identification or DNA extraction was performed were identified as *Campylobacter spp.*

Multilocus sequence typing. Among the 219 human *C. jejuni* cases included in the study, 178 (81%) isolates were available for MLST typing. Typed isolates from other sources were equally distributed among sampling period and sampling area. MLST was carried out with the high-resolution melting (HRM) system as previously described (29) or by the conventional method as described by Dingle et al. (11) with modified amplification conditions (28). For some isolates, primers of the extended MLST system (38) or new primers designed in a previous study (31) were used. Sequences were compared and analyzed with BioNumerics program version 5.0. Allele numbers, sequence types (STs) and clonal complexes (CCs) were assigned by submitting DNA sequence to the *C. jejuni* MLST database website (http://pubmlst.org/campylobacter).

Data analysis. Minimum spanning trees were constructed within BioNumerics, using the allelic data set. New STs not assigned to a CC within the *C. jejuni* MLST database were examined for CCs by use of eBURST3 (15, 55). Members of a CC were defined as groups of two or more independent isolates with an ST that shared identical alleles

at four or more loci, as defined by Dingle et al. (11). CCs were constructed using a maximum neighbor distance of two changes and a minimum size of two STs.

Assignment of human isolates to putative sources based on MLST allelic profile was performed using STRUCTURE, a Bayesian model-based clustering method designed to infer population structure and assign individuals to populations using multilocus genotype data (47). Differences in genotype frequencies between populations in the reference data set allow probabilistic assignment of isolates to these populations, even if there is some sharing of genotypes between the reference data set populations. This model had shown to provide a greater probability of correct assignment of an isolate to its origin (47). Analyses were performed with 10,000 iterations following a 10,000-iteration burn-in using the no-admixture model of STRUCTURE, and the isolate collection to be assigned was distinguished from the reference data set populations using the "usepopinfo" flag. Human *C. jejuni* isolates were assigned to putative sources of infection by comparing their MLST profile to data sets comprising all the animal and environmental isolate genotype data typed in the same time frame as human campylobacteriosis cases.

Statistical analysis. For the case-case analysis, we compared the incidence rate ratios (IRR) and the risk factors for campylobacteriosis among patients living in rural vs urban areas, as defined in the Results Section. Risk factors for campylobacteriosis were compared by conditional logistic regression for data adjusted for age and sex. All risk factors with a $p< 0.10$ by univariate analysis were included in a multivariate logistic regression, stepwise selection model. Association between chicken positivity rates and the campylobacteriosis incidence rates was calculated with Spearman's correlation. Seasonal differences in chicken positivity rates were calculated with Kruskall-Wallis for independent samples. Differences between STRUCTURE sources

attribution were calculated with the Mann-Whitney U test for independent samples. All statistical analyses were performed using SAS version 18 (SAS, Cary, NC).

RESULTS

Cases of campylobacteriosis. From July 2005 to December 2007, 352 cases of campylobacteriosis were reported, of which 111 were excluded: 41 cases acquired their infection outside Québec, 19 resided outside the Eastern Townships, 49 could not be interviewed or declined to participate and two reinfection cases were rejected. Consequently, 350 cases were used to calculate the crude incidence rate and 241 cases of campylobacteriosis were included in the final data set for the case-case comparison.

Overall, 219 (90.9%) of the isolates were identified as *C. jejuni*; the remainder included 11 *C. coli*, 1 *C. lari* subsp. *concheus*, 2 *C. fetus*, 3 *C. upsaliensis* and 5 *Campylobacter* spp. Essentially all (99%) isolates were obtained from stool cultures, except for the two *C. fetus* isolates which were obtained from blood cultures. Clinical presentation of cases is shown in supplemental material.

Demographics. During the study period, the cumulative crude incidence rate of campylobacteriosis was 117.2/100,000 inhabitants in the Eastern Townships, compared to 80.6/100,000 inhabitants in the remainder of Québec province (p=0.01). The incidence rate for the cases included in the data set was 39.2/100,000 inhabitants from 07/01/2005 to 06/30/2006 compared to 26.1/100,000 inhabitants from 07/01/2006 to 06/30/2007 (p=0.0052) (Table S1).

Among the 89 municipalities, one was categorized as a small metropolitan area (hereinafter called urban zone), one as a small non metropolitan city area, 15 as small town areas – these two categories were combined and defined as semi-rural areas –, and 72 as rural areas. The cumulative incidence rates varied from 55.6/100,000 inhabitants in the urban zone, 110.7/100,000 inhabitants in the semi-rural zone and 96.6/100,000 in the rural zone; this gap was observed for both years of the study (data

not shown). The risk of campylobacteriosis was 1.89-fold higher in rural and semi-rural areas than in the urban zone ($p<0.0001$). Since there was no statistical difference in the incidence rates (both global and by age group) between rural and semi-rural areas, these two categories were combined and hereinafter called rural areas. This combination facilitated the univariate and multivariate analyses since the size of the population in both the rural (n=151 255) and urban (n=147 430) areas was about the same.

Most cases occurred during the months of July, August and September (Figure 1). However, in 2006, a surprisingly high rate of *Campylobacter* infections occurred from January to April compared to the same period in 2007 ($p=0.0004$). The median age of the cases was 34 years (range: 9 months-85 years). Overall, 116 (48.1%) cases were female.

The incidence rates of campylobacteriosis varied considerably by age (Figure 2) and by sex (Figure 3), with the highest rates occurring among 0-4 year-old children (149.2/100, 000) in both urban and rural areas, and 15-34 year-old young adults (mean = 148.0/100,000), in the rural areas. In the urban area, only 0-4 year-old children had an incidence rate that stood out from other age groups. Campylobacter incidence was higher in rural than in urban areas for both the 15-34 year age group (IRR=2.8 [1.6-4.9]; $p=0.0001$), and the adult $\geq$ 55 years (IRR=2.3 [1.0-5.6]; $p=0.003$). There was no statistical difference between rural and urban areas for the 0-4 year-old children category (IRR=1.46 [0.58-4.0]; $p=0.38$).

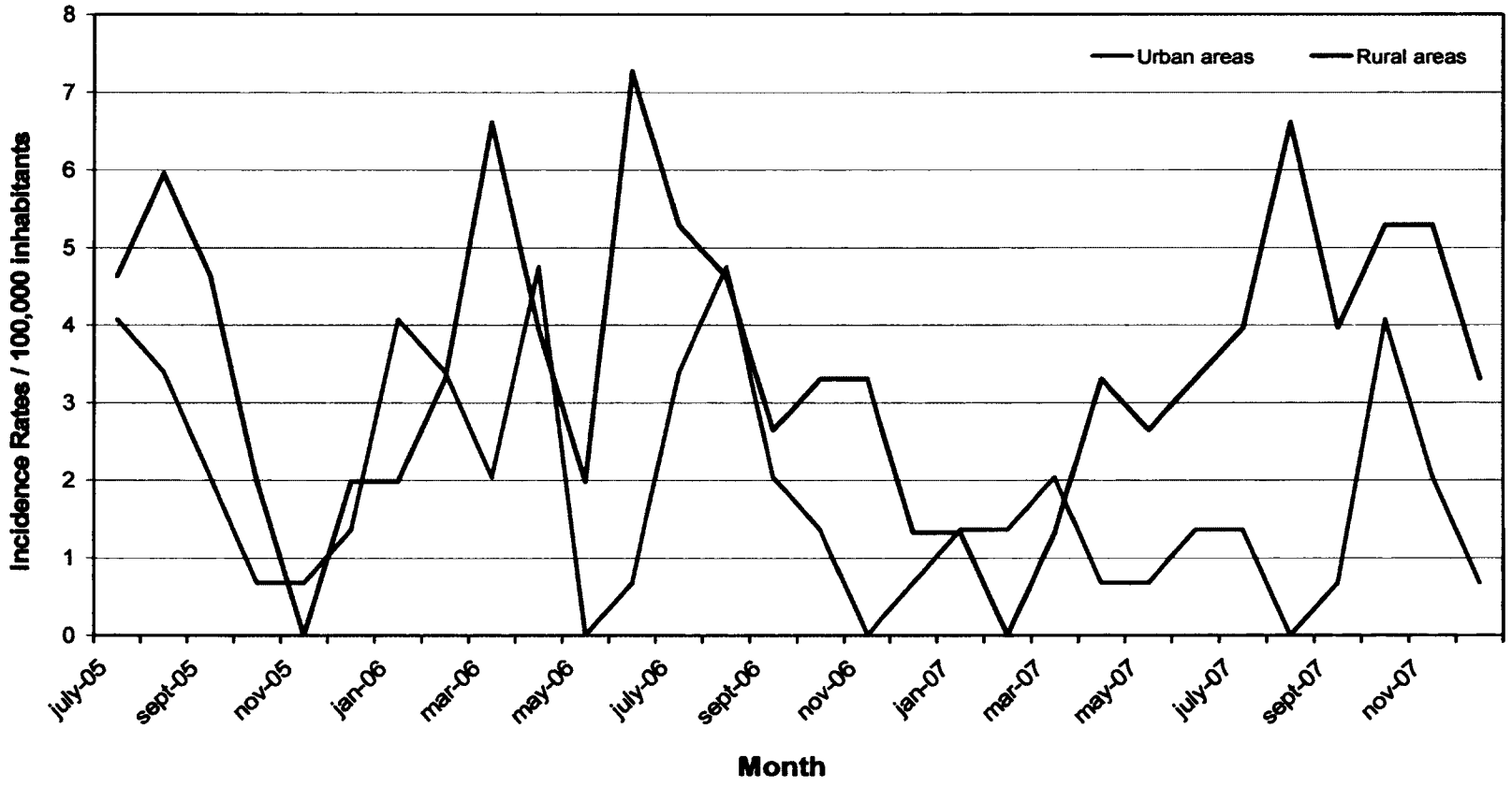


Figure 1. Monthly distribution of incidence rates of *Campylobacter* infections from July 2005 to December 2007 for urban and rural areas.

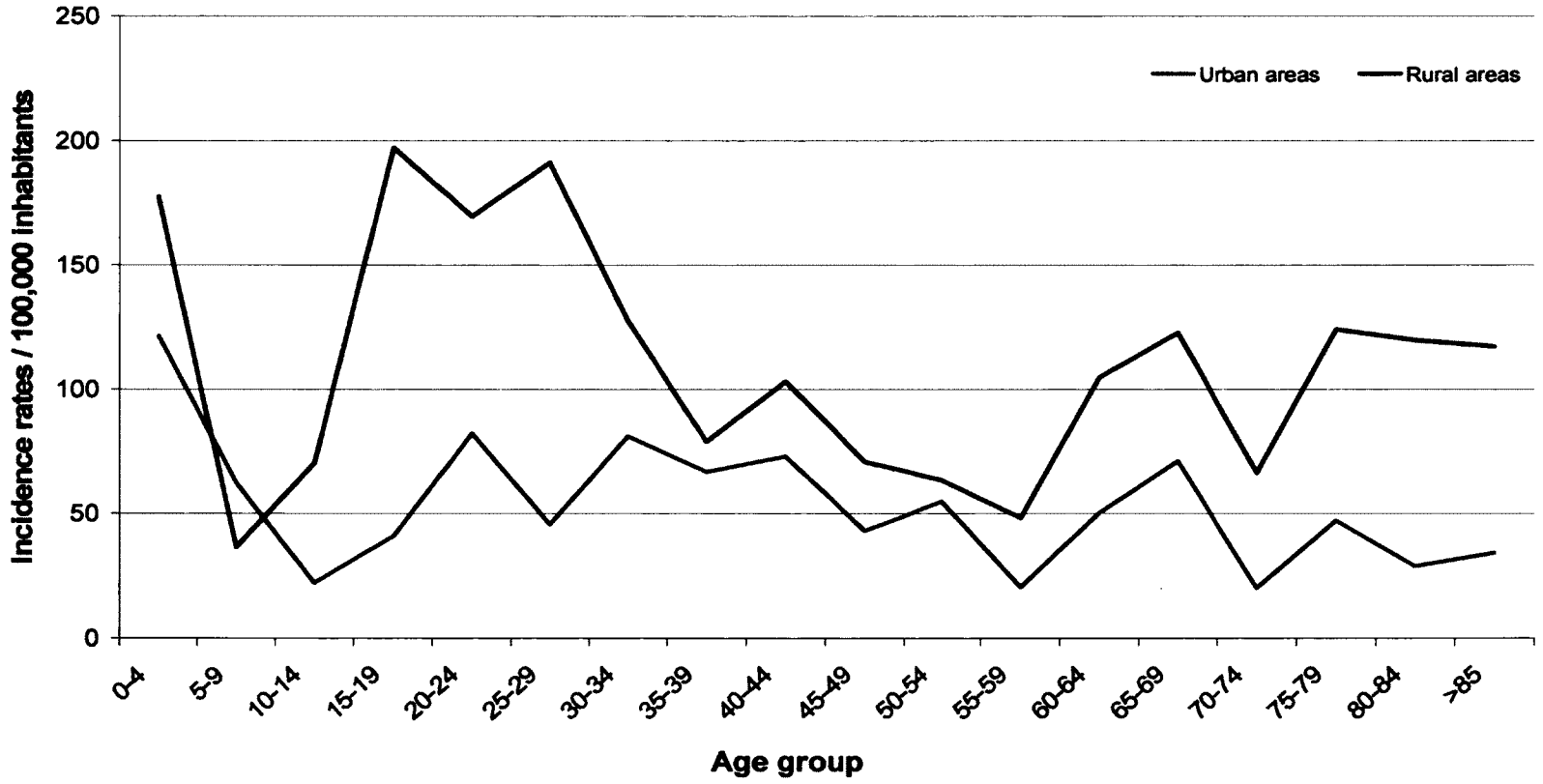
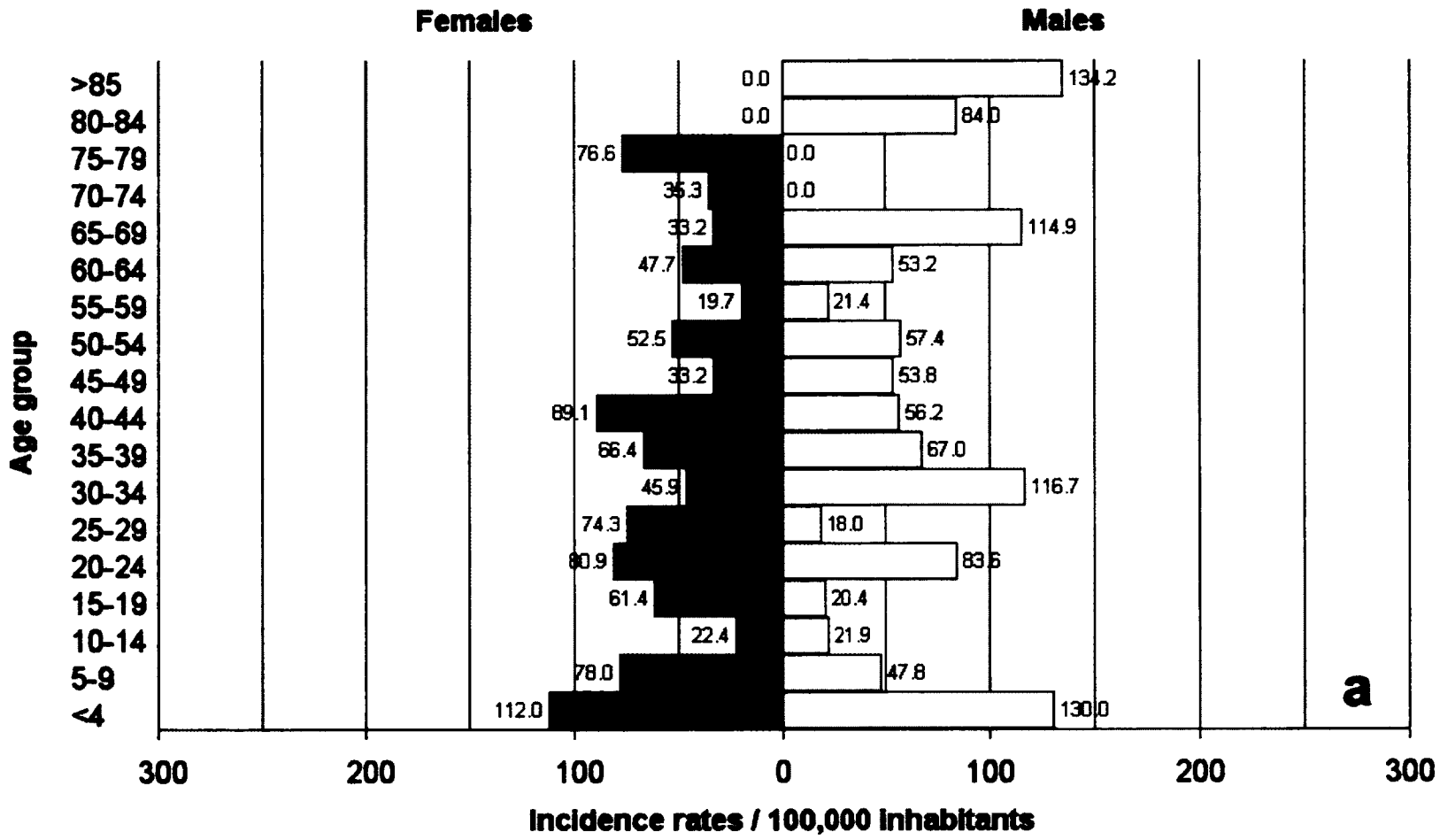
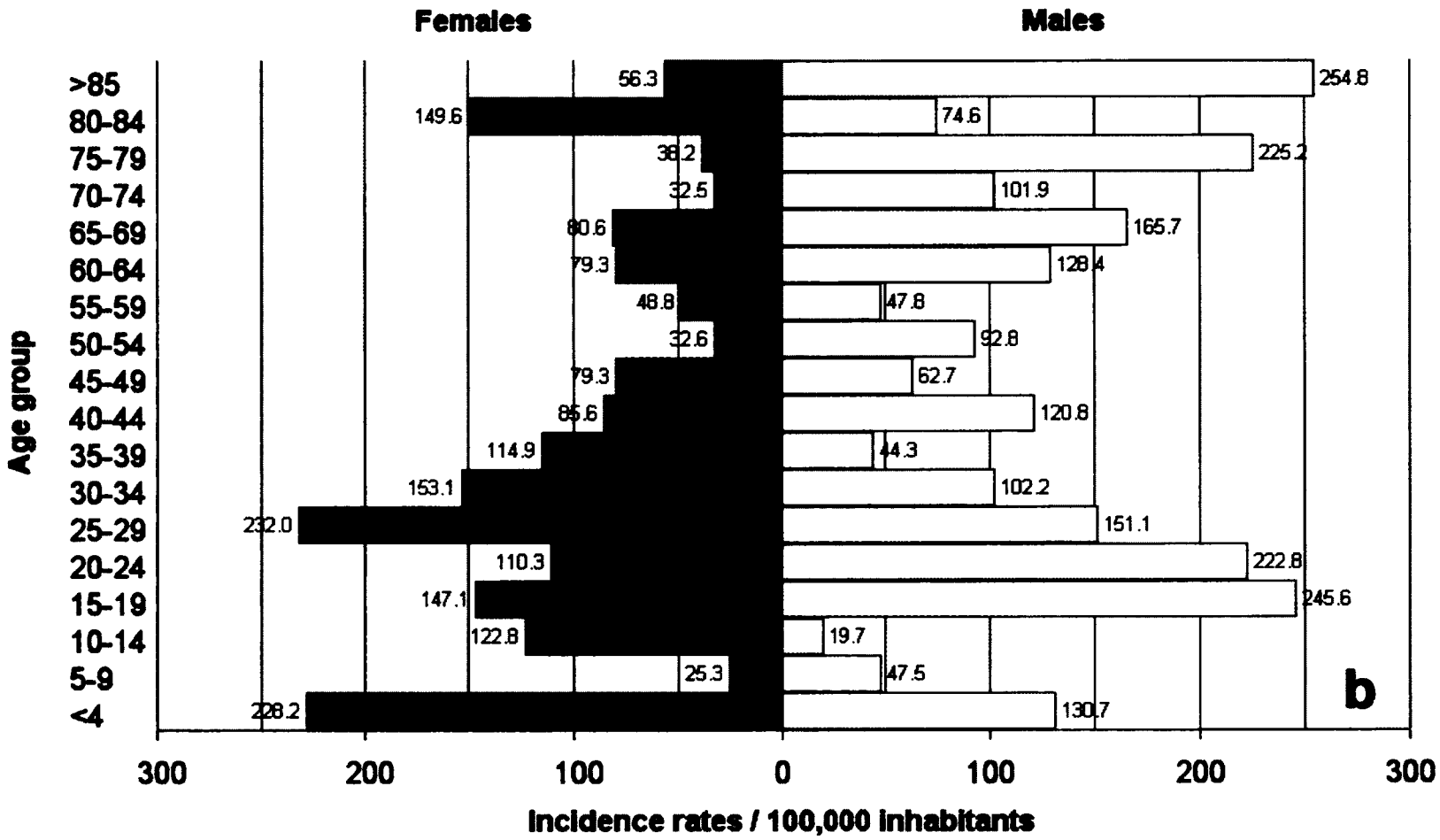


Figure 2. Distribution of incidence rates of *Campylobacter* infections by age group for urban and rural areas.


Females
Males
>85
80-84
75-79
70-74
65-69
60-64
55-59
50-54
45-49
40-44
35-39
30-34
25-29
20-24
15-19
10-14
5-9
<4
Age group
0.0
0.0
76.6
35.3
33.2
47.7
19.7
52.5
33.2
89.1
66.4
45.9
74.3
80.9
61.4
22.4
78.0
112.0
134.2
84.0
0.0
0.0
114.9
53.2
21.4
57.4
53.8
56.2
67.0
116.7
18.0
83.6
20.4
21.9
47.8
130.0
a
300
200
100
0
100
200
300
Incidence rates / 100,000 inhabitants


Females
Males
>85
80-84
75-79
70-74
65-69
60-64
55-59
50-54
45-49
40-44
35-39
30-34
25-29
20-24
15-19
10-14
5-9
<4
Age group
56.3
149.6
38.2
32.5
80.6
79.3
48.8
32.6
79.3
85.6
114.9
153.1
232.0
110.3
147.1
122.8
25.3
228.2
254.8
74.6
225.2
101.9
165.7
128.4
47.8
92.8
62.7
120.8
44.3
102.2
151.1
222.8
245.6
19.7
47.5
130.7
b
300
200
100
0
100
200
300
Incidence rates / 100,000 inhabitants

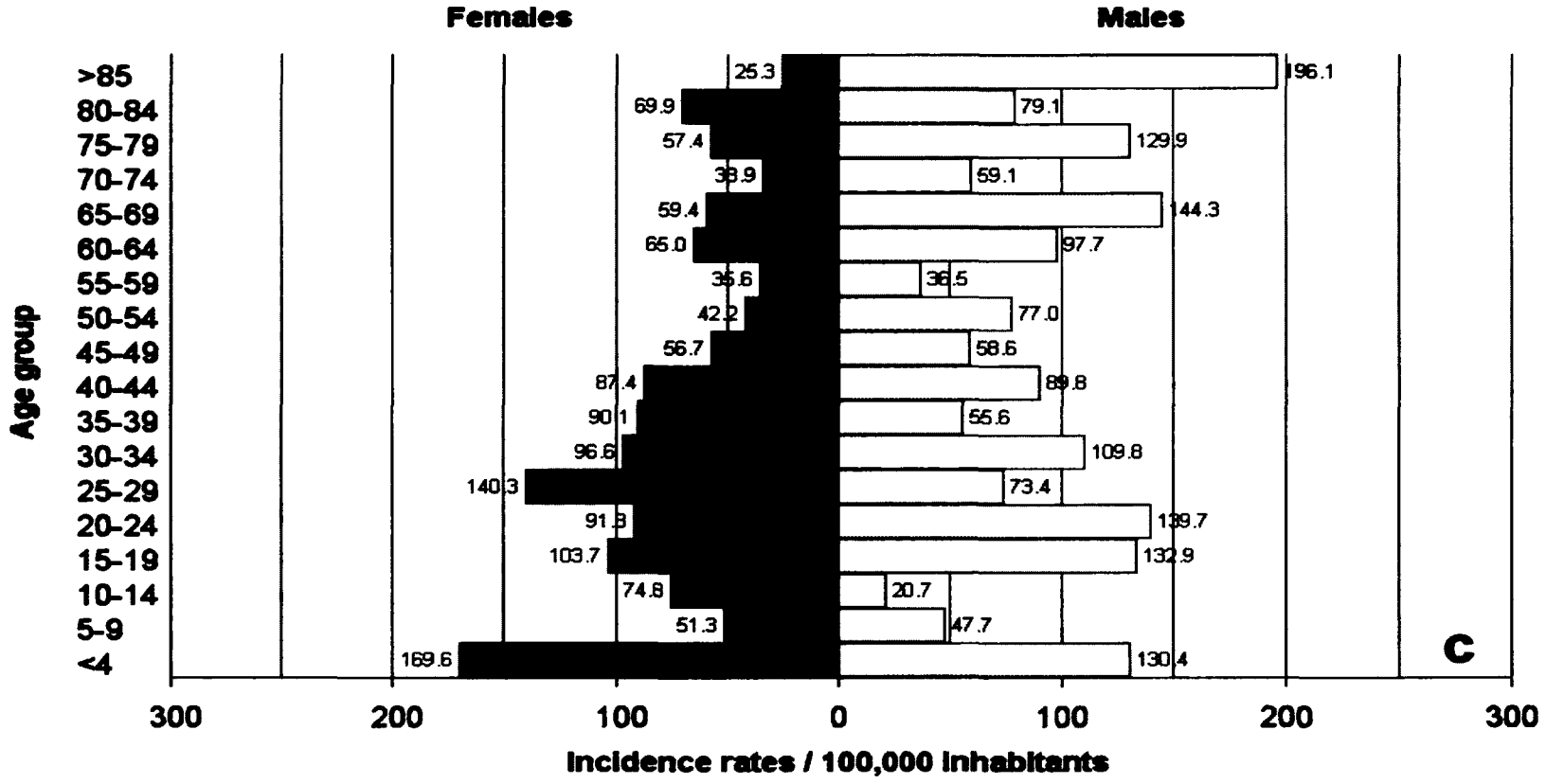


Figure 3. Incidence rates of *Campylobacter* infections by age group and sex for (a) urban zone, (b) rural zone and for (c) global Eastern Townships.

Case-case comparison. Among the 45 exposure factors evaluated by univariate conditional logistic regression and adjusted for age and sex, the following 7 variables were significantly more frequent ($p<0.05$) among the cases acquired in rural areas compared to those acquired in urban areas (Table 1): living on a farm (OR=13.3, $p=0.0005$), professional exposure to animals (pet shop, farm, zoo or veterinary clinic) (OR=11.4, $p=0.02$), water source at home coming from a well (OR=10.0, $p<0.0001$), living near a farm (OR=9.2, $p<0.0001$), not having disinfected the well within the past 6 months (OR=5.0, $p=0.03$), drinking pitcher or faucet filtered water (OR=3.2, $p=0.01$) and consuming raw milk (OR=2.9, $p=0.03$). Drinking bulk water was significantly less frequent among cases acquired in rural areas (OR=0.5, $p=0.04$). There was no statistical difference between urban and rural cases among activities related to consuming or handling poultry. Of note, 81% of rural cases who reported drinking filtered water (either directly at the faucet or in a pitcher) had their water source at home coming from a well. Univariate analysis restricted to the 15-34 year old age group showed that water source at home coming from a well (OR=30.2, $p<0.0002$) and living near a farm (OR=9.2, $p=0.007$) were significantly more frequent among the cases acquired in rural areas compared to those acquired in urban areas. However, in the same age group, consuming undercooked chicken (OR=0.2, $p=0.018$) was significantly less frequent among the cases acquired in rural areas compared to those acquired in urban areas.

Table 1. Comparison of exposure factors for campylobacteriosis among rural vs urban cases.

Risk factors	Rural area		Urban area		Univariate analysis: rural versus urban area		
					Adjusted for age and sex		
	Cases	Rates (%)	Cases	Rates (%)	OR	95% CI	p value
Living on a farm	39/155	25.2	2/78	2.6	13.3	3.1 - 56.8	0.0005
Working in a petshop, farm, zoo or veterinary clinic	17/135	12.6	1/69	1.5	11.4	1.5 - 88.9	0.0201
Water at home coming from a well	92/151	60.9	10/75	13.3	10.0	4.8 - 21.1	< 0.0001
Living near a farm	57/157	36.3	3/79	6.2	9.2	3.5 - 24.2	< 0.0001
Not having disinfected the well in the last 6 months	77/88	87.5	6/10	60.0	5.0	1.2 - 21.1	0.0298
Consuming filtered water	32/157	20.4	6/81	7.4	3.2	1.3 - 8.1	0.0123
Consuming raw milk	13/48	21.3	6/82	7.3	2.9	1.1 - 7.5	0.0288
Consuming bulk water	17/151	11.3	18/82	22.0	0.5	0.2 - 0.9	0.0414

Risk factor	Multivariate analysis adjusted for age and sex: rural versus urban area		
	OR	95% CI	p value
Working in a petshop, farm, zoo or veterinary clinic	10.7	1.2 – 91.0	0.0320
Water at home coming from a well	8.3	3.4 – 20.4	< 0.0001

Conditional multivariate analysis adjusted for age and sex identified two independent factors associated with rural area (Table 1): a professional exposure to animals (pet shop, farm, zoo or veterinary clinic) (OR=10.6, p=0.032) and a water source at home coming from a well (OR=8.3, p<0.0001). The correlations between independent variables in multivariate logistic regression were performed to eliminate colinearity between variables. All correlations were less than 0.3.

Animal and environmental isolates. A total of 879 chickens from 59 different food stores were analysed: 371 (42%) chickens were positive for *Campylobacter* spp. with 332 *C. jejuni*, 37 *C. coli* and 2 *Campylobacter* spp. The monthly prevalence of *Campylobacter* varied between 15.6% and 72.2% (Figure 4). There was no clear seasonal variation (p=0.246) even if rates tended to be higher in summer and fall. However, there was a weak to moderate association between variation in the chicken positivity rates and campylobacteriosis incidence rates in humans (Spearman's ρ coefficient=0.27; p=0.008) (Figure 4). There was no difference in the prevalence rates in chicken purchased in grocery stores between urban and rural areas (p=0.7).

Among the 485 bovine feces sampled, 170 (34.3%) were positive for *Campylobacter*, with 123 (70.7%) *C. jejuni*, 38 (21.8%) *C. fetus*, 6 (3.4%) *C. lari*, 5 (2.9%) *C. coli* and 2 (1.1%) *Campylobacter* spp. In general, Campylobacters were more frequently found among dairy cows (39.2%) than in beef cattle (20.8%) (p=0.0001). *C. jejuni* was significantly more frequent among dairy cows (77.2% versus 48.3% among beef cattle, p=0.001), in contrast with *C. fetus* which was significantly more present among beef cattle (41.4% versus 15.9% among dairy cows, p=0.002).

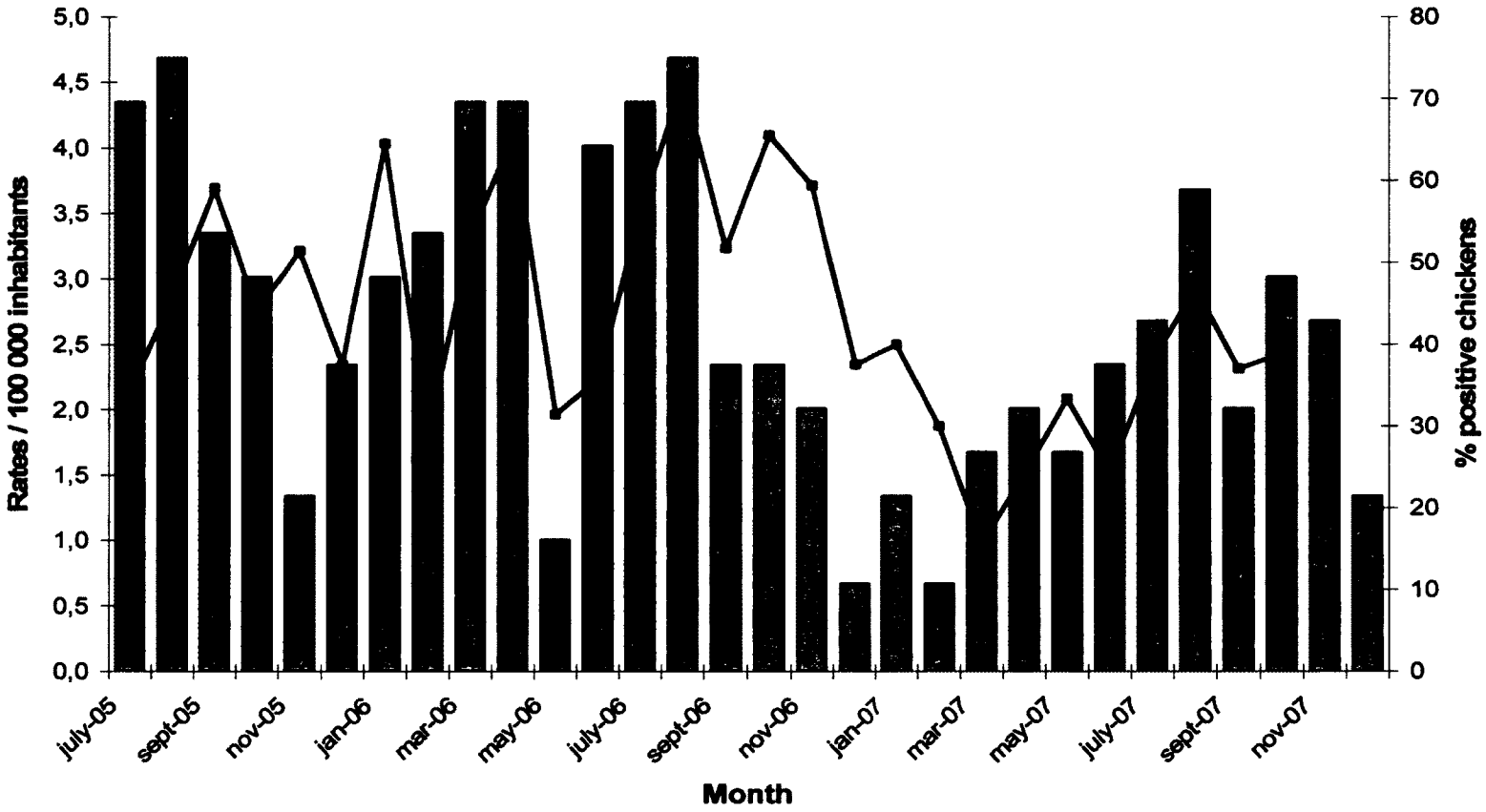


Figure 4. Monthly distribution of the incidence rates of *Campylobacter* infections in humans from July, 2005 to December, 2007 (columns) and of the prevalence of *Campylobacter* in whole retail chickens from July, 2005 to October 2007 (line graph).

Among the 639 wild bird feces sampled, 108 (16.9%) were positive for *Campylobacter* with 78 (72.2%) *C. jejuni*, 14 (13%) *C. lari*, 9 (8.3%) *C. coli*, 1 (0.9%) *C. fetus*, 1 (0.9%) *C. upsaliensis* and 5 (4.6%) *Campylobacter* spp. Snow geese had the highest positivity rate with 13 (46.4%) isolates, followed by gulls with 78 (33.2%) isolates, Canada geese with 6 (25%) isolates and ducks with 11 (3.1%) isolates. Except for *C. jejuni* which was the most prevalent *Campylobacter* species in each type of birds and the only one present in ducks, *C. lari*, *C. fetus* and *C. upsaliensis* were only isolated in gulls while *C. coli* was isolated from gulls, snow geese and Canada geese.

Multi-locus sequence typing. A total of 851 *C. jejuni* isolates (178 human, 257 chicken, 87 bovine, 266 water and 63 wild bird isolates) were typed by MLST. A total of 262 STs were identified (Table S2), of which 188 STs, representing 743 (87%) isolates, were assigned to 31 previously described CCs. The remaining 108 isolates were distributed among 74 STs which could not be assigned to any of the known lineages. The most frequent STs identified were ST-45 (96 isolates), ST-1212 (74 isolates) and ST-21 (66 isolates). Overall, 204 STs (77.9% of the STs) were associated with one or two isolates only. The most prevalent CCs identified were CC ST-21 (152 isolates), CC ST-45 (138 isolates) and CC ST-607 (83 isolates), which together represented 43.8% of typed isolates. Only the two largest CCs (CCs ST-21 and ST-45) and in CC ST-42 were found in all five sources (Figure 5). Twenty one of the 31 CCs observed in this study included isolates from humans and at least one other source.

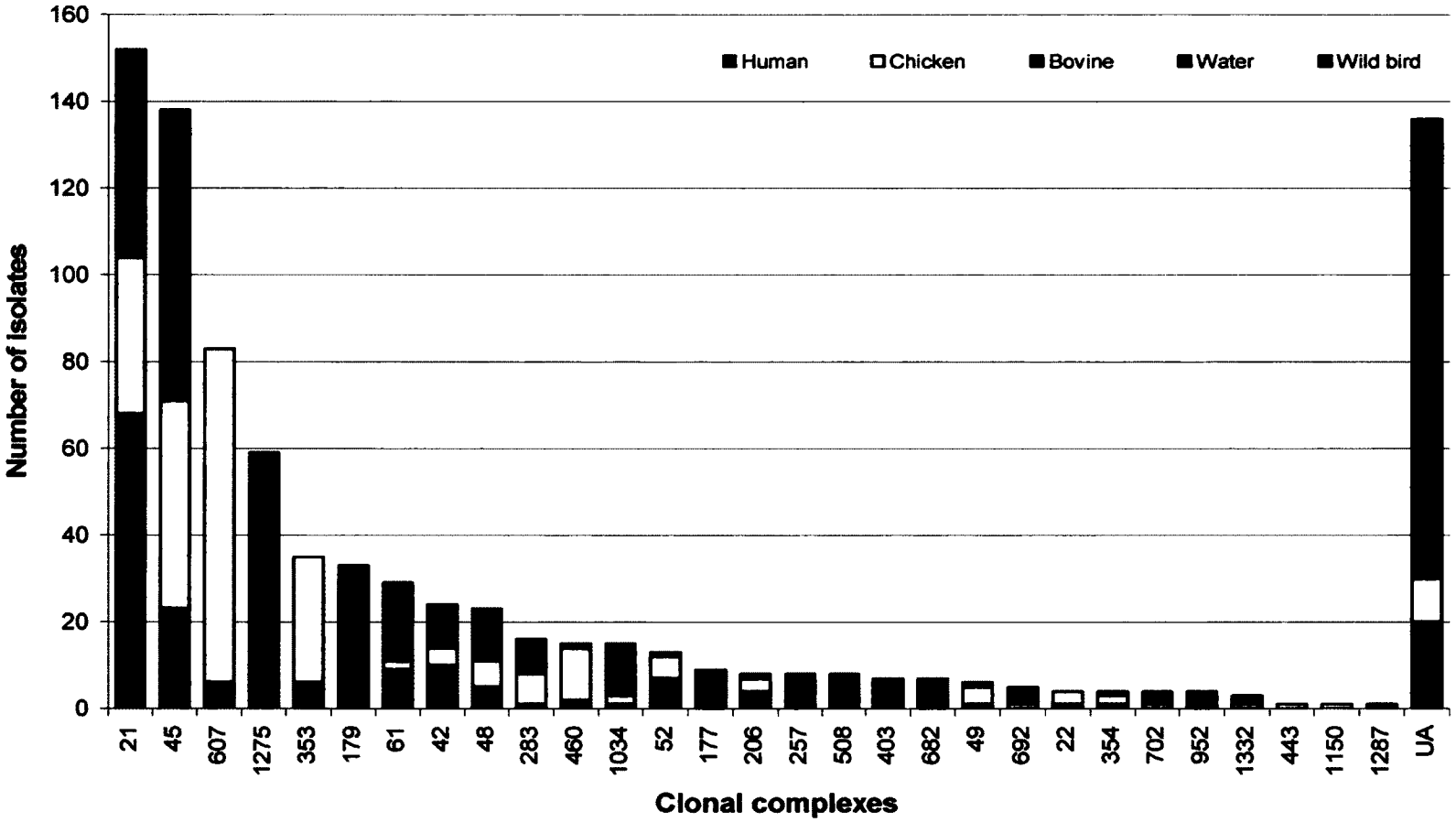


Figure 5. Distribution of sources for *C. jejuni* isolates among clonal complexes. Human isolates are in black, chicken isolates are in yellow, bovine isolates are in red, water isolates are in blue and wild bird isolates are in green. The remaining 108 isolates were distributed among 74 STs which could not be assigned to any of the known lineages (unassigned STs; UA).

Among the 262 STs identified, 160 (61.1%) were previously unreported in the international database, but 108 (67.5%) of them could be assigned to known CCs (Table S2). Forty-three (26.9%) of the new STs resulted from new allele sequences, and the remainder resulted from new combinations of previously described alleles (data not shown). Of note, three water isolates had new allele sequences detected in all seven genes. As mentioned before, one isolate had a deletion at position 461 of the *aspA* allele and was not submitted to the international database (31). Most (87.5%) of the new STs were represented by a single isolate. New STs were found principally among water isolates (93/266 isolates; 34.9%), followed by wild bird (18/63 isolates; 28.6%), human (19/178 isolates; 10.7%), chicken (27/257 isolates; 10.5%), and bovine isolates (3/87 isolates; 3.5%).

Among the human isolates, 113 rural and 65 urban isolates were typed by MLST. The four most frequent CCs identified among human isolates were CC ST-21 (38.2% of the human isolates), CC ST-45 (12.9%), CC ST-42 (5.6%) and CC ST-61 (5.1%). The incidence rates of CC ST-21, CC ST-45 and CC ST-61 were higher among the rural than the urban isolates. However, only CC ST-61 and CC ST-21 for the 15-34 year-old age group had significant differences between both areas (Table 2). When we analysed if a CC was more frequently related to a particular exposure factor, the only two significant associations found were for CC ST-61 which was associated with working in a pet shop, a farm, a zoo or a veterinarian clinic ($p=0.03$) and with consuming raw milk ($p=0.003$).

Table 2. Proportion of CCs from human isolates among age groups and areas.

Clonal complex	Age group	Isolates		Incidence rate per 100,000		Incidence ratio	95% incidence intervals	p value
		Rural area	Urban Area	Rural area	Urban area			
ST-21	0-14	8	6	31.4	24.5	1.3	0.4 - 4.5	-
	15-34	25	12	75.8	28.9	2.6	1.3 - 5.7	0.004
	35-54	7	5	15.3	11.8	1.3	0.4 - 5.2	-
	≥ 55	2	3	4.3	7.7	0.6	0.1 - 4.8	-
	Total	42	26	27.8	17.6	1.6	0.9 - 2.7	-
ST-45	0-14	0	3	0.0	12.3	-	-	-
	15-34	2	0	6.1	0.0	-	-	-
	35-54	5	3	10.9	7.1	1.6	0.3 – 10.0	-
	≥ 55	8	2	17.0	5.1	3.3	0.7 - 32.0	-
	Total	15	8	9.9	5.4	1.8	0.7 – 5.0	-
ST-42	0-14	0	0	0.0	0.0	-	-	-
	15-34	2	1	6.1	2.4	2.5	0.1 - 148.5	-
	35-54	2	0	4.4	0.0	-	-	-
	≥ 55	2	3	4.3	7.7	0.6	0.1 - 4.8	-
	Total	6	4	4.0	2.7	1.5	0.4 - 7.0	-
ST-61	0-14	3	0	11.8	0.0	-	-	-
	15-34	4	0	12.1	0.0	-	-	-
	35-54	0	1	0.0	2.4	-	-	-
	≥ 55	1	0	2.1	0.0	-	-	-
	Total	8	1	5.3	0.7	7.8	1.1 - 346.0	0.02
UA	0-14	3	2	11.8	8.2	1.4	0.2 - 17.3	-
	15-34	4	2	12.1	4.8	2.5	0.4 - 27.8	-
	35-54	2	3	4.4	7.1	0.6	0.1 - 5.4	-
	≥ 55	4	0	8.5	0.0	-	-	-
	Total	13	7	8.6	4.7	1.8	0.7 - 5.4	-

The annual distribution of the CCs in human isolates is shown in Figure S1. While CC ST-21 was present all over the year, most CC ST-45, CC ST-42 and unassigned ST isolates occurred in late spring, summer and early fall. The distribution of CCs among age groups in each area is shown in Table 2. CCs ST-21 and ST-61 were more frequent in the rural 15-34 year old age group, while the presence of CC ST-45 was more frequent in rural 35-54 year old and >55 year old age groups. However, only in the CC ST-21 for rural 15-34 year-old age group was this difference statistically significant.

Source attribution of campylobacteriosis. As shown in Figure 5, some frequent genotypes in non-human isolates were associated with particular sources. For example, CC ST-607 and ST-353 were only associated with chicken, CC ST-1275 with water and wild birds, CC ST-179 mostly with water and CC ST-61 was common in bovine. The same associations were observed at the level of individual loci.

The assignment probability for each putative source was calculated for each human isolate individually (Figure 6) and the percentage of all clinical isolates attributed to each source was calculated as the sum of these probabilities (Figure 7). The human *C. jejuni* isolates were attributed to sources as follows: 64.5% to chicken, 25.8% to bovine, 7.4% to water and 2.3% to wild birds. The source attribution for clinical isolates was also compared between rural and urban areas globally and for age groups. Chicken was the most frequently associated source of human infection, being attributed to 62.3% of cases in rural zone and to 68.3% of cases in urban zone ($p=0.92$), followed by bovine with 28% of cases in rural zone and to 21.9% of cases in urban zone ($p=0.80$). Significant differences in the involvement of sources in rural vs urban area was observed only for water (6.9% vs 8.2%, $p=0.025$). Only 2.7% of cases in the rural zone and 1.5% of cases in the urban zone ($p=0.74$) were associated with

wild birds (Figure 7). Analysis restricted to age groups showed that for 15-34 years old, human cases was more frequently associated with bovine in the rural zone than in the urban zone (19,5% vs 8,2%, p=0.02). Among putative sources, only bovine showed a clear seasonal variation, since the majority of the cases were found during summer and fall, particularly in 2006 (Figure 6). Human cases associated to chicken were equally distributed all over the year.

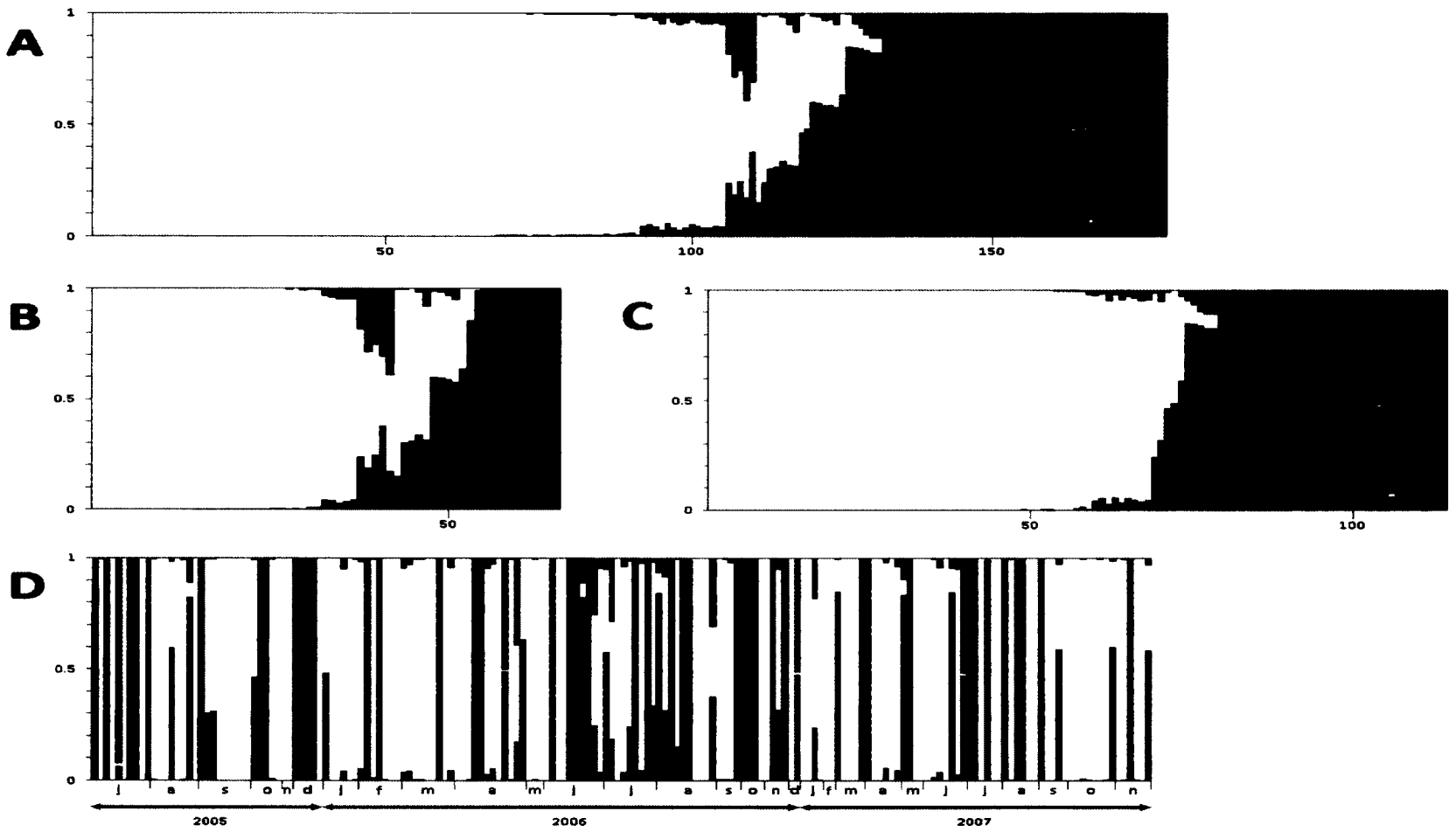
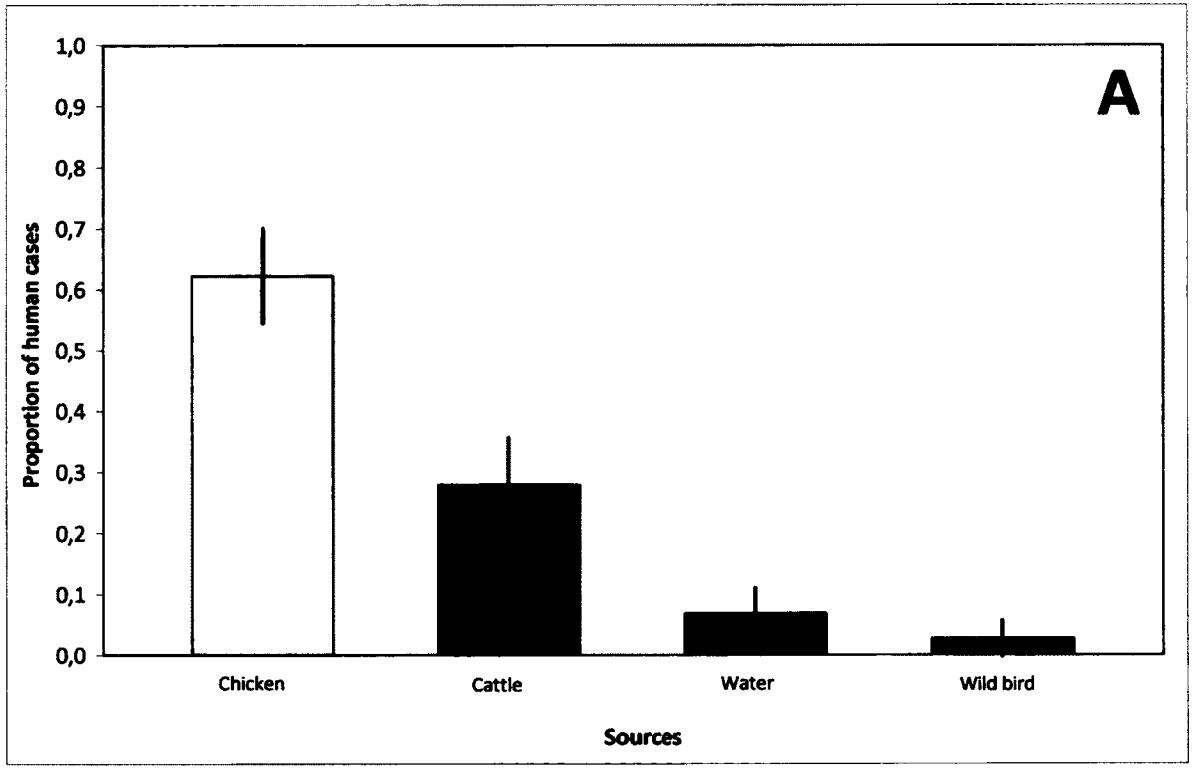
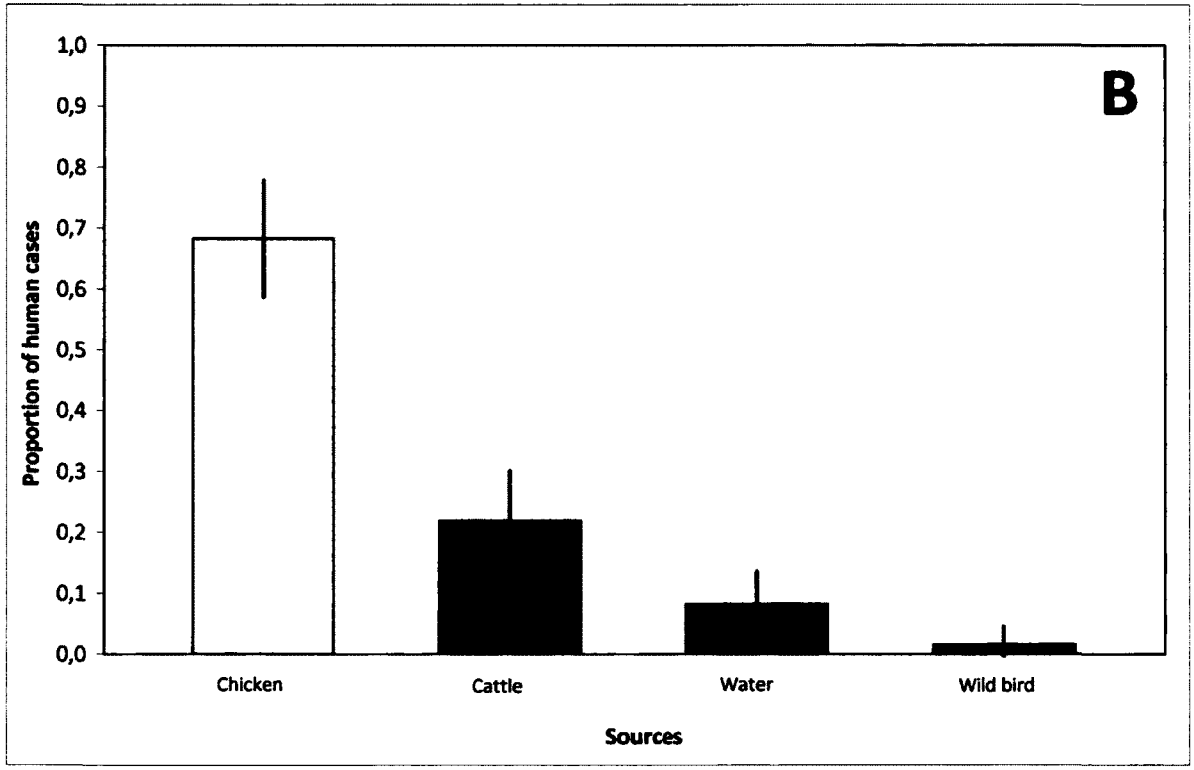


Figure 6. The source probability assignment (y axis) of human campylobacteriosis cases (x axis) using the asymmetric island algorithm. Each isolate is represented by a vertical bar, showing the estimated probability that it comes from each of the putative sources. Sources for *Campylobacter jejuni* were chickens (yellow), cattle (red), water (blue) and wild birds (green). Panel A showed all the 178 human cases typed, panel B cases from urban area and panel C from rural area. Panel D showed all the human cases sorted in the time by month of positive sampling. The first letter of each month is indicated, starting at July, 2005.


A
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
Proportion of human cases
Chicken
Cattle
Water
Wild bird
Sources


B
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
Proportion of human cases
Chicken
Cattle
Water
Wild bird
Sources

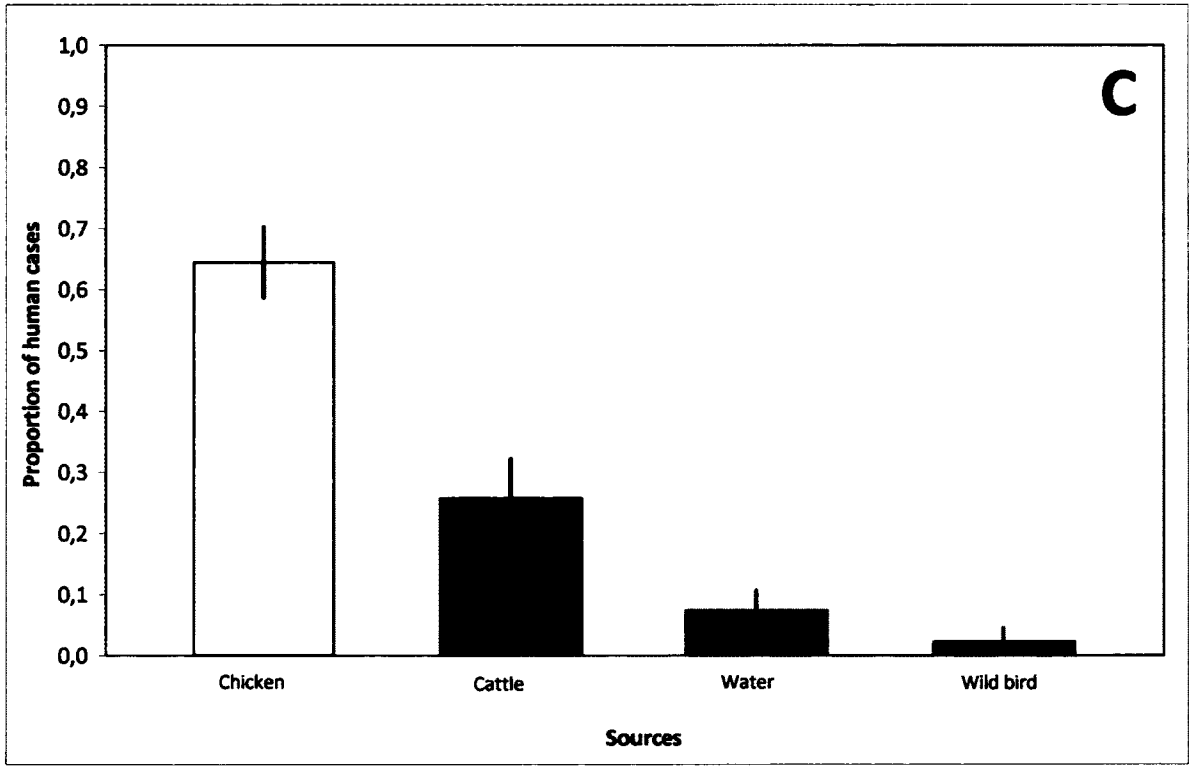


Figure 7. The origin of human campylobacteriosis cases in rural area (a), urban area (b) and in Eastern Townships (c) was determined using the asymmetric island model.

DISCUSSION

Our study confirms that chicken is the most important source for campylobacteriosis, accounting for 65% of cases globally, with no significant difference between urban and rural areas, nor among age groups. From the case-case study data, there were no significant differences for all chicken exposure factors for the population zone, indicating that even if chicken is an important risk factor for campylobacteriosis, it does not explain the higher incidence rates in the rural zone. Association with chicken is the primary risk factor associated with sporadic campylobacter cases in the majority of recent case-control studies (4, 9, 12, 14, 58). Our results are consistent with those observed in United Kingdom (49, 63), in the Netherlands (41) and in New-Zealand (42) with a similar proportion of chicken putative associated source. However, in the Netherlands chicken exposure was highest in cases from urbanized areas and also for young children aged 0–4 years living in urban areas (41).

Significantly higher incidence rates of campylobacteriosis were found in rural area, both globally and at the age group level. We demonstrated that this can be explained by particular contamination sources and risk factors particular to this zone. Bovine was the second most frequent putative source for campylobacteriosis in our study, accounting for 26% of cases. Our data suggests that bovine exposure might explain the excess number of cases in rural compared to urban areas. Only one clonal complex, CC ST-61, was found significantly more frequently among human isolates from rural than urban areas. In previous studies, only CC ST-45 had been shown to be associated with increased rural incidence rate (53). CC ST-61 is also the only one clonal complex associated with two farm risk factors in the rural zone. This observation is consistent with the fact that CC ST-61 is found principally among

bovine isolates and that the majority of farming in the Eastern Townships is dairy or beef farming [80% of animal husbandry are bovine and only 0.8% are chicken in the Eastern Townships(39)]. This finding confirms that bovine is an important reservoir and has an underestimated potential to infect humans.

The seasonal presence of CC ST-45 and ST-42 indicated that human contamination source from these two CCs mainly occurred during late spring, summer and early fall. Those periods of time coincided with the highest water contamination period in the Eastern Townships and with manure spread (56) and we shown that those CCs are mainly associated with water and bovine, respectively. The fact that human isolates related to bovine had a clear summer and fall seasonality distribution also suggest that bovine is responsible for the excess number of cases in the rural zone. The seasonality of incidence for the two years is similar to that previously described (27, 35, 44, 53) with a sharp increase from May until October.

During the highest incidence period of the year, incidence was higher in rural zones than in urban zones. This difference was seen for most age groups, but the difference was only significant for 15 to 34-year-olds. As suggested by others, these observations may indicate increased exposure of the more rural population to sources of *Campylobacter* and true differences in distribution season between areas, perhaps indicating distinct transmission routes (53).

Professional exposure was already known as an important campylobacteriosis risk factor for dairy farm and poultry abattoir workers (7, 18). This risk factor is one of the two which remained significant by multivariate analysis. Out of the 18 cases that had professional exposure to animals, 16 worked in bovine farms, one in a poultry farm and one in a veterinary clinic and 13 belonged to the 15 to 34-year-old age group. This professional exposure correlated with the finding of assignment sources using the

molecular data (mainly CC ST-21 and ST-61). This finding emphasised the hypothesis of Mullner et al. that bovine-derived cases are commonly assumed to be the result of direct environmental and occupational, rather than food-borne exposures in contrast with poultry sources where the dominant transmission pathways are believed to be the handling and consumption of under-cooked meat (42).

Another significant risk factor, consumption of raw milk, has already been associated with campylobacteriosis (24, 35, 45). Since the Eastern Townships agricultural zone is mainly composed of dairy farms, raw milk is often consumed by the farmer's family and neighbours. An interesting fact is that when the 0-4 year old age group was removed from the analysis, this risk factor was no longer significant. Thus raw milk consumption could be a major factor for explaining the high campylobacteriosis prevalence in this age group and could explain why others have found an association between high campylobacter incidence in 0-4 age group in agricultural settings (16, 19, 25). However, contact with farm animals has been recently identified as a significant risk factor in this age group and could remain an important source of contamination in rural area (12). Both professional exposure and raw milk consumption risk factors are reinforced with the fact that CC ST-61, a well known bovine associated CC, is significantly associated with those risk factors.

Another important risk factor for campylobacteriosis in rural areas is the use of wells, which remained significant by multivariate analysis. Not having disinfected the well within 6 months was also a rural associated risk factor. It is well known that wells may be contaminated with pathogenic bacteria and water quality is not often monitored (61). Especially in rural area, fecal contamination of the water source has been observed to occur by several different mechanisms, including runoff of surface water from farms after rain, especially after flood conditions and the distribution of

manure sludge to land (1, 5, 22, 26, 40, 59). Surface water is more prone to contamination from livestock and other sources than ground water, but since ground water is often consumed without any treatment, even a small amount of contamination can give rise to infections in humans.

One of our findings in this study is that human Campylobacter isolates predominantly represent particular genetic lineages (genotypes) that are a limited subset of the genotypes found among all animal and environmental sources. Some non human isolates were never found among human clonal complexes. One hypothesis is that these isolates are well adapted to their ecological niche and may not be able to infect humans. It has been shown that isolates obtained from particularly niches were unable to colonize chickens (20, 23, 62). Hepworth et al. (23) recently showed that some regions specifically absent from the genome of particular strains may play an important role in the process of *Campylobacter* infection of humans. They suggested that the genomic divergence observed constitutes evidence of adaptation leading to niche specialization. Recently, Gripp et al. (20) suggested that some of the rarer sequence types, such as isolates from wild animals which rarely mix with other niches, have irreversibly adapted to a single host species.

In this study, the most frequent CCs and STs were the same as those frequently identified in other studies (8, 10, 11, 28, 34, 37, 48, 53). In addition to the frequently observed strains, a wide genetic variability was observed among isolates in this study since 78% of STs were associated with only one or two isolates. This fact is due in majority to water isolates which represent more than the half (110/204) of those isolates. Water isolates were also associated with the majority of the STs that were not previously reported. As shown in our previous study (28) and from other parts of the world (6, 54) a wide range of ST have been identified among water isolates and the

majority could not be assigned to a known CC. From isolates associated with a CC, the three major CCs were CC ST-45, CC ST-1275 and CC ST-179. ST-45, the founder sequence type of CC ST-45, was the second most common ST identified in our study (the first among water isolates) and also from river water isolates in northwest England (54). It was also an abundant type isolated in water isolates from New Zealand (6). Among the river water CCs, only CC ST-45 also contained numerous human isolates. However, it was not the principal CC found among human isolates in the Eastern Townships, since CC ST-21 was the CC most commonly associated with human infections. CC ST-21 was underrepresented among water isolates, mitigating the hypothesis that sewage could be an important source of *Campylobacter* in river water (6). Since bovine is the most important ecological niche containing CC ST-21 after human in the Eastern Townships, it is surprising not to find a higher proportion of this type among water isolates; because of the high agricultural pressure (principally cattle and dairy farms) present in the catchment area of sampling sites.

The hypothesis that ST-45 is an environmentally well-adapted type which can survive under stress better than other STs (54) is supported by our findings. A recent study also found that isolates from CC ST-21 and ST-45 had different survival patterns after being submitted to various stresses (21). CC ST-45 was found to be the second most common CC in the wild bird population in our study and the first in another study (17). The two other major CCs identified among water isolates were CC ST-1275 and CC ST-179. These CCs are mainly associated with wild birds in our study and others (6, 54), but only a few were reported in human infections.

This study has some limitation in addition to the declaration bias mentioned above. First, some significant Campylobacter sources may have been missed because of the small number of campylobacteriosis cases studied. Also, a memory bias could

be involved in the answers obtained since the cases were being asked to recall potential exposures that occurred often more than one or two weeks prior to the interview. Finally, the fact that we used isolates from only four potential sources could have masked other potential associated sources for human isolates, such as sheep or pig which are commonly sampled in other studies. However, pigs are known to be mainly the reservoir of *C. coli* and sheep farming is not frequent in the Eastern Townships.

We showed with this study an urban-rural gradient of campylobacteriosis. The excess number of cases in rural area seems to be caused by bovine exposition, particularly in the 15-34 years old group, probably due to professional exposition and water consumption from a well. Bovine exposure is probably the source of campylobacteriosis seasonality. Chicken consumption is the major risk factor and our model associates 65% of cases to chicken, independently of zone, sex and age. The case-case approach combined with molecular source attribution analysis was a unique approach to determine the differences in campylobacteriosis incidence of two different population zones. This study design attempted to validate with molecular data the finding of the case-case report and the hypothesis raised by many other studies. With these results, we believed that public education program should be addressed according to the main risk factors shown in this study and effective, targeted preventive measures should be developed.

.

ACKNOWLEDGMENTS

This study was funded by the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada and the Centre de Recherche Clinique Étienne Le-Bel du Centre Hospitalier Universitaire de Sherbrooke. The Campylobacter Multilocus Sequence Typing website (http://campylobacter.mlst.net) used in the analysis was developed by Man-Suen Chan and sited at the University of Oxford. We thank Roberto Toffoli from Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec for providing contact with the farmers who collaborated in our study. We thank Patrice Charbonneau from Sherbrooke City for giving us access to the waste management sites. We thank Philippe Bélanger from Ministère de la Santé et des Services Sociaux du Québec for providing Campylobacteriosis data for Québec province. We thank the other members of the CampyloGIS project (Goze Bénié, Ferdinand Bonn, Olivier Thomas, Sylvain Quessy, François Milord, Suzanne Ménard, Jacques Désilets, Michel Pomerleau, André Lavoie, Rémi Desbiens, Djoan Bonfils, Alexandre Chammat and Karen St-Pierre). We finally thank all the trainees who work on this project.

REFERENCES

1. 2000. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May-June 2000. Can Commun Dis Rep **26**:170-3.
2. **Altekruse, S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow.** 1999. Campylobacter jejuni--an emerging foodborne pathogen. Emerg Infect Dis **5**:28-35.
3. **Arsenault, J., A. Ravel, P. Michel, O. Berke, and P. Gosselin.** 2011. Do patients with recurrent episodes of campylobacteriosis differ from those with a single disease event? BMC Public Health **11**:32.
4. **Buettner, S., B. Wieland, K. D. Staerk, and G. Regula.** 2010. Risk attribution of Campylobacter infection by age group using exposure modelling. Epidemiol Infect **138**:1748-61.
5. **Carter, A. M., R. E. Pacha, G. W. Clark, and E. A. Williams.** 1987. Seasonal occurrence of Campylobacter spp. in surface waters and their correlation with standard indicator bacteria. Appl Environ Microbiol **53**:523-6.
6. **Carter, P. E., S. M. McTavish, H. J. Brooks, D. Campbell, J. M. Collins-Emerson, A. C. Midwinter, and N. P. French.** 2009. Novel clonal complexes with an unknown animal reservoir dominate Campylobacter jejuni isolates from river water in New Zealand. Appl Environ Microbiol **75**:6038-46.
7. **Cawthraw, S. A., L. Lind, B. Kaijser, and D. G. Newell.** 2000. Antibodies, directed towards Campylobacter jejuni antigens, in sera from poultry abattoir workers. Clin Exp Immunol **122**:55-60.
8. **Colles, F. M., K. Jones, R. M. Harding, and M. C. Maiden.** 2003. Genetic diversity of Campylobacter jejuni isolates from farm animals and the farm environment. Appl Environ Microbiol **69**:7409-13.
9. **Danis, K., M. Di Renzi, W. O'Neill, B. Smyth, P. McKeown, B. Foley, V. Tohani, and M. Devine.** 2009. Risk factors for sporadic Campylobacter infection: an all-Ireland case-control study. Euro Surveill **14**.
10. **de Haan, C. P., R. Kivisto, M. Hakkinen, H. Rautelin, and M. L. Hanninen.** 2010. Decreasing trend of overlapping multilocus sequence types between human and chicken Campylobacter jejuni isolates over a decade in Finland. Appl Environ Microbiol **76**:5228-36.
11. **Dingle, K. E., F. M. Colles, D. R. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J. Bootsma, R. J. Willems, R. Urwin, and M. C. Maiden.** 2001. Multilocus sequence typing system for Campylobacter jejuni. J Clin Microbiol **39**:14-23.
12. **Doorduyn, Y., W. E. Van Den Brandhof, Y. T. Van Duynhoven, B. J. Breukink, J. A. Wagenaar, and W. Van Pelt.** 2010. Risk factors for indigenous Campylobacter jejuni and Campylobacter coli infections in The Netherlands: a case-control study. Epidemiol Infect **138**:1391-404.
13. **Ethelberg, S., J. Simonsen, P. Gerner-Smidt, K. E. Olsen, and K. Molbak.** 2005. Spatial distribution and registry-based case-control analysis of Campylobacter infections in Denmark, 1991-2001. Am J Epidemiol **162**:1008-15.
14. **Fajo-Pascual, M., P. Godoy, M. Ferrero-Cancer, and K. Wymore.** 2010. Case-control study of risk factors for sporadic Campylobacter infections in northeastern Spain. Eur J Public Health **20**:443-8.

15. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol **186:**1518-30.
16. **Fitzenberger, J., H. Uphoff, S. Gawrich, and A. M. Hauri.** 2010. Urban-rural differences of age- and species-specific campylobacteriosis incidence, Hesse, Germany, July 2005 - June 2006. Euro Surveill **15.**
17. **French, N. P., A. Midwinter, B. Holland, J. Collins-Emerson, R. Pattison, F. Colles, and P. Carter.** 2009. Molecular epidemiology of Campylobacter jejuni isolates from wild-bird fecal material in children's playgrounds. Appl Environ Microbiol **75:**779-83.
18. **Gilpin, B. J., P. Scholes, B. Robson, and M. G. Savill.** 2008. The transmission of thermotolerant Campylobacter spp. to people living or working on dairy farms in New Zealand. Zoonoses Public Health **55:**352-60.
19. **Green, C. G., D. O. Krause, and J. L. Wylie.** 2006. Spatial analysis of campylobacter infection in the Canadian province of Manitoba. Int J Health Geogr **5:**2.
20. **Gripp, E., D. Hlahla, X. Didelot, F. Kops, S. Maurischat, K. Tedin, T. Alter, L. Ellerbroek, K. Schreiber, D. Schomburg, T. Janssen, P. Bartholomaus, D. Hofreuter, S. Woltemate, M. Uhr, B. Brenneke, P. Gruning, G. Gerlach, L. Wieler, S. Suerbaum, and C. Josenhans.** 2011. Closely related Campylobacter jejuni strains from different sources reveal a generalist rather than a specialist lifestyle. BMC Genomics **12:**584.
21. **Habib, I., M. Uyttendaele, and L. De Zutter.** 2010. Survival of poultry-derived Campylobacter jejuni of multilocus sequence type clonal complexes 21 and 45 under freeze, chill, oxidative, acid and heat stresses. Food Microbiol **27:**829-34.
22. **Hanninen, M. L., H. Haajanen, T. Pummi, K. Wermundsen, M. L. Katila, H. Sarkkinen, I. Miettinen, and H. Rautelin.** 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:**1391-6.
23. **Hepworth, P. J., K. E. Ashelford, J. Hinds, K. A. Gould, A. A. Witney, N. J. Williams, H. Leatherbarrow, N. P. French, R. J. Birtles, C. Mendonca, N. Dorrell, B. W. Wren, P. Wigley, N. Hall, and C. Winstanley.** 2011. Genomic variations define divergence of water/wildlife-associated Campylobacter jejuni niche specialists from common clonal complexes. Environ Microbiol **2011.**
24. **Heuvelink, A. E., C. van Heerwaarden, A. Zwartkruis-Nahuis, J. J. Tilburg, M. H. Bos, F. G. Heilmann, A. Hofhuis, T. Hoekstra, and E. de Boer.** 2009. Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk. Int J Food Microbiol **134:**70-4.
25. **Kabore, H., P. Levallois, P. Michel, P. Payment, P. Dery, and S. Gingras.** 2010. Association between potential zoonotic enteric infections in children and environmental risk factors in Quebec, 1999-2006. Zoonoses Public Health **57:**e195-205.
26. **Koenraad, P. M. F. J., F. M. Rombouts, and S. H. W. Notermans.** 1997. Epidemiological aspects of thermophilic Campylobacter in water-related environments: A review. Water environment research **69:**52-63.

27. **Kovats, R. S., S. J. Edwards, D. Charron, J. Cowden, R. M. D'Souza, K. L. Ebi, C. Gauci, P. Gerner-Smidt, S. Hajat, S. Hales, G. Hernandez Pezzi, B. Kriz, K. Kutsar, P. McKeown, K. Mellou, B. Menne, S. O'Brien, W. van Pelt, and H. Schmid.** 2005. Climate variability and campylobacter infection: an international study. Int J Biometeorol **49**:207-14.

28. **Levesque, S., E. Frost, R. D. Arbeit, and S. Michaud.** 2008. Multilocus sequence typing of Campylobacter jejuni isolates from humans, chickens, raw milk, and environmental water in Quebec, Canada. J Clin Microbiol **46**:3404-11.

29. **Levesque, S., S. Michaud, R. D. Arbeit, and E. H. Frost.** High-Resolution Melting System to Perform Multilocus Sequence Typing of Campylobacter jejuni. PLoS One **6**:e16167.

30. **Levesque, S., K. St-Pierre, E. Frost, R. D. Arbeit, and S. Michaud.** 2011. Determination of the optimal culture conditions for detecting thermophilic campylobacters in environmental water. J Microbiol Methods.

31. **Levesque, S., K. St-Pierre, E. Frost, R. D. Arbeit, and S. Michaud.** 2012. Use of Amplified-Fragment Length Polymorphism to Study the Ecology of Campylobacter Jejuni in Environmental Water and to Predict Multi-Locus Sequence Typing Clonal Complexes. Appl Environ Microbiol.

32. **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**:6370-9.

33. **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**:267-72.

34. **McTavish, S. M., C. E. Pope, C. Nicol, D. Campbell, N. French, and P. E. Carter.** 2009. Multilocus sequence typing of Campylobacter jejuni, and the correlation between clonal complex and pulsed-field gel electrophoresis macrorestriction profile. FEMS Microbiol Lett **298**:149-56.

35. **Michaud, S., S. Menard, and R. D. Arbeit.** 2004. Campylobacteriosis, Eastern Townships, Quebec. Emerg Infect Dis **10**:1844-7.

36. **Michaud, S., S. Menard, C. Gaudreau, and R. D. Arbeit.** 2001. Comparison of SmaI-defined genotypes of Campylobacter jejuni examined by KpnI: a population-based study. J Med Microbiol **50**:1075-81.

37. **Mickan, L., R. Doyle, M. Valcanis, K. E. Dingle, L. Unicomb, and J. Lanser.** 2007. Multilocus sequence typing of Campylobacter jejuni isolates from New South Wales, Australia. J Appl Microbiol **102**:144-52.

38. **Miller, W. G., S. L. On, G. Wang, S. Fontanoz, A. J. Lastovica, and R. E. Mandrell.** 2005. Extended multilocus sequence typing system for Campylobacter coli, C. lari, C. upsaliensis, and C. helveticus. J Clin Microbiol **43**:2315-29.

39. **Ministère de l'Agriculture, d. P. e. d. l. A., Direction régionale de l'Estrie.** 2010. Agriculture et agroalimentaire en Estrie. Gouvernement du Québec. Avalable at: http://www.mapaq.gouv.qc.ca/SiteCollectionDocuments/Regions/Estrie/ProfilRegion/Estrie_profil.pdf.

40. **Moreno, Y., S. Botella, J. L. Alonso, M. A. Ferrus, M. Hernandez, and J. Hernandez.** 2003. Specific detection of Arcobacter and Campylobacter strains in water and sewage by PCR and fluorescent in situ hybridization. Appl Environ Microbiol **69**:1181-6.

41. **Mughini Gras, L., J. H. Smid, J. A. Wagenaar, A. G. de Boer, A. H. Havelaar, I. H. Friesema, N. P. French, L. Busani, and W. van Pelt.** 2012. Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis. PLoS One 7:e42599.

42. **Mullner, P., S. E. Spencer, D. J. Wilson, G. Jones, A. D. Noble, A. C. Midwinter, J. M. Collins-Emerson, P. Carter, S. Hathaway, and N. P. French.** 2009. Assigning the source of human campylobacteriosis in New Zealand: a comparative genetic and epidemiological approach. Infect Genet Evol **9**:1311-9.

43. **Nachamkin, I.** 2003. *Campylobacter* and *Arcobacter*, p. 902-914. *In* R. H. Yolken (ed.), Manual of Clinical Microbiology, 8th ed. American Society for Microbiology, Washington, D.C.

44. **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**:383-90.

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

46. **Popovic-Uroic, T.** 1989. Campylobacter jejuni and Campylobacter coli diarrhoea in rural and urban populations in Yugoslavia. Epidemiol Infect **102**:59-67.

47. **Pritchard, J. K., M. Stephens, and P. Donnelly.** 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-59.

48. **Sails, A. D., B. Swaminathan, and P. I. Fields.** 2003. Clonal complexes of Campylobacter jejuni identified by multilocus sequence typing correlate with strain associations identified by multilocus enzyme electrophoresis. J Clin Microbiol **41**:4058-67.

49. **Sheppard, S. K., J. F. Dallas, N. J. Strachan, M. MacRae, N. D. McCarthy, D. J. Wilson, F. J. Gormley, D. Falush, I. D. Ogden, M. C. Maiden, and K. J. Forbes.** 2009. Campylobacter genotyping to determine the source of human infection. Clin Infect Dis **48**:1072-8.

50. **Silva, J., D. Leite, M. Fernandes, C. Mena, P. A. Gibbs, and P. Teixeira.** 2011. Campylobacter spp. as a Foodborne Pathogen: A Review. Front Microbiol **2**:200.

51. **Skirrow, M. B.** 1987. A demographic survey of campylobacter, salmonella and shigella infections in England. A Public Health Laboratory Service Survey. Epidemiol Infect **99**:647-57.

52. **Slater, E. R., and R. J. Owen.** 1997. Restriction fragment length polymorphism analysis shows that the hippuricase gene of Campylobacter jejuni is highly conserved. Lett Appl Microbiol **25**:274-8.

53. **Sopwith, W., A. Birtles, M. Matthews, A. Fox, S. Gee, M. Painter, M. Regan, Q. Syed, and E. Bolton.** 2006. Campylobacter jejuni multilocus sequence types in humans, northwest England, 2003-2004. Emerg Infect Dis **12**:1500-7.

54. **Sopwith, W., A. Birtles, M. Matthews, A. Fox, S. Gee, M. Painter, M. Regan, Q. Syed, and E. Bolton.** 2008. Identification of potential environmentally adapted Campylobacter jejuni strain, United Kingdom. Emerg Infect Dis **14**:1769-73.

55. **Spratt, B. G., W. P. Hanage, B. Li, D. M. Aanensen, and E. J. Feil.** 2004. Displaying the relatedness among isolates of bacterial species -- the eBURST approach. FEMS Microbiol Lett **241:**129-34.

56. **St-Pierre, K., S. Levesque, E. Frost, N. Carrier, R. D. Arbeit, and S. Michaud.** 2009. Thermotolerant coliforms are not a good surrogate for Campylobacter spp. in environmental water. Appl Environ Microbiol **75:**6736-44.

57. **Studahl, A., and Y. Andersson.** 2000. Risk factors for indigenous campylobacter infection: a Swedish case-control study. Epidemiol Infect **125:**269-75.

58. **Tam, C. C., C. D. Higgins, K. R. Neal, L. C. Rodrigues, S. E. Millership, and S. J. O'Brien.** 2009. Chicken consumption and use of acid-suppressing medications as risk factors for Campylobacter enteritis, England. Emerg Infect Dis **15:**1402-8.

59. **Thomas, C., H. Gibson, D. J. Hill, and M. Mabey.** 1998. Campylobacter epidemiology: an aquatic perspective. J Appl Microbiol **85 Suppl 1:**168S-177S.

60. **Thompson, J. S., F. E. Cahoon, and D. S. Hodge.** 1986. Rate of Campylobacter spp. isolation in three regions of Ontario, Canada, from 1978 to 1985. J Clin Microbiol **24:**876-8.

61. **Weir, E.** 2005. Well-water maintenance. Cmaj **172:**1438.

62. **Williams, N. J., T. R. Jones, H. J. Leatherbarrow, R. J. Birtles, A. Lahuerta-Marin, M. Bennett, and C. Winstanley.** 2010. Isolation of a novel Campylobacter jejuni clone associated with the bank vole, Myodes glareolus. Appl Environ Microbiol **76:**7318-21.

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

SUPPLEMENTAL MATERIAL

MATERIAL AND METHODS

River water analysis. We identified 32 sampling sites on 13 rivers and 12 streams across the 7 counties of the Eastern Townships, Quebec, Canada and sampled weekly from July 17, 2005 to November 25, 2007. For each sampling site, ~ 3000 ml of water were collected weekly from the nearshore areas or from a bridge passing over the site, using a horizontal alpha water sampler (Geneq, Montreal, Canada) at a depth about 15 to 30 cm below the surface. The sample was transported on ice to the laboratory, held at 4°C and tested within 24 h. Water was filtered through a 0.45 µm pore-size membrane filter and Preston selective enrichment broth (Oxoid, Nepean, Ontario, Canada) and Karmali agar (Oxoid) were used to isolate *Campylobacter* (1, 3).

Retailed whole fresh chicken analysis. The chickens were stored at 4°C overnight and washed vigorously with 250 mL of nutrient broth. The broth was filtered through cheesecloth and centrifuged at 16,300 x g for 15 min. The sediment was suspended in 5 mL of Brucella broth (Oxoid); 100 mL of Preston broth were added to the suspension, gently mixed, and incubated under microaerobic atmosphere at 37°C for 4 h, then at 42°C for 48 h. Next, 200 µl of the suspension were plated on Karmali agar and incubated at 42°C for 48 h under microaerobic conditions.

Bovine feces analysis. Feces samples were transported in Enteric Plus medium (Meridian Bioscience Inc, Ohio, USA) and processed on the same day. About 1-2 g of each feces sample were transferred to 25 ml of Preston broth and incubated 3-4 h at 37°C and then transferred to 42°C to complete 48 h of incubation. After incubation of this

enrichment broth, 200 µl were streaked on a Karmali plate and incubated at 42°C for 48 h.

Wild bird feces analysis. Feces samples were transported in Enteric Plus medium and processed on the same day. About 1-2 g of each feces sample were transferred to 25 ml of Preston broth and incubated 3-4 h at 37°C and transferred to 42°C until 48 h of incubation. Cloacae swabs were directly inoculated in Preston broth and processed in the same way as fecal samples. After incubation of the enrichment broth, 200 µl were streaked on a Karmali plate and incubated at 42°C for 48 h.

Suspected *Campylobacter* isolates. After incubation, each Karmali plate was visually screened for suspected *Campylobacter* isolates. From each plate, one suspected colony was fully identified to species. In the case of morphologically different colonies on the same plate, more that one colony could be identified to species. Species identification was made as described previously (2, 3).

RESULTS

Regional incidence rates. The rates varied from 49.2/100,000 in Granit to 211.1/100,000 in Coaticook; these interregional differences were observed for both years of the study (Table 1). The relative risk (RR) represents the incidence rate of campylobacteriosis in one county compared to the incidence rate in the other counties taken as a whole. The risk of campylobacteriosis was 2.93-fold higher in Coaticook ($p<0.0001$) and 1.62-fold higher in Haut St-François ($p=0.02$) than elsewhere in the Eastern Townships.

Clinical presentation of cases. Diarrhoea was a presenting complaint in 100% of cases, with a median duration of 7 days (range: 1-180 days). Other presenting findings included abdominal pain (89%), fever (75%), nausea (47%), bloody stools (40%) and vomiting (28%). There was appreciable morbidity; 35% of the cases were hospitalised (median duration: 2 day; range: 1-11 days), and 53% of cases missed work or school (median duration: 4 days; range 1-21 days). One case was hospitalised due to a *C. fetus* bacteremia with septic thrombophlebitis of both legs. There were no deaths.

The median interval from the onset of symptoms until stool culture was 4 days (range: 0-167 days; 90^{th}%ile, 9 days), with 5 additional days (range: 1-29 days; 90^{th}%ile, 9 days) before notification of the Public Health Department, and 5 more days (range: 0-29 days; 90^{th}%ile, 14 days) until the epidemiological investigation. Thus, the median total interval from the onset of symptoms to the interview of the cases was 15 days (range: 6-200 days; 90^{th}%ile, 26 days). The majority of intervals over 26 days was explained by the delay between the onset of symptoms and the stool culture.

According to answers to the whole questionnaire, the interviewers tried to determine the probable source of infection. The principal risk factors were chicken (17%), contaminated water (19%), an animal contact (15%), raw milk (16%), other food (10%), an infectious contact (4%), and other sources (2%). In 17%, interviewers could not identify a putative source of infection.

References for supplemental material

1. **Levesque, S., K. St-Pierre, E. Frost, R. D. Arbeit, and S. Michaud.** 2011. Determination of the optimal culture conditions for detecting thermophilic campylobacters in environmental water. J Microbiol Methods **86:**82-8.
2. **Levesque, S., K. St-Pierre, E. Frost, R. D. Arbeit, and S. Michaud.** 2012. Use of Amplified-Fragment Length Polymorphism to Study the Ecology of Campylobacter Jejuni in Environmental Water and to Predict Multi-Locus Sequence Typing Clonal Complexes. Appl Environ Microbiol.
3. **St-Pierre, K., S. Levesque, E. Frost, N. Carrier, R. D. Arbeit, and S. Michaud.** 2009. Thermotolerant coliforms are not a good surrogate for Campylobacter spp. in environmental water. Appl Environ Microbiol **75:**6736-44.

Table S1. Incidence rates of campylobacteriosis in each county and for each municipality category in the Eastern Townships [a].

	Total population	2005-2006		2006-2007		All the study period			
		Number of cases	Incidence rate per 100,000	Number of cases	Incidence rate per 100,000	Number of cases	Incidence rate per 100,000	RR	p value
Eastern Townships	298685	117	39.2	78	26.1	241	80.7	—	—
County									
Asbestos	14485	8	55.2	4	27.6	15	103.6	1.30	NS
Val St-François	29005	12	41.4	9	31.0	30	103.4	1.32	NS
Sherbrooke	147430	40	27.1	29	19.7	82	55.6	0.99	NS
Coaticook	18475	15	81.2	16	86.6	39	211.1	2.93	< 0.0001
Memphrémagog	45330	18	39.7	13	28.7	37	81.6	1.01	NS
Haut St-François	21615	18	83.3	4	18.5	27	124.9	1.62	0.0175
Granit	22345	6	26.9	3	13.4	11	49.2	0.59	NS
Zone									
Rural and Semi-rural	151255	77	50.9	49	32.4	159	105.1	1.89	< 0.0001
Urban	147430	40	27.1	29	19.7	82	55.6	0.53	

[a] Incidence rates were calculated with the cases in the final data set (n = 241).
[b] The study period is from 07-2010 to 12-2007.

Table S2. Distribution of 851 *C. jejuni* isolates among clonal complexes, sequence types (ST) and isolation sources. New STs identified in this study and in our previous one (2) are in boldface and new CCs are italicized.

		Source of isolation					
CC	**ST**	**Human**	**Chicken**	**Bovine**	**Water**	**Wild bird**	**Total**
ST-21	8	6	6			1	13
	19	2				1	3
	21	31	10	19	5	1	66
	50	4	17	1			22
	86		1				1
	169	1					1
	806	5		9			14
	982	10		1			11
	1209	2		4	2		8
	2038	1					1
	2862	2	1				3
	3857			1			1
	3875	1					1
	3877	1					1
	4018	1					1
	4026			1			1
	4110		1				1
	4377	1					1
	4379			1			1
	NEW				1		1
Total ST-21		**68**	**36**	**37**	**8**	**3**	**152**
ST-22	22	1	3				4
Total ST-22		**1**	**3**				**4**
ST-42	42	5	1	5	1	1	13
	459	4	2	1			7
	2371			1			1
	3880	1					1
	4109		1				1
	4206				1		1
Total ST-42		**10**	**4**	**7**	**2**	**1**	**24**
ST-45	45	18	38	1	36	3	96
	137	3	3		5		11
	168				1		1
	241		1		3	1	5
	408					1	1
	538	1					1
	583					1	1
	679	1					1
	766			1			1
	1228		1				1
	1363					1	1
	2197				4		4
	2219				3		3
	3730		1				1
	3883		1				1

	3887		1				1
	4024		1				1
	4025		1				1
	4094				1		1
	4114				1		1
	4135				1		1
	4136				1		1
	4191				1		1
	4381				1		1
Total ST-45		**23**	**48**	**2**	**58**	**7**	**138**
	38	3		9			12
ST-48	48	1	6				7
	918	1		3			4
Total ST-48		**5**	**6**	**12**			**23**
	3			1			1
ST-49	**2868**		3				3
	4020	1					1
	4181		1				1
Total ST-49		**1**	**4**	**1**			**6**
	52	7	3				10
ST-52	1812		2				2
	4127				1		1
Total ST-52		**7**	**5**		**1**		**13**
	61	6	2	13	1		22
	432				1		1
	955	1					1
ST-61	1244	1					1
	4017	1					1
	4120				2		2
	4348				1		1
Total ST-61		**9**	**2**	**13**	**5**		**29**
	177				3		3
	563				1		1
ST-177	2539				1		1
	4141				2		2
	4212					1	1
	4213					1	1
Total ST-177					**7**	**2**	**9**
	1207	1			2		3
	3889	2		1	19		22
	4027				1		1
ST-179	**4031**				1		1
	4121				1		1
	4186				2		2
	4189				2		2
	4380				1		1
Total ST-179		**3**		**1**	**29**		**33**
ST-206	222	3	1	1			5
	4090	1	2				3
Total ST-206		**4**	**3**	**1**			**8**

ST-257	929	3	1	2		6
	4022	1		1		2
Total ST-257		**4**	**1**	**3**		**8**
ST-283	267	1	1		8	10
	3884		1			1
	4180		4			4
	4347		1			1
Total ST-283		**1**	**7**		**8**	**16**
ST-353	353	1	6			7
	939		3			3
	1210	1	11			12
	1218	1				1
	1898	1				1
	3879	1				1
	3888		1			1
	4019	1				1
	4023		1			1
	4092		1			1
	4182		5			5
	4184		1			1
Total ST-353		**6**	**29**			**35**
ST-354	354				1	1
	3874	1				1
	4091		2			2
Total ST-354		**1**	**2**		**1**	**4**
ST-403	933	3		4		7
Total ST-403		**3**		**4**		**7**
ST-443	443		1			1
Total ST-443			**1**			**1**
ST-460	460	1	2			3
	535		7			7
	3881		2			2
	3882		1			1
	4099	1				1
	4112				1	1
Total ST-460		**2**	**12**		**1**	**15**
ST-508	132			4		4
	508	1				1
	4111			1		1
	4183		1			1
	4194				1	1
Total ST-508		**1**	**1**	**5**	**1**	**8**
ST-607	607	1	2			3
	924		4			4
	1212	4	70			74
	4178	1				1
	4179		1			1
Total ST-607		**6**	**77**			**83**
ST-682	682				5	5
	1027				1	1

	4203			1		1
Total ST-682				**7**		**7**
	699			3		3
ST-692	**4188**			1		1
	4378		1			1
Total ST-692			**1**	**4**		**5**
	702		1			1
ST-702	**4132**			1		1
	4214				2	2
Total ST-702			**1**	**1**	**2**	**4**
	4028			1		1
ST-952	**4190**			1		1
	4202			1		1
	4208			1		1
Total ST-952				**4**		**4**
	694			1		1
	1709		1			1
	1956		1			1
	3891				1	1
	4071	1		1	1	3
	4079				1	1
ST-1034	**4106**				1	1
	4113			1		1
	4124			1		1
	4129			1		1
	4133			1		1
	4193			1		1
	4383			1		1
Total ST-1034		**1**	**2**	**8**	**4**	**15**
ST-1150	**4360**		1			1
Total ST-1150			**1**			**1**
	1224			6		6
	4029			1		1
	4074			1		1
	4076			1		1
ST-1224	**4104**			1		1
	4105			1		1
	4130			3		3
	4134			1		1
	4197			1		1
	4352			1		1
Total *ST-1224*				**17**		**17**
	637			16	14	30
	1223				2	2
	1225			1	2	3
ST-1275	1268			2		2
	1275				1	1
	1283			1		1
	1292			4		4
	4021	1				1

	4034				1	1
	4096			1		1
	4101			1		1
	4119			1		1
	4177	1				1
	4200			1		1
	4205			1		1
	4210				5	5
	4215				1	1
	4279			1		1
	4362				1	1
Total ST-1275		**2**		**30**	**27**	**59**
ST-1287	**4032**			1		1
Total ST-1287				**1**		**1**
	1276				1	1
ST-1332	**3886**		1			1
	4078				1	1
Total ST-1332			**1**		**2**	**3**
	1226			3		3
	1230			2		2
ST-4102	**2866**	1	1	2		4
	4102			1		1
	4118			1		1
Total *ST-4102*		**1**	**1**	**9**		**11**
	448			1		1
	464	1				1
	468			1	2	3
	922	14	2	1		17
	995		1			1
	996			1		1
	997			1		1
	1030	1				1
	1289			1	2	3
	1351			1		1
	1479			3		3
	1748	1				1
Unassigned	1911		1			1
	1938				1	1
	1959			1		1
	1961			2		2
	1972		2			2
	2090			1		1
	2349			1		1
	2514			2		2
	2875	1			3	4
	3644		1			1
	3885		1			1
	3890			1		1
	4030			1		1
	4033			2		2

4069	1					1
4073				1		1
4075				1		1
4077					1	1
4080					1	1
4093				5		5
4095				1		1
4097				1		1
4098				1		1
4100				1		1
4103				1		1
4115				1		1
4116				1		1
4117				1		1
4122				1		1
4123				1		1
4125				1		1
4126				1		1
4128				1		1
4131				1		1
4137				1		1
4138				1		1
4139				1		1
4140				1		1
4185		1				1
4187				1		1
4192				1		1
4195				1		1
4196				1		1
4198				1		1
4199				1		1
4201				1		1
4204				2		2
4207				1		1
4209				1		1
4211					1	1
4216					1	1
4217					1	1
4349				1		1
4350				1		1
4351				1		1
4353					1	1
4361				1		1
4364				1		1
4365				1		1
4366				1		1
4367				1		1
4382					1	1
Total unassigned	**19**	**9**	**1**	**64**	**15**	**108**
Total	**178**	**257**	**87**	**266**	**63**	**851**

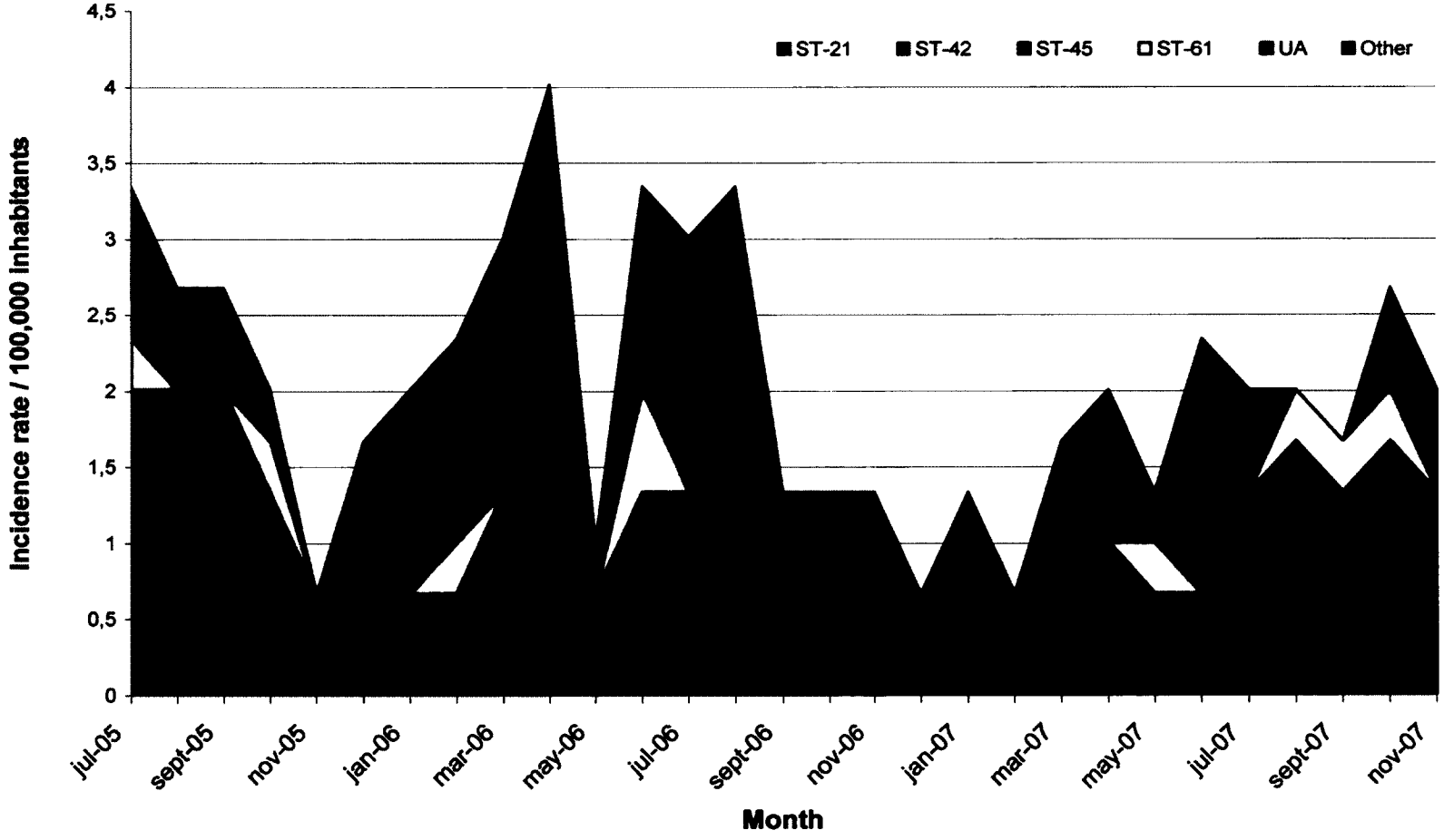


Figure S1. Monthly distribution of clonal complexes among human isolates typed by MLST.

DISCUSSION

7.1 Distribution de la résistance aux antibiotiques des isolats de *C. jejuni* obtenus des différentes niches écologiques

La détermination du profil de sensibilité aux antibiotiques des microorganismes est une méthode phénotypique de base pour la caractérisation des bactéries. Quoique grossière, cette méthode permet de suivre le transfert de pathogènes d'un hôte à l'autre. En effet, en fonction de la pression sélective qu'exerce un antibiotique administré à un hôte sur sa population bactérienne, la résistance développée par la population bactérienne de cet hôte peut servir de marqueur. Cette technique phénotypique était largement utilisée dans les années 80 et au début des années 90. Parfois en combinaison avec des techniques moléculaires, cette technique ciblait particulièrement l'investigation d'éclosions suite à l'émergence de la résistance chez plusieurs espèces bactériennes. Ce choix était également influencé par le fait que les différentes techniques de détermination du profil de sensibilité aux antibiotiques sont facilement réalisables et utilisées de routine dans tous les laboratoires hospitaliers (Blanc *et al.*, 1994, Flournoy, 1982, Hartmann et West, 1997). Encore aujourd'hui, de vastes programmes de surveillance sont basés sur les tendances de résistance aux antibiotiques de différentes espèces bactériennes, par exemple le Programme intégré canadien de surveillance de la résistance aux antimicrobiens (PICRA).

Par ailleurs, la détermination de la sensibilité aux antibiotiques des bactéries est principalement effectuée pour choisir le traitement approprié lors d'une infection chez un patient. Le suivi des tendances de résistance des microorganismes est donc primordial pour la validation du traitement empirique des infections bactériennes. Le premier objectif de mon projet de recherche était d'étudier les niveaux et la distribution de la résistance aux antibiotiques des isolats de *C. jejuni* obtenus des différentes niches écologiques, afin de déterminer les niveaux de résistance des souches de l'Estrie et d'établir la provenance des isolats humains résistants. Comme le Campylobacter peut se transmettre de l'animal à l'humain via un contact direct, la nourriture ou l'eau contaminée, l'émergence de souches multirésistantes aux fluoroquinolones et aux macrolides chez les animaux, soulève des inquiétudes face au traitement futur des infections chez l'humain.

Nous avons comparé les résultats de sensibilité à trois classes d'antibiotiques utilisés pour le traitement des campylobactérioses chez des souches isolées chez l'humain où l'infection était acquise localement versus à l'étranger. Nous avons démontré que, pour la ciprofloxacine, 50% des souches acquises à l'étranger étaient résistantes, comparativement à 5,9% pour les souches acquises localement. Ceci est donc un élément important à prendre en considération lors du traitement empirique des voyageurs souffrant d'une campylobactériose. De 2001 à 2003, le niveau de résistance aux antibiotiques est demeuré stable parmi les souches d'origine humaine. Cependant, parmi les souches isolées chez le poulet, le taux de résistance à l'érythromycine a plus que doublé et a augmenté de 38,3% pour la tétracycline. Ces augmentations sont probablement le reflet de l'usage vétérinaire de ces substances. Par contre, le taux de résistance des souches de poulet à la ciprofloxacine est très faible avec seulement 1,8%

des souches. Ceci est probablement le reflet de l'effet du retrait de l'utilisation des fluoroquinolones en milieu vétérinaire au Canada en 1997 (Engberg, 2001). Les données du PICRA de 2009 montrent une diminution de la résistance aux antimicrobiens depuis 2004 chez les souches de Campylobacter isolées de poulet au Québec ainsi qu'en Ontario (Gouvernement du Canada, 2010). Cependant, la tendance inverse est observée pour la Colombie-Britannique et la Saskatchewan. Il sera intéressant de comparer l'utilisation des antibiotiques pour l'usage vétérinaire dans ces différentes provinces lors de l'analyse finale du PICRA. Comme notre étude est la plus récente sur le profil de résistance des souches humaines de Campylobacter au Québec, nous ne pouvons pas vérifier si cette diminution de la résistance chez les souches de poulet s'est reflétée parmi les souches humaines.

La résistance aux quinolones parmi les souches humaines de Campylobacter a été observée pour la première fois au début des années 1990 en Asie et dans certains pays européens. L'apparition de la résistance chez ces souches coïncide avec le début de l'utilisation des fluoroquinolones et de l'enrofloxacine dans la production animale dans ces pays (Allos, 2001, Endtz, 1991).

Plusieurs pays ont vérifié l'effet d'une interdiction de l'usage d'antibiotiques en agriculture sur les taux de résistance des souches de Campylobacter. Aux États-Unis, les fluoroquinolones ont été bannies dans l'élevage du poulet en 2005. Deux groupes ont analysé des souches isolées dans les fermes de producteurs de poulet une année avant et une année après l'interdiction et aucune baisse significative du taux de résistance des souches n'a été observée (Nannapaneti *et al.*, 2009, Price *et al.*, 2007). Ils ont cependant rapporté que des souches isolées de producteurs n'ayant jamais fait l'usage de

fluoroquinolones présentaient un taux de résistance significativement plus faible que les souches obtenues chez les producteurs conventionnels. Les auteurs ont donc conclu que l'arrêt seul de l'utilisation des antibiotiques n'était pas une mesure suffisante pour observer une diminution des taux de résistance des souches de Campylobacter. Cependant, trois limites importantes soulèvent un questionnement sur ces deux études. Premièrement, très peu de producteurs de poulet ont été inclus dans l'étude, ce qui limite l'extrapolation des données sur la situation globale de la résistance aux États-Unis. Deuxièmement, les souches analysées ont été isolées seulement une année après l'interdiction ; l'effet à long terme n'a donc pas pu être mesuré. Troisièmement, aucune information n'est donnée par rapport au *fitness cost* de la bactérie en fonction de la persistance du mécanisme de résistance. Ce dernier aspect serait particulièrement intéressant à analyser pour expliquer le maintien de la résistance aux quinolones chez le Campylobacter en l'absence de pression sélective par des antibiotiques.

Contrairement aux États-Unis, la France a pour sa part observé une diminution du taux de résistance aux quinolones de 2002 à 2004 chez les souches isolées de poulet et chez l'humain, suite à la restriction de l'utilisation de ces molécules dans l'élevage animal en 1999 par l'Union Européenne (Gallay *et al.*, 2007). Les auteurs suggèrent que la diminution du taux observée 2 à 3 ans après l'instauration de la mesure restrictive pourrait être attribuable à sa lente installation, ou encore que son effet sur les taux de résistance ne soit pas immédiat. Au Danemark, entre 1997 et 2007 aucune baisse significative du taux de résistance aux quinolones ne fut observée chez les souches de poulet (Skjot-Rasmussen *et al.*, 2009). Parallèlement, la résistance des souches chez l'humain a continué d'augmenter. Les auteurs ont conclu que l'acquisition de souches résistantes chez l'humain était possiblement due à l'importation de viande de poulet

d'autres pays (où les taux de résistance sont plus élevés) et aux voyages à l'étranger. L'Australie pour sa part, où les fluoroquinolones n'ont jamais été utilisées dans l'élevage animal, ne présente aucune résistance chez les souches humaines acquises localement analysées jusqu'en 2002 (Unicomb *et al.*, 2003).

Le haut taux de résistance à l'érythromycine chez les souches isolées de poulet est préoccupant et est sans doute le reflet de l'utilisation des macrolides dans cette industrie (Health Canada, 2002). Cependant, le faible taux de résistance à l'érythromycine des souches humaines donne une indication quand à la provenance de ces souches. En effet, selon ces résultats, le poulet serait peut-être moins important comme source de campylobactérioses chez l'humain que mentionné dans la littérature, et l'apport des autres sources potentielles de contamination, tels le bovin et l'eau où la résistance à l'érythromycine est absente, serait potentiellement sous-évalué. Cependant, des études moléculaires plus poussées sont nécessaires pour la confirmation de ces observations, ainsi que l'évaluation du *fitness cost* de cette résistance chez la bactérie.

Une étude intéressante sur la connaissance et l'attitude des producteurs de poulet du Canada en matière de sécurité des aliments et des bonnes pratiques de production a révélé que seulement 21,2% des répondants avaient indiqué que le Campylobacter pouvait être transmis de la viande de poulet contaminée aux humains, et que seulement 26,6% croyaient que l'usage des antibiotiques dans cette industrie était relié à la résistance des souches chez l'humain (Younq *et al.*, 2010). Ceci démontre que des programmes d'éducation des producteurs doivent cibler davantage la connaissance des implications des antibiotiques dans l'élevage.

Certains auteurs ont suggéré que la résistance des souches humaines proviendrait de l'utilisation des ces antibiotiques pour le traitement des infections chez l'homme. Bien que pour l'érythromycine, le développement de la résistance en cours de traitement ait été rapporté peu fréquemment (Funke *et al.,* 1994), l'apparition de la résistance aux fluoroquinolones en cours de traitement est beaucoup plus fréquente (Engberg *et al.,* 2001, Nachamkin *et al.,* 2000). Cependant, comme la transmission interhumaine du Campylobacter n'est rapportée que de façon anecdotique, le potentiel de transmission de souches résistantes est peu probable (Engberg *et al.,* 2004).

Puisque notre étude date d'environ 10 ans, il serait pertinent d'effectuer un état de la situation de la résistance aux antibiotiques des souches humaines au Québec. Ceci permettrait d'en suivre l'évolution pour la détermination des traitements empiriques, et d'effectuer la corrélation avec les taux actuels de résistance des souches en milieu agricole et l'utilisation des antibiotiques. Si l'évolution de la résistance des souches humaines est à la hausse, il serait temps pour une réflexion sur l'utilisation des antibiotiques comme promoteurs de croissance dans l'industrie agricole et ses implications, non seulement en médecine humaine, mais aussi en médecine vétérinaire. Également, comme les pratiques de prescription des antibiotiques sont différentes en milieu agricole qu'en milieu vétérinaire chez les animaux de compagnie, il serait intéressant d'en étudier l'impact chez les souches de ces animaux et aussi le potentiel de transmission de ces souches aux humains.

7.2 Épidémiologie moléculaire des souches de Campylobacter

Pour la deuxième partie de mon projet de recherche, nous avons voulu comparer trois méthodes de typage moléculaire pour le Campylobacter, la première basée sur des profils de restriction et les deux autres sur le séquençage de gènes afin de déterminer l'utilité de ces méthodes pour étudier l'épidémiologie du Campylobacter et leur applicabilité aux populations bactériennes du Québec. Nous avons également vérifié si les résultats des différentes méthodes de typage moléculaire pour le Campylobacter étaient comparables et établi la concordance des résultats entre les trois méthodes. Finalement, la distribution des profils de résistance a également été comparée aux résultats de typage moléculaire afin de vérifier si la résistance aux antibiotiques est attribuable à une niche écologique en particulier, ou encore à des génotypes bien précis.

Nous avons analysé par les méthodes de typage du gène *fla* et par MLST des isolats de *C. jejuni* isolées d'humain, de poulet, de lait cru et d'eau, entre 1998 et 2003. Parmi cette collection, nous avons également analysé un sous-groupe d'isolats par EGCP. Finalement, nous avons regardé la distribution de la résistance aux antibiotiques parmi les regroupements générés par le MLST.

Plusieurs méthodes de typage ont été décrites pour l'analyse des souches de Campylobacter, mais aucune ne s'est encore imposée comme étant la plus efficace. Plusieurs facteurs entrent en jeu lors du choix d'une méthode de typage moléculaire. La question de recherche initiale, le type de microorganisme, le nombre d'isolats à analyser, le coût de la méthode, sa rapidité et sa complexité, la reproductibilité de la méthode et la capacité de comparaison avec les autres études ne sont que quelques

exemples de facteurs à prendre en considération. Pour l'instant, aucune méthode de typage ne remplit tous les critères. Il est donc primordial d'évaluer différentes méthodes avant de choisir celle qui sera la plus appropriée pour notre question de recherche. En analysant trois différentes méthodes de typage sur un grand nombre d'isolats de Campylobacter de sources différentes, nous avons démontré qu'il n'existe pas de relations simples et linéaires entre ces trois méthodes. En d'autres termes, les génotypes regroupés par une méthode ne correspondent pas à ceux regroupés par une autre méthode. Il est effectivement très rare que les résultats de deux méthodes de typage moléculaire soient concordants, ou encore que les résultats d'une méthode prédisent ceux d'une autre, probablement dû au fait que chaque méthode de typage cible un aspect ou une section différente du génome bactérien. Un des rares exemples où une méthode prédit les résultats d'une autre est pour le *Staphylococcus aureus* résistant à la méthicilline où le typage du gène *spa* permet de prédire adéquatement le type épidémique établit par EGCP (Golding *et al.*, 2008).

L'EGCP est une méthode de typage basée sur l'analyse du génome complet de la bactérie. C'est encore aujourd'hui la méthode de référence pour le typage de plusieurs microorganismes, principalement en contexte d'éclosion. C'est une méthode qui est très discriminante, tel que confirmé par nos résultats. Puisque notre devis de recherche était longitudinal et que nous voulons évaluer la distribution génétique des isolats de Campylobacter à travers le temps et les espèces, nous avons démontré que l'EGCP était peu utile pour ce genre d'étude. Ceci s'explique par le fait que l'EGCP ne détecte pas seulement les changements nucléotidiques qui affectent les sites de restrictions, mais principalement les changements au niveau du chromosome (insertions, délétions et réarrangements chromosomiques) (Clinical and laboratory standards institute, 2007).

Comme nous, ainsi que d'autres auteurs, l'avons démontré, le Campylobacter est un microorganisme peu clonal (où ce type de changement est fréquent), il est donc normal que l'EGCP ne soit pas adéquat pour ce genre de devis de recherche. L'EGCP a un *clock speed* plus élevé que les autres méthodes, c'est-à-dire que l'EGCP est apte à détecter les changements chromosomiques qui surviennent plus rapidement et a donc un pouvoir de discrimination plus grand.

Le typage du gène *fla* est une méthode qui est basée sur le séquençage d'un gène du flagelle du Campylobacter. Parce que la région séquencée est la région hypervariable, cette méthode offre un bon taux de discrimination (Meinersmann *et al.*, 1997). Cependant, ce gène peut être soumis à de multiples variations dues au fait que les flagelles bactériens sont immunogènes et donc soumis à une grande pression sélective (Gilbreath *et al.*, 2011). Même si cette méthode est rapide, simple et peu coûteuse, son utilisation doit se faire principalement dans un contexte d'éclosion.

Le MLST s'est imposé comme étant la méthode de référence pour l'étude de l'évolution des populations bactériennes. Grâce à nos données, nous avons confirmé que le MLST est la seule méthode capable d'attribuer des souches à des niches écologiques particulières, tels le poulet, le lait cru et l'eau. Outrepassant son utilité que pour un contexte d'éclosion (Clark *et al.*, 2005), cette méthode a donc le potentiel d'attribuer une source à un isolat humain et par le fait même, d'établir les routes de transmission du Campylobacter entre les différents hôtes. Étant basée sur l'analyse de gènes métaboliques, non soumis à une pression sélective accrue, l'évolution de ces gènes est plus lente, ce qui permet le suivi des populations bactériennes sur de longues périodes et à travers différents hôtes. Par cette méthode, nous avons démontré que certains

génotypes de souches humaines se retrouvaient dans les complexes clonaux regroupant exclusivement ou à forte prédominance des souches de poulet, confirmant ainsi que le poulet est une source importante de contamination par le Campylobacter chez l'humain. De plus, l'eau et le lait cru forment des complexes clonaux exclusifs où des souches humaines se retrouvent également, suggérant que ces deux sources peuvent aussi contaminer l'humain et être une source significative d'infections sporadiques. Nous avons donc démontré que le MLST est la méthode de choix pour l'association des souches humaines à une source de contamination particulière et qu'elle est applicable pour l'analyse de la structure des populations de Campylobacter du Québec. Nous avons également identifié des isolats qui sont dans des CCs exclusivement composés d'isolats en provenance de l'eau. Cette observation appuie l'hypothèse que certaines souches n'auraient pas la capacité d'infecter les humains. Cependant, des études de génomique comparative, couplées à des études *in vivo* à l'aide de modèles d'infection chez l'animal sont nécessaires pour la démonstration de cette hypothèse.

Finalement, nous avons vérifié si la distribution de la résistance aux antibiotiques était associée à des génotypes en particulier où davantage à une niche écologique. Nous avons démontré que la résistance était distribuée de façon aléatoire parmi les différents complexes clonaux et semblait être associée davantage à la source de l'isolat qu'au complexe clonal. La même observation fut faite par d'autres groupes ayant étudié des isolats humains et de poulets en Afrique et en Suisse (Kinana *et al.*, 2006, Kittl *et al.*, 2011). Kinana a suggéré que l'émergence de la résistance aux quinolones n'était pas reliée à la diffusion d'un clone unique, mais bien au résultat de la pression sélective des quinolones sur les différents clones d'une même niche écologique, dans leur cas le poulet (Kinana *et al.*, 2006).

7.3 Développement d'un système plus performant pour effectuer le MLST.

Bien que nous ayons montré que le MLST soit la méthode de choix pour répondre à nos questions de recherche, cette méthode demeure très coûteuse (environ 100$ par isolats) et fastidieuse (amplification de 7 gènes et 14 réactions de séquençage par isolat à analyser). Nous avons donc voulu développer un système plus rapide et moins coûteux pour nous permettre d'identifier les différents allèles composant le ST d'un isolat. Nous avons utilisé la technologie du *high-resolution melting* (HRM) afin de développer un tel système.

Le HRM est une méthode simple, s'appuyant sur une PCR, pour la détection des variations dans des séquences d'ADN en mesurant la température de fusion de brins d'ADN (Taylor, 2009). La température de fusion (T_m) d'une molécule d'ADN en double brin à deux molécules simple brin est influencée par plusieurs facteurs, tels que la longueur des fragments, la teneur en bases GC et même la séquence des fragments (Ririe *et al.*, 1997). Une molécule d'ADN est dénaturée progressivement, d'abord les régions riches en AT, puis finalement les régions riches en GC. Chaque séquence d'ADN double brin possède son propre profil de dénaturation/renaturation et c'est ce que le HRM exploite. L'utilisation d'un fluorophore qui lie à saturation l'ADN double brin, combiné à un changement de température lent et précis (de l'ordre de 0,02°C par seconde) et à de nombreuses acquisitions du signal de fluorescence, permet une détection très précise du profil de dénaturation d'une séquence d'ADN. Après l'amplification par PCR du fragment à analyser, la lecture de la perte de fluorescence est effectuée sans qu'aucun réactif ne soit ajouté. Principalement utilisé pour détecter des mutations génétiques chez les eucaryotes, où des hétéroduplexes peuvent être

formés à partir d'une population de fragments homoduplexes sauvages et mutants (Taylor, 2009), le HRM peut également s'appliquer à la comparaison de fragments d'ADN de procaryotes. Peu d'études utilisant cette technologie ont été réalisées chez les procaryotes et nous avons été les premiers à l'utiliser pour l'épidémiologie moléculaire.

Comme le MLST est basé sur la détection de mutations ponctuelles (SNP) dans des fragments d'ADN de longueur fixe, la technologie HRM était applicable pour la détermination des allèles des gènes composant les STs des souches de Campylobacter. Nous avons développé un système complémentaire à la caractérisation classique par séquençage des 7 gènes qui compose le système MLST du Campylobacter en identifiant les allèles les plus fréquents, à un coût plus faible et plus rapidement. Nous avons démontré que le HRM pouvait détecter tous les types de SNPs, peu importe le nucléotide qui diffère d'une séquence à l'autre. Une différence mesurable dans le T_m des fragments était observable avec aussi peu qu'un seul nucléotide de différence et peu importe la position de ce nucléotide dans le fragment d'ADN.

Ce système comporte plusieurs avantages, soit la rapidité de détermination des allèles du MLST, la diminution considérable des coûts d'analyse par souche (de l'ordre de 70 à 80 % par rapport à la méthode traditionnelle par séquençage) et la diminution des risques de contamination due au fait que toute la réaction (étape d'amplification et de HRM) se fait dans un même tube fermé. Comme l'analyse des fragments par HRM nécessite la comparaison avec des fragments de référence (des fragments dont la séquence est déjà connue), un fragment inconnu lors d'une réaction précédente peut par la suite devenir un fragment de référence une fois sa séquence déterminée. Ce système est particulièrement performant lorsque la diversité allélique est faible, comme dans les

niches écologiques très clonales, tels que le poulet ou le bovin par exemple. Une connaissance approfondie des populations génétiques qui composent une niche écologique permet une sélection judicieuse des fragments de référence. Par l'utilisation du HRM pour le MLST, nous avons démontré que cet outil moléculaire pourrait être utilisable pour d'autres applications impliquant de l'ADN bactérien dans le futur, telles que la détection de mutations ponctuelles qui confèrent la résistance aux antibiotiques.

7.4 Analyse du Campylobacter dans l'eau.

Le Campylobacter ne se multiplie pas dans l'eau. Sa présence est donc due à une contamination de l'eau par la bactérie. Comme le Campylobacter colonise une très grande variété d'animaux et d'oiseaux, les sources de contamination de l'eau environnementale peuvent être nombreuses. Lors d'une étude pilote sur le Campylobacter dans l'eau en Estrie, nous avions constaté que le taux de positivité des échantillons pour la présence de Campylobacter était élevé et que les génotypes étaient assez variés (Lévesque *et al.*, 2005, Lévesque *et al.*, 2008). Parallèlement à une étude cas-cas en Estrie sur les cas de campylobactériose (le sujet du dernier chapitre de ma thèse), une étude de prévalence fut conduite de 2005 à 2007 dans le but d'obtenir des isolats de Campylobacter de différentes niches écologiques (poulet, bovin, oiseaux sauvages et eau) afin de comparer les génotypes de ces isolats à ceux obtenus d'infection chez l'humain. Le second objectif était de vérifier, en fonction de la variabilité génétique des souches obtenues, si la contamination de l'eau par du Campylobacter en Estrie était causée par une source principale, ou par de multiples sources. Près de 1000 isolats de *C. jejuni* furent obtenus de l'eau durant cette étude de prévalence (St-Pierre *et al.*, 2009). Considérant la grande quantité d'isolats à génotyper,

nous ne pouvions pas tous les analyser par MSLT. Comme nous avions démontré précédemment que les isolats de Campylobacter dans l'eau étaient très diversifiés génétiquement (Lévesque *et al.*, 2008), l'utilisation de notre système HRM n'aurait pas été optimale pour le typage de cette grande quantité d'isolats. Nous avons décidé d'utiliser l'AFLP comme méthode de typage des souches d'eau. Cette méthode possède plusieurs avantages : elle est rapide, peu coûteuse, permet d'analyser un grand nombre d'isolats et a un très bon pouvoir de discrimination, comparable à l'EGCP et au MLST selon certaines études (Duim *et al.*, 2003, Lindstedt *et al.*, 2000). Nous avons émis l'hypothèse que nous pourrions déterminer à l'aide du AFLP, un sous-groupe d'isolats à typer par MLST qui serait représentatif des génotypes en circulation dans l'eau. Cette hypothèse était basée sur deux études qui avaient démontré une bonne corrélation entre les résultats de AFLP et de MLST (Duim *et al.*, 2003, Shouls *et al.*, 2003).

Grâce à l'AFLP, nous avons pu déterminer que la diversité génétique les isolats de *C. jejuni* dans l'eau était élevée. Cette haute variabilité génétique peut être le résultat de plusieurs sources différentes de contamination de l'eau, ou encore de la contamination par peu de sources différentes, mais avec des populations faiblement clonales de Campylobacter. Une étude effectuée en France en 2006 a démontré également une très grande diversité génétique parmi les souches obtenues de l'eau environnementale (Denis *et al.*, 2009). Tout comme nous, les auteurs ont isolé des souches de Campylobacter sur toute l'année, quoique les variations de température en France soient moins importantes qu'au Québec. Les auteurs ont émis l'hypothèse que la variabilité génétique des souches observée tout au long de l'année pourrait être liée à la présence ponctuelle d'animaux et d'activités agricoles autour des rivières. La même observation fut effectuée en Angleterre à l'été 2000 où les auteurs ont observé une plus

grande diversité génétique des souches de *C. jejuni* que *C. coli*. Ils ont attribué ce phénomène au fait que plusieurs espèces d'animaux d'élevage et d'animaux sauvages soient porteuses de *C. jejuni*, contrairement à *C. coli* où sa distribution est limitée (KEMP *et al.*, 2005).

À partir des résultats d'AFLP, nous avons choisi un sous-groupe de 266 isolats pour analyse par MLST. Le choix des isolats s'est fait sur la base de la diversité génétique (parmi les agrégats déterminés par AFLP, ainsi que des génotypes uniques), de la distribution parmi les sites d'échantillonnage et de la distribution dans le temps. Contrairement à ce que les études précédentes avaient démontré, la corrélation fut très faible entre les génotypes déterminés par AFLP et ceux par MLST, tant au niveau des ST que des CC. Seulement quatre études avant la nôtre avaient comparé les résultats de typage obtenus par AFLP et MLST (trois sur *C. jejuni* et une sur *C. fetus*) (Duim *et al.*, 2003, Islam *et al.*, 2009, Shouls *el al.*, 2003, van Bergen *et al.*, 2005). Chacune de ces études avait montré une bonne corrélation entre les résultats d'AFLP et de MLST. Pour le *C. fetus*, il a été démontré que les souches de cette espèce ont un faible niveau de diversité génétique et qu'elles sont génétiquement homogènes, comparativement aux autres espèces de Campylobacter (van Bergen *et al.*, 2005). Ceci peut expliquer en partie la bonne corrélation des génotypes par AFLP et MLST pour le *C. fetus*. Les études sur *C. jejuni* ont été effectuées sur de petits nombres de souches et majoritairement sur des isolats humains, qui classiquement ont moins de diversité génétique que les souches provenant de l'environnement (Carter *et al.*, 2009). Comme l'AFLP et le MLST ne ciblent pas les mêmes déterminants génétiques, il est normal d'obtenir une faible concordance des génotypes parmi un groupe d'isolats très diversifié génétiquement. Nous avons donc démontré une fois de plus que le MLST est la

méthode de choix pour suivre l'évolution génétique des populations peu clonales telles que le Campylobacter.

7.5 Les sources de la campylobactériose.

La majorité des cas de campylobactériose sont des infections sporadiques dont la source est rarement identifiée. Les principales sources de contamination sont connues, mais l'importance de chacune ainsi que la cause de la variation des taux d'incidence selon l'âge et le niveau de ruralité de résidence des cas sont encore hypothétiques. Plusieurs groupes de recherche ont étudié les sources des infections sporadiques, principalement par des études cas-témoins, ou encore en étudiant rétrospectivement la distribution des cas. Bien que plusieurs facteurs de risque de campylobactériose aient été identifiés, aucune de ces études n'a pu déterminer la proportion de risque attribuable à chaque source de contamination potentielle. La majorité des devis d'étude utilisés par le passé servaient à générer des hypothèses sur les modes de transmission du Campylobacter. Nous avons utilisé le devis d'étude cas-cas, pour lequel la réduction des biais de sélection potentiels fut démontré, puisque les cas et les témoins (aussi des cas) sont sélectionnés à partir du même système de surveillance (Krumkamp *et al.*, 2008). Notre approche unique fut de combiner les données épidémiologiques aux données moléculaires déterminées par MLST, afin de renforcer les résultats obtenus par l'analyse des données cliniques.

Le poulet est reconnu comme la source principale de campylobactériose à travers le monde, principalement par la consommation de viande mal cuite ou encore via la contamination croisée. Cependant, le pourcentage de cas attribuable au poulet

varie selon les études, en fonction de l'épidémiologie locale et de la localisation géographique de l'étude. Les autres réservoirs du Campylobacter (animaux d'élevage, animaux de compagnie, animaux sauvages) peuvent aussi être des sources de contamination via l'alimentation, l'exposition par contact direct ou encore via l'ingestion d'eau contaminée. L'apport en nombre de campylobactériose de ces sources de contamination dites secondaires varie également d'une étude à l'autre. Ces sources ont été majoritairement suspectées comme facteur de risque. Des variations du taux d'incidence de campylobactériose ont été observées, ainsi qu'un gradient de cas entre les régions urbaines et rurales dans plusieurs études (Popovic-Uroic, 1989, Skirrow, 1987, Sopwith *et al.*, 2006, Studahl et Andersson, 1986, Thompson *et al.*, 1986), dont une en Estrie (Michaud *et al.*, 2004). Un gradient urbain-rural a également été observé au niveau de différents groupes d'âge, entre autres au Manitoba. Dans cette province, le taux d'incidence de campylobactériose était sept fois plus élevé dans les régions rurales par rapport à la région métropolitaine de Winnipeg (Green *et al.*, 2006).

Notre hypothèse de départ était que le taux de campylobactériose attribuable au poulet était le même en milieu urbain qu'en milieu rural, et que d'autres sources de contamination pourraient expliquer le gradient urbain-rural. En Estrie, une variation importante du taux d'incidence à travers les différentes MRC rurales versus la région de Sherbrooke (zone urbaine) avait été observée par le passé (Michaud *et al.*, 2004). Nous avons voulu déterminer quelle était la proportion des cas qui était attribuable aux sources de contamination autres que le poulet et déterminer quelles étaient les sources de l'excédant de cas de campylobactériose en milieu rural. Enfin, comme mentionné précédemment, une étude de prévalence du Campylobacter dans les différents réservoirs a été effectuée parallèlement à l'étude cas-cas, afin d'obtenir des isolats à caractériser,

dans le but d'établir la corrélation avec les isolats humains. Afin d'associer un isolat humain de Campylobacter à une source potentielle, nous avons utilisé une méthode de classification bayésienne basée sur un modèle conçu pour déduire la structure démographique et assigner les individus (isolats bactériens) à des populations (niches écologiques) en utilisant les données du MLST. Cette méthode a démontré son utilité pour la détermination de la source d'origine d'un isolat bactérien (Pritchard *et al.*, 2000).

Nous avons confirmé que la majorité des cas d'infections sporadiques étaient associés au poulet avec près de 65 % des cas, ce qui confirme ce qui avait été observé dans plusieurs pays industrialisés (Bessell *et al.*, 2012, Mullner *et al.*, 2009, Mughini Gras *et al.*, 2012, Sheppard *et al.*, 2009, Wilson *et al.*, 2008). L'exposition au poulet sous ses multiples formes a été identifiée comme le facteur de risque majoritaire des infections sporadiques d'une multitude d'études cas-témoins à travers le monde (Domingues *et al.*, 2012). Un aspect important de notre étude est qu'il n'y ait pas de différence entre la proportion des cas reliés au poulet entre le milieu urbain et rural. Ceci s'explique par le fait que le pourcentage de contamination des poulets dans les épiceries était le même à travers l'Estrie. Comme il y a très peu d'élevages de poulet en Estrie (0,8 % des élevages) (MAPAQ, 2010), les infections liées au poulet sont presque en totalité dues à une exposition alimentaire. Les habitudes et les pratiques alimentaires associées à la consommation de poulet des gens vivant en milieu rural ne diffèrent donc pas de celles des personnes vivant en milieu urbain.

Nous avons démontré que les différents modes d'exposition aux bovins pouvaient expliquer environ 26 % des cas de campylobactériose. Bien que le

pourcentage global soit plus élevé en milieu rural qu'en milieu urbain, la différence n'est significative que pour les 15-34 ans. Par contre, le gradient urbain-rural du taux d'incidence des campylobactérioses était plus prononcé pour certains groupes d'âge, soit chez les 0-4 ans, les 15-34 ans et chez les plus de 55 ans, où une incidence significativement plus élevée a été observée en milieu rural. Parmi les facteurs de risque significatifs identifiés par l'étude cas-cas, trois facteurs reliés aux bovins étaient associés à un risque plus élevé de développer une campylobactériose en milieu rural. Pour la première fois, nous avons confirmé ces observations à l'aide de données de typage moléculaire. En effet, le nombre de cas humain appartenant au CC ST-61 était significativement plus élevé en milieu rural, ce ST étant connu pour être le principal ST retrouvé chez les bovins, particulièrement chez les bovins laitiers (Oporto *et al.*, 2011). Au niveau de l'attribution des sources potentielles, le groupe d'âge des 15-34 ans était significativement plus associé à une exposition aux bovins. L'exposition aux bovins dans un cadre d'emploi est particulièrement significative pour ce groupe d'âge. Une étude récente au Québec avait d'ailleurs soulevé cette observation pour le même groupe d'âge en milieu rural (Arsenault *et al.*, 2012).

Plusieurs études ont montré un risque significativement plus élevé de campylobactériose chez le groupe d'âge 0-4 ans ou encore chez les moins de 15 ans associé au milieu rural et à la présence élevée de ruminants (Ethelberg *et al.*, 2005, Fitzenderger *et al.*, 2010, Green *et al.*, 2006). Pour notre part, outre le fait qu'un nombre significativement plus élevé de cas ait été retrouvé en milieu rural pour ce groupe d'âge, aucun autre facteur de risque ou ST ne fut significativement associé à ce groupe d'âge. Le fait que nous ayons peu de cas pour ce groupe d'âge pourrait expliquer le manque de puissance statistique et l'absence de relation. Cependant, lorsque nous analysons les

données sans ce groupe d'âge, l'exposition au lait cru ne demeure pas significativement associée au milieu rural. Ceci indique que la consommation de lait cru pourrait être une source importante de campylobactériose pour ce groupe d'âge en milieu rural.

Nous avons observé des variations saisonnières au niveau de certains génotypes particulièrement associés aux bovins et à l'eau. Le taux d'incidence des campylobactériose présente également une saisonnalité, avec une hausse à la fin du printemps et une baisse en automne. Cette saisonnalité des campylobactérioses a été observée dans plusieurs études (Kovats *et al.*, 2005, Nylan *et al.*, 2002, Sopwitj *et al.*, 2006). Une étude récente a suggéré, à l'aide d'un devis cas-cas entre les cas attribuables aux poulets et ceux attribuables aux bovins, que ces derniers présentaient une saisonnalité plus marquée que ceux associés au poulet (Bessell *et al.*, 2012). Ils ont également montré que les cas attribuables aux bovins étaient davantage retrouvés en milieu rural. Finalement, l'utilisation d'un puits comme source d'approvisionnement en eau et le fait de ne pas l'avoir désinfecté sont des facteurs de risque significativement associés à la campylobactériose en milieu rural. Une étude effectuée en Colombie-Britannique a étudié spécifiquement cet aspect et a démontré que le risque de développer une infection entérique était 5,2 fois plus élevé pour les individus obtenant leur eau d'un puits (Uhlmann *et al.*, 2009). La contamination via l'eau en provenance d'un puits s'ajoute probablement au phénomène de la saisonnalité des cas de campylobactériose.

CONCLUSION

Dans le cadre de mes études graduées, je me suis penché sur plusieurs aspects touchant les campylobactérioses et le Campylobacter chez ses différents hôtes. Nous avons établi les taux de résistance aux antibiotiques d'importance médicale pour l'humain des souches de Campylobacter de différentes niches écologiques. Notre étude est la plus récente au Québec et il serait important de suivre l'évolution de la résistance des souches, particulièrement pour la détermination du traitement empirique adéquat. Nous avons confirmé, en comparant différentes méthodes de typage moléculaire, que le MLST est la méthode de choix pour suivre l'évolution génétique des populations de Campylobacter et pour l'attribution de source à une niche écologique particulière. Nous avons également développé un système plus performant et moins coûteux pour effectuer le MLST. Par ce système, nous avons démontré que le HRM pouvait être utilisé pour d'autres applications chez les bactéries. Nous avons par la suite établi que les isolats de Campylobacter en provenance de l'eau étaient très diversifiés génétiquement, probablement dû à de multiples sources de contamination. Finalement, nous avons été les premiers à utiliser un devis épidémiologique cas-cas en combinaison avec des données de typage moléculaire, afin de démontrer que l'excédant de cas de campylobactériose en milieu rural était dû au bovin, via un contact direct, la consommation de lait cru et l'eau. Le groupe d'âge des 15-34 ans est particulièrement affecté en milieu rural, dû entre autres à une exposition agricole en milieu de travail.

Par ces études, nous avons confirmé les différentes sources sporadiques de campylobactérioses par des données moléculaires. Par la détermination des sources

d'infections, nous avons établi des pistes d'intervention utilisable par les autorités de santé publique, afin de diminuer l'incidence de la campylobactériose au Québec. En effet, différentes mesures de prévention pourraient être mises en place afin de cibler les sources principales de campylobactériose, et des campagnes de prévention ciblées pour les groupes d'âge les plus à risque pourraient être instaurées.

Finalement, le Campylobacter demeure une bactérie fascinante de par sa capacité d'adaptation aux différentes niches écologiques qu'elle colonise et de son ubiquité parmi les différentes espèces animales et dans l'environnement. Nous avons démontré que certains génotypes ne sont jamais retrouvés chez l'humain et n'auraient donc pas la capacité de l'infecter. Avec l'avènement et l'accessibilité grandissante du séquençage complet des génomes bactérien, le Campylobacter demeure un modèle de choix pour l'étude de la pathogénèse bactérienne et des facteurs de virulence liés à l'hôte. Je suis convaincu que, dans un avenir rapproché, les différents mécanismes de virulence liés à l'infection du Campylobacter seront élucidés et que des traitements préventifs, tels un vaccin efficace, ou encore un procédé d'éradication de la bactérie des différents hôtes pourront être développés, afin de diminuer considérablement le fardeau de santé publique que représentent la campylobactériose.

REMERCIEMENTS

Le parcours d'un étudiant gradué est un long cheminement durant lequel des rencontres déterminantes viennent enrichir votre formation et votre personnalité. Voici quelques-unes de ces rencontres.

Je tiens tout d'abord à remercier mes directeurs de recherche, le Dr Sophie Michaud et Dr Eric Frost pour leur support, leurs encouragements, leur enseignement et pour m'avoir transmis leur curiosité et leur goût pour la recherche. Merci à Éric d'être un puits sans fond de connaissances scientifiques et une machine à fournir des idées de recherche. Merci pour ta confiance, tes encouragements et ton soutien indéfectible. Un merci tout spécial à Sophie pour m'avoir donné la chance de prouver ma valeur en tant qu'étudiant gradué et pour l'amitié que nous avons développée au cours de ces années. Merci de m'avoir transmis ta persévérance, ta volonté de réussir, ta rigueur scientifique et ton grand souci du travail bien fait. Merci de m'avoir encouragé et aidé à aller au-delà de mes limites et à devenir une meilleure personne.

Merci à Mme Suzanne Brouillette, coordonnatrice académique au programme de biologie sans qui je n'aurais jamais pu faire des études graduées. Merci Suzanne pour ton support et tes encouragements. Merci à Mme Sylvie Deslandes d'avoir été un modèle d'organisation et de m'avoir enseigné une méthode de travail infaillible. Merci pour ta disponibilité, tes connaissances intarissables et ton écoute. Merci à Karen St-Pierre, ma collègue de laboratoire, pour les échanges passionnants sur le

Campylobacter, pour ta bonne humeur et pour ton grand souci d'autrui. Merci aux membres de l'équipe CAMPYLOGIS pour leur support et leur aide. Un remerciement particulier à Dr Robert D. Arbeit pour son esprit critique et son apport envers chacun de mes manuscrits. Merci à tous les étudiants d'été, stagiaires et professionnels de recherche qui sont passés dans notre laboratoire. Vous avez été mes mains durant de nombreuses heures et je vous en remercie profondément.

Merci à M. Roberto Toffoli du MAPAQ pour sa grande disponibilité et des contacts dans le milieu agricole. Merci à M. Patrice Charbonneau de la Ville de Sherbrooke d'avoir été ma porte d'entrée dans les sites d'enfouissement sanitaire. Merci à M. Jean Rodrigue du Service Canadien de la Faune pour l'échantillonnage des canards lors des campagnes de bagage sur la rivière Magog. Merci à Mme Josée Bernard de la corporation de gestion CHARMES pour ses précieux conseils et pour la disponibilité de son équipe. Un grand merci au personnel du laboratoire de microbiologie du CHUS Fleurimont pour leur enseignement, leur disponibilité et leur collaboration.

Je voudrais également remercier tous les membres du département pour leur contribution intellectuelle et scientifique. Un merci spécial à Carole Picard, Sylvie Carrier, Mylène Ledoux, Normand Laframboise, Sherry Bellam, Lise Imbault et Catherine Desrosiers. Merci à Dave, Tim, Mart et Fizz pour leur amitié et les discussions passionnantes sur les sujets les plus farfelus et non scientifiques qu'il peut exister. Un merci spécial à Marie pour son amitié infaillible et pour sa grande générosité.

J'aimerais également adresser un merci profond à ma famille pour leur support de tous les instants et leur encouragement sans limites. Vous avez été pour moi une source de réconfort durant les épreuves et une inspiration pour mes études. Merci à Mylène et à Audrée de partager votre vie avec la mienne.

J'aimerais finalement remercier les membres de mon jury de thèse pour leurs commentaires et leur apport à cet ouvrage.

RÉFÉRENCES

Acar JF, Goldstein FW (1997) Trends in bacterial resistance to fluoroquinolones. *Clin Infect Dis* 24(Suppl 1):S67-73.

Agence de santé publique du Canada. 20 novembre 2009, date de révision. Maladies à déclaration obligatoire en direct. [En ligne]. Agence de santé publique du Canada, Ottawa, Canada. http://dsol-smed.phac-aspc.gc.ca/dsol-smed/ndis/index_f.html.

Allos BM, Lippy FT, Carlsen A, Washburn RG, Blaser MJ (1998) *Campylobacter jejuni* strains from patients with Guillain-Barre syndrome. *Emerg Infect Dis* 4(2):263-8.

Allos BM (2001) *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin Infect Dis* 32(8):1201-6.

Applied Biosystems (2005) Microbial AFLP fingerprint protocol. Applied Biosystems, Foster City, CA, USA.

Arsenault J, Michel P, Berke O, Ravel A, Gosselin P (2012) Environmental characteristics associated with campylobacteriosis: accounting for the effect of age and season. *Epidemiol Infect* 140(2):311-22.

Atabay HI, Corry JE (1998) The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. *J Appl Microbiol* 84(5):733-40.

Bessell PR, Rotariu O, Innocent GT, Smith-Palmer A, Strachan NJ, Forbes KJ, Cowden JM, Reid SW, Matthews L (2012) Using sequence data to identify alternative routes and risk of infection: a case-study of *Campylobacter* in Scotland. *BMC Infect Dis* 12:80. doi: 10.1186/1471-2334-12-80.

Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ (1988) Experimental *Campylobacter jejuni* infection in humans. *J Infect Dis* 157(3):472-9.

Blanc DS, Lugeon C, Wenger A, Siegrist HH, Francioli P (1994) Quantitative antibiogram typing using inhibition zone diameters compared with ribotyping for epidemiological typing of methicillin-resistant *Staphylococcus aureus*. *J Clin Microibol* 32(10):2505-9.

Blaser MJ, Taylor DN, Feldman RA (1983) Epidemiology of *Campylobacter jejuni* infections. *Epidemiol Rev* 5:157-76.

Buswell CM, Herlihy YM, Lawrence LM, McGuiggan JT, Marsh PD, Keevil CW, Leach SA (1998) Extended survival and persistence of *Campylobacter spp.* in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. *Appl Environ Microbiol* 64(2):733-41.

Carter PE, McTavish SM, Brooks HJ, Campbell D, Collins-Emerson JM, Midwinter AC, French NP (2009) Novel clonal complexes with an unknown animal reservoir dominate *Campylobacter jejuni* isolates from river water in New Zealand. *Appl Environ Microbiol* 75(19):6038-46.

Centre d'expertise en analyse environnementale du Québec (2005) Recherche et dénombrement des coliformes fécaux (thermotolérants) et confirmation à l'espèce *Escherichia coli*: méthode par filtration sur membrane. Dans: Ministère du Développement durable, de l'Environnement et des Parcs du Québec, MA. 700-Fec. Ec 1.0, Rév. 2. 20p.

Center for disease control and prevention (CDC). 01 décembre 2009, date de révision. PulseNet "Least Wanted" Pathogens. [En ligne]. Centers for Disease Control and Prevention, Atlanta, U.S.A. http://www.cdc.gov/pulsenet/pathogens.htm

Center for disease control and prevention (CDC). 01 décembre 2009, date de révision. What is PulseNet?. [En ligne]. Centers for Disease Control and Prevention, Atlanta, U.S.A. http://www.cdc.gov/PULSENET/images/pfge_process.gif

Chaban B, Musil KM, Himsworth CG, Hill JE (2009) Development of cpn60-based real-time quantitative PCR assays for the detection of 14 *Campylobacter* species and application to screening of canine fecal samples. *Appl Environ Microbiol* 75(10):3055-61.

Clark CG, Bryden L, Cuff WR, Johnson PL, Jamieson F, Ciebin B, Wang G (2005) Use of the oxford multilocus sequence typing protocol and sequencing of the flagellin short variable region to characterize isolates from a large outbreak of waterborne *Campylobacter* sp. strains in Walkerton, Ontario, Canada. *J Clin Microbiol* 43(5):2080-91.

Clinical and Laboratory Standards Institute (CLSI) (2007) Molecular methods for bacterial strain typing; proposed guideline. CLSI document MM11-P. CLSI, Wayne, Pa.

Colles FM, Jones K, Harding RM, Maiden MC (2003) Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. *Appl Environ Microbiol* 69(12):7409-13.

Connell SR, Trieber CA, Stelzl U, Einfeldt E, Taylor DE, Nierhaus KH (2002) The tetracycline resistance protein Tet(o) perturbs the conformation of the ribosomal decoding centre. *Mol Microbiol* 45(6):1463-72.

Debruyne L, Broman T, Bergström S, Olsen B, On SL, Vandamme P (2010) *Campylobacter volucris* sp. nov., isolated from black-headed gulls (*Larus ridibundus*). *Int J Syst Evol Microbiol* 60(Pt 8):1870-5.

Denis M, M Tanguy, Chidaine B, Laisney MJ, Mégraud F, Fravalo P (2011) Description and sources of contamination by *Campylobacter* spp. of river water destined for human consumption in Brittany, France. *Pathol Biol (Paris)* 59(5):256-63.

Djordjevic SP, Unicomb LE, Adamson PJ, Mickan L, Rios R; Australian Campylobacter Subtyping Study Group (2007) Clonal complexes of *Campylobacter jejuni* identified by multilocus sequence typing are reliably predicted by restriction fragment length polymorphism analyses of the flaA gene. *J Clin Microbiol* 45(1):102-8.

Dingle KE, Maiden MCJ (2005) Population genetics of *Campylobacter jejuni*, p. 43-57. Dans: J. M. Ketley, M. E. Konkel (ed), *Campylobacter, molecular and cellular biology.* Horizon Bioscience. Norfolk, U.K.

Dingle KE, Colles FM, Ure R, Wagenaar JA, Duim B, Bolton FJ, Fox AJ, Wareing DR, Maiden MC (2002) Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. *Emerg Infect Dis* 8(9):949-55.

Domingues AR, Pires SM, Halasa T, Hald T (2012) Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. *Epidemiol Infect* 140(6):970-81.

Drlica, K (1999) Mechanism of fluoroquinolone action. *Curr Opin Microbiol* 2(5):504-8.

Duim B, Wassenaar TM, Rigter A, Wagenaar J (1999) High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with amplified fragment length polymorphism fingerprinting. *Appl Environ Microbiol* 65(6):2369-75.

Duim B, Godschalk PC, van den Braak N, Dingle KE, Dijkstra JR, Leyde E, van der Plas J, Colles FM, Endtz HP, Wagenaar JA, Maiden MC, van Belkum A (2003) Molecular evidence for dissemination of unique *Campylobacter jejuni* clones in Curacao, Netherlands Antilles. *J Clin Microbiol* 41(12):5593-7.

Engberg J (2006) Contributions to the epidemiology of *Campylobacter* infections. A review of clinical and microbiological studies. *Dan Med Bull* 53(4):361-89.

Engberg J, Neimann J, Nielsen EM, Aerestrup FM, Fussing V (2004) Quinolone-resistant *Campylobacter* infections: risk factors and clinical consequences. *Emerg Infect Dis* 10(6):1056–1063.

Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I (2001) Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. *Emerg Infect Dis* 7(1):24-34.

Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP (1991) Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *J Antimicrob Chemother* 27(2):199–208.

Ethelberg S, Simonsen J, Gerner-Smidt P, Olsen KE, Mølbak K (2005) Spatial distribution and registry-based case-control analysis of *Campylobacter* infections in Denmark, 1991-2001. *Am J Epidemiol* 162(10):1008-15.

Evans MR, Roberts RJ, Ribeiro CD, Gardner D, Kembrey D (1996) A milk-borne *Campylobacter* outbreak following an educational farm visit. *Epidemiol Infect* 117(3):457-62.

Eyles R, Niyogi D, Townsend C, Benwell G, Weinstein P (2003) Spatial and temporal patterns of *Campylobacter* contamination underlying public health risk in the Taieri River, New Zealand. *J Environ Qual* 32(5):1820-8.

Finch MJ, Blake PA (1985) Foodborne outbreaks of campylobacteriosis: the United States experience, 1980-1982. *Am J Epidemiol* 122(2):262-8.

Fitzenberger J, Uphoff H, Gawrich S, Hauri AM (2010) Urban-rural differences of age- and species-specific campylobacteriosis incidence, Hesse, Germany, July 2005 - June 2006. *Euro Surveill* 15(42).

Flournoy D J (1982) Quantitative antibiogram as a potential tool for epidemiologycal typing. *Infect Control* 3(5):384-7.

Friedman CR, Neimann J, Wegener HC, Tauxe RV (2000) Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations, p.121-38. Dans : I. Nachamkin, M. J. Blaser (ed), *Campylobacter*. ASM Press. 2nd ed. Washington, D. C.

Funke G, Baumann R, Penner JL, Altwegg M (1994) Development of resistance to macrolide antibiotics in an AIDS patient treated with clarithromycin for *Campylobacter jejuni* diarrhea. *Eur J Clin Microbiol Infect Dis* 13(7):612–615.

Gallay A, Prouzet-Mauléon V, Kempf I, Lehours P, Labadi L, Camou C, Denis M, de Valk H, Desenclos JC, Mégraud F (2007) *Campylobacter* antimicrobial drug resistance among humans, broiler chickens, and pigs, France. *Emerg Infect Dis* 13(2):259-66.

Gaudreau C, Gilbert H (1998) Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. *Antimicrob Agents Chemother* 42(8):2106-8.

Gibbens JC, Pascoe SJ, Evans SJ, Davies RH, Sayers AR (2001) A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. *Prev Vet Med* 48(2):85-99.

Gibreel A, Kos VN, Keelan M, Trieber CA, Levesque S, Michaud S, Taylor DE (2005) Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*: molecular mechanism and stability of the resistance phenotype. *Antimicrob Agents Chemother* 49(7):2753-9.

Gibreel A, Taylor DE (2006) Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J Antimicrob Chemother* 58(2):243-55.

Gilbreath JJ, Cody WL, Merrell DS, Hendrixson DR (2011) Change is good: variations in common biological mechanisms in the epsilonproteobacterial genera *Campylobacter* and *Helicobacter*. *Microbiol Mol Biol Rev* 75(1):84-132.

Golding GR, Campbell JL, Spreitzer DJ, Veyhl J, Surynicz K, Simor A, Mulvey MR; Canadian Nosocomial Infection Surveillance Program (2008) A preliminary guideline for the assignment of methicillin-resistant *Staphylococcus aureus* to a Canadian pulsed-field gel electrophoresis epidemic type using spa typing. *Can J Infect Dis Med Microbiol* 19(4):273-81.

Gouvernement du Canada (2010) Programme intégré canadien de surveillance de la résistance aux antimicrobiens (PICRA) 2009 – Résultats préliminaires. Guelph (Ontario): Agence de la santé publique du Canada.

Green CG, Krause DO, Wylie JL (2006) Spatial analysis of *Campylobacter* infection in the Canadian province of Manitoba. *Int J Health* Geogr 5:2.

Gudmundson J, Chirino-Trejo JM (1993) A case of bovine mastitis caused by *Campylobacter jejuni. Zentralbl Veterinarmed B* 40(5):326-8.

Guévremont E, Normand V, Lamoureux L, Côté C (2008) Genetic detection of *Campylobacter lanienae* in fecal matter and stored manure from swine and dairy cattle. *Foodborne Pathog Dis* 5(3):361-4.

Gupta A, Nelson JM, Barrett TJ, Tauxe RV, Rossiter SP, Friedman CR, Joyce KW, Smith KE, Jones TF, Hawkins MA, Shiferaw B, Beebe JL, Vugia DJ, Rabatsky-Ehr T, Benson JA, Root TP, Angulo FJ; NARMS Working Group (2004) Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. *Emerg Infect Dis* 10(6):1102-9.

Hartmann FA, West SE (1997) Utilization of both phenotypic and molecular analyses to investigate an outbreak of multidrug-resistant *Salmonella* anatum in horses. *Can J Vet Res* 61(3):173-81.

Health Canada (2002) Report of the advisory committee on animal uses of antimicrobials and impact on resistance and human health, p. 62–79.

Hernández J, Fayos A, Alonso JL, Owen RJ (1996) Ribotypes and AP-PCR fingerprints of thermophilic campylobacters from marine recreational waters. *J Appl Bacteriol* 80(2):157-64.

Inglis GD, Morck DW, McAllister TA, Entz T, Olson ME, Yanke LJ, Read RR (2006) Temporal prevalence of antimicrobial resistance in *Campylobacter spp.* from beef cattle in Alberta feedlots. *Appl Environ Microbiol* 72(6):4088-95.

Islam Z, van Belkum A, Wagenaar JA, Cody AJ, de Boer AG, Tabor H, Jacobs BC, Talukder KA, Endtz HP (2009) Comparative genotyping of *Campylobacter jejuni* strains from patients with Guillain-Barre syndrome in Bangladesh. *PLoS One* 4(9):e7257.

Jacobs-Reitsma W (2000) *Campylobacter* in the food supply, p.467-81. Dans : Nachamkin I, Blaser MJ (ed), *Campylobacter*. ASM Press. 2nd ed. Washington, D. C.

Jones PH, Willis AT, Robinson DA, Skirrow MB, Josephs DS (1981) *Campylobacter* enteritis associated with the consumption of free school milk. *J Hyg (Lond)* 87(2):155-62.

Kehoe PG, Ambrosone CB, Arnett DK, Cerhan JR, Pedersen N, Wei Q, Wang D (2010) The Launch of International Journal of Molecular Epidemiology and Genetics. *Int J Mol Epidemiol Genet* 1(1):i-ii.

Kemp R, Leatherbarrow AJ, Williams NJ, Hart CA, Clough HE, Turner J, Wright EJ, French NP (2005) Prevalence and genetic diversity of *Campylobacter* spp. in environmental water samples from a 100-square-kilometer predominantly dairy farming area. *Appl Environ Microbiol* 71(4):1876-82.

Kinana AD, Cardinale E, Tall F, Bahsoun I, Sire JM, Garin B, Breurec S, Boye CS, Perrier-Gros-Claude JD (2006) Genetic diversity and quinolone resistance in *Campylobacter jejuni* isolates from poultry in Senegal. *Appl Environ Microbiol* 72(5):3309-13.

Kittl S, Kuhnert P, Hächler H, Korczak BM (2011) Comparison of genotypes and antibiotic resistance of *Campylobacter jejuni* isolated from humans and slaughtered chickens in Switzerland. *J Appl Microbiol* 110(2):513-520.

Klena JD, Konkel ME (2005) Methods for epidemiological analysis of *Campylobacter jejuni*, p. 165-79. Dans : Ketley JM, Konkel ME (ed), *Campylobacter, molecular and cellular biology*. Horizon Bioscience. Norfolk, U.K.

Kornblatt AN, Barrett T, Morris GK, Tosh FE (1985) Epidemiologic and laboratory investigation of an outbreak of *Campylobacter* enteritis associated with raw milk. *Am J Epidemiol* 122(5):884-9.

Kovats RS, Edwards SJ, Charron D, Cowden J, D'Souza RM, Ebi KL, Gauci C, Gerner-Smidt P, Hajat S, Hales S, Hernández Pezzi G, Kriz B, Kutsar K, McKeown P, Mellou K, Menne B, O'Brien S, van Pelt W, Schmid H (2005) Climate variability and *Campylobacter* infection: an international study. *Int J Biometeorol* 49(4):207-14.

Krumkamp R, Reintjes R, Dirksen-Fischer M (2008) Case-case study of a *Salmonella* outbreak: an epidemiologic method to analyse surveillance data. *Int J Hyg Environ Health* 211(1-2):163-7.

Lehtola MJ, Pitkänen T, Miebach L, Miettinen IT (2006) Survival of *Campylobacter jejuni* in potable water biofilms: a comparative study with different detection methods. *Water Sci Technol* 54(3):57-61.

Lévesque S, Martel J, Frost E, Michaud S (2005) Prevalence of *Campylobacter* in river and tributary water in the Eastern Townships, Québec, Canada. Abstract. 13th Int. Workshop *Campylobacter, Helicobacter*, and Related Organisms, abstract. A52.

Lévesque S, Frost E, Arbeit RD, 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(10):3404–3411.

Lin J, Michel LO, Zhang Q (2002) CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrob Agents Chemother* 46(7):2124-31.

Lindmark H, Harbom B, Thebo L, Andersson L, Hedin G, Osterman B, Lindberg T, Andersson Y, Westöö A, Olsson Engvall E (2004) Genetic characterization and antibiotic resistance of *Campylobacter jejuni* isolated from meats, water, and humans in Sweden. *J Clin Microbiol* 42(2):700-6.

Lindstedt BA, Heir E, Vardund T, Melby KK, Kapperud G (2000) Comparative fingerprinting analysis of *Campylobacter jejuni* subsp. *jejuni* strains by amplified-fragment length polymorphism genotyping. *J Clin Microbiol* 38(9):3379–3387.

MacFaddin J F (2000) Biochemical test for identification of medical bacteria. Lippincott Williams & Wilkins. 3rd ed. USA. 912 pages.

Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A* 95(6):3140-5.

Manning G, Dowson CG, Bagnall MC, Ahmed IH, West M, Newell DG (2003) Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. *Appl Environ Microbiol* 69(11):6370-9.

MAPAQ (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec), Direction régionale de l'Estrie (2010) Agriculture et agroalimentaire en Estrie. Gouvernement du Québec. Disponible à: http://www.mapaq.gouv.qc.ca/SiteCollectionDocuments/Regions/Estrie/ProfilRegion/Estrie_profil.pdf. Page consultée le 9 décembre 2012.

Matsuda M, Tai K, Moore JE, Millar BC, Murayama O (2004) Nucleotide sequence analysis of the recA gene and discrimination of the three isolates of urease-positive thermophilic *Campylobacter* (UPTC) isolated from seagulls (*Larus spp.*) in Northern Ireland. *J Basic Microbiol* 44(3):215-9.

McDermott PF, Bodeis SM, English LL, White DG, Walker RD, Zhao S, Simjee S, Wagner DD (2002) Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. *J Infect Dis* 185(6):837-40.

McEwen SA, Fedorka-Cray PJ (2002) Antimicrobial use and resistance in animals. *Clin Infect Dis* 34(Suppl 3):S93-S106.

McTavish SM, Pope CE, Nicol C, Sexton K, French N, Carter PE (2008) Wide geographical distribution of internationally rare *Campylobacter* clones within New Zealand. *Epidemiol Infect* 136(9):1244-52.

McTavish SM, Pope CE, Nicol C, Campbell D, French N, Carter PE (2009) Multilocus sequence typing of *Campylobacter jejuni*, and the correlation between clonal complex and pulsed-field gel electrophoresis macrorestriction profile. *FEMS Microbiol Lett* 298(2):149-56.

Meinersmann RJ, Helsel LO, Fields PI, Hiett KL (1997) Discrimination of *Campylobacter jejuni* isolates by fla gene sequencing. *J Clin Microbiol* 35(11):2810-4.

Ménard S (2006) Rapport sur les maladies infectieuses à déclaration obligatoire en Estrie – Année 2005. Sherbrooke : Régie Régionale de la Santé et des Services Sociaux de l'Estrie.

Michaud S, Menard S, Gaudreau C, Arbeit RD (2001) Comparison of SmaI-defined genotypes of *Campylobacter jejuni* examined by KpnI: a population-based study. *J Med Microbiol* 50(12):1075-81.

Michaud S, Ménard S, Arbeit RD (2004) Campylobacteriosis, Eastern Townships, Quebec. *Emerg Infect Dis* 10(10):1844-7.

Miller WG, Mandrell RE (2005) Prevalence of *Campylobacter* in the food and water supply: incidence, outbreaks, isolation and detection, p. 101-63. Dans: Ketley JM, Konkel ME (ed), *Campylobacter, molecular and cellular biology.* Horizon Bioscience. Norfolk, U.K.

Moreno GS, Griffiths PL, Connerton IF, Park RW (1993) Occurrence of campylobacters in small domestic and laboratory animals. *J Appl Bacteriol* 75(1):49-54.

MSSS (Bureau de surveillance et vigie) (2010) Nombre (n) de cas déclarés et taux d'incidence (Ti) annuelle par 100 000 habitants de campylobactériose selon l'espèce, province de Québec, 2000-2009. MSSS, 1 mars 2010.

Mughini Gras L, Smid JH, Wagenaar JA, de Boer AG, Havelaar AH, Friesema IH, French NP, Busani L, van Pelt W (2012) Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis. *PLoS One* 7(8):e42599.

Mullner P, Spencer SE, Wilson DJ, Jones G, Noble AD, Midwinter AC, Collins-Emerson JM, Carter P, Hathaway S, French NP (2009) Assigning the source of human campylobacteriosis in New Zealand: a comparative genetic and epidemiological approach. *Infect Genet Evol* 9(6):1311-9.

Nachamkin I, Bohachick K, Patton CM (1993) Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *J Clin Microbiol* 31(6):1531-6.

Nachamkin I, Engberg J, Aarestrup FM (2000) Diagnosis ans anti,icrobial susceptibility of *Campylobacter* species, p. 45-66. Dans : Nachamkin I, Blaser MJ (ed), *Campylobacter*. ASM Press. 2nd ed. Washington, D. C.

Nakajima Y (1999) Mechanisms of bacterial resistance to macrolide antibiotics. *J Infect Chemother* 5(2):61-74.

Nannapaneni R, Chalova VI, Story R, Wiggins KC, Crandall PG, Ricke SC, Johnson MG (2009) Ciprofloxacin-sensitive and ciprofloxacin-resistant *Campylobacter jejuni* are equally susceptible to natural orange oil-based antimicrobials. *J Environ Sci Health B* 44(6):571-7.

Nygård K, Andersson Y, Røttingen JA, Svensson A, Lindbäck J, Kistemann T, Giesecke J (2004) Association between environmental risk factors and *Campylobacter* infections in Sweden. *Epidemiol Infect* 132(2):317-25.

Nylen G, Dunstan F, Palmer SR, Andersson Y, Bager F, Cowden J, Feierl G, Galloway Y, Kapperud G, Megraud F, Molbak K, Petersen LR, Ruutu P (2002) The seasonal distribution of campylobacter infection in nine European countries and New Zealand. *Epidemiol Infect* 128(3):383-90.

On SLW (2005) Taxonomy, phylogeny, and methods for the identification of *Campylobacter* species, p. 13-42. Dans : Ketley MJ, Konkel ME (ed), *Campylobacter, molecular and cellular biology*. Horizon Bioscience. Norfolk, U.K.

Oporto B, Juste RA, López-Portolés JA, Hurtado A (2011) Genetic diversity among *Campylobacter jejuni* isolates from healthy livestock and their links to human isolates in Spain. *Zoonoses Public Health* 58(5):365-75.

Orr KE, Lightfoot NF, Sisson PR, Harkis BA, Tweddle JL, Boyd P, Carroll A, Jackson CJ, Wareing DR, Freeman R (1995) Direct milk excretion of *Campylobacter jejuni* in a dairy cow causing cases of human enteritis. *Epidemiol Infect* 114(1):15-24.

Payment P, Richardson L, Siemiatycki J, Dewar R, Edwardes M, Franco E (1991) A randomized trial to evaluate the risk of gastrointestinal disease due to consumption of drinking water meeting current microbiological standards. *Am J Public Health* 81(6):703-8.

Payot S, Avrain L, Magras C, Praud K, Cloeckaert A, Chaslus-Dancla E (2004) Relative contribution of target gene mutation and efflux to fluoroquinolone and erythromycin resistance, in French poultry and pig isolates of *Campylobacter coli*. *Int J Antimicrob Agents* 23(5):468-72.

Payot S, Bolla JM, Corcoran D, Fanning S, Mégraud F, Zhang Q (2006) Mechanisms of fluoroquinolone and macrolide resistance in *Campylobacter spp. Microbes Infect* 8(7):1967-71.

Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M, 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(3):281-7.

Pigrau C, Bartolome R, Almirante B, Planes AM, Gavalda J, Pahissa A (1997) Bacteremia due to *Campylobacter* species: clinical findings and antimicrobial susceptibility patterns. *Clin Infect Dis* 25(6):1414-20.

Popovic-Uroic T (1989) *Campylobacter jejuni* and *Campylobacter coli* diarrhoea in rural and urban populations in Yugoslavia. *Epidemiol Infect* 102(1):59-67.

Price LB, Lackey LG, Vailes R, Silbergeld E (2007) The persistence of fluoroquinolone-resistant *Campylobacter* in poultry production. *Environ Health Perspect* 115(7):1035-9.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155(2):945-59.

Quessy S, Messier S (1992) Prevalence of *Salmonella spp.*, *Campylobacter spp.* and *Listeria spp.* in ring-billed gulls (*Larus delawarensis*). *J Wildl Dis* 28(4):526-31.

Ririe KM, Rasmussen RP, Wittwer CT (1997) Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal Biochem* 245(2):154-160.

Robinson DA (1981) Infective dose of *Campylobacter jejuni* in milk. *Br Med J (Clin Res Ed)* 282(6276):1584.

Rosenquist H, Boysen L, Galliano C, Nordentoft S, Ethelberg S, Borck B (2009) Danish strategies to control *Campylobacter* in broilers and broiler meat: facts and effects. *Epidemiol Infect* 137(12):1742-50.

Salfield NJ, Pugh EJ (1987) *Campylobacter* enteritis in young children living in households with puppies. *Br Med J (Clin Res Ed)* 294(6563):21-2.

Shanker S, Lee A, Sorrell TC (1990) Horizontal transmission of *Campylobacter jejuni* amongst broiler chicks: experimental studies. *Epidemiol Infect* 104(1):101-10.

Schouls LM, Reulen S, Duim B, Wagenaar JA, Willems RJ, Dingle KE, Colles FM, Van Embden JD (2003) Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymrphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. *J Clin Microbiol* 41(1):15-26.

Sheppard SK, Dallas JF, Strachan NJ, MacRae M, McCarthy ND, Wilson DJ, Gormley FJ, Falush D, Ogden ID, Maiden MC, Forbes KJ (2009) *Campylobacter* genotyping to determine the source of human infection. *Clin Infect Dis* 48(8):1072-8.

Shreeve JE, Toszeghy M, Pattison M, Newell DG (2000) Sequential spread of *Campylobacter* infection in a multipen broiler house. *Avian Dis* 44(4):983-8.

Skirrow MB (1987). A demographic survey of *campylobacter*, *salmonella* and *shigella* infections in England. A Public Health Laboratory Service Survey. *Epidemiol Infect* 99(3):647-57.

Skirrow MB, Butzler JP (2000) Foreword, p. XVII-XXIII. Dans: Nachamkin I, Blaser MJ (ed), *Campylobacter*. ASM Press. 2nd ed. Washington, D. C.

Skirrow MB, Blaser MJ (2000) Clinical aspects of *Campylobacter* infection, p.69-88. Dans: Nachamkin I, Blaser MJ (ed), *Campylobacter*. ASM Press. 2nd ed. Washington, D. C.

Skirrow MB (2006) John McFadyean and the centenary of the first isolation of *Campylobacter* species. *Clin Infect Dis* 43(9):1213-7.

Skjøt-Rasmussen L, Ethelberg S, Emborg HD, Agersø Y, Larsen LS, Nordentoft S, Olsen SS, Ejlertsen T, Holt H, Nielsen EM, Hammerum AM (2009) Trends in occurrence of antimicrobial resistance in *Campylobacter jejuni* isolates from broiler chickens, broiler chicken meat, and human domestically acquired cases and travel associated cases in Denmark. *Int J Food Microbiol* 131(2-3):277-9.

Smith KE, Besser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH, Johnson BP, Moore KA, Osterholm MT (1999) Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. Investigation Team. *N Engl J Med* 340(20):1525-32.

Sopwith W, Birtles A, Matthews M, Fox A, Gee S, Painter M, Regan M, Syed Q, Bolton E (2006) *Campylobacter jejuni* multilocus sequence types in humans, northwest England, 2003-2004. *Emerg Infect Dis* 12(10):1500-7.

Spratt BG (1999) Multilocus sequence typing: molecular typing of bacterial pathogens in an era of rapid DNA sequencing and the internet. *Curr Opin Microbiol* 2(3):312-6.

St-Pierre K, Lévesque S, Frost E, Carrier N, Arbeit RD, Michaud S (2009) Thermotolerant coliforms are not a good surrogate for *Campylobacter spp.* in environmental water. *Appl Environ Microbiol* 75(21):6736-44.

Studahl A, Andersson Y (2000) Risk factors for indigenous *Campylobacter* infection: a Swedish case-control study. *Epidemiol Infect* 125(2):269-75.

Taylor CF (2009) Mutation scanning using high-resolution melting. *Biochem Soc Trans* 37(Pt 2):433-7.

Taylor DE, Mandrell RE (2005) Prevalence of *Campylobacter* in the food and water supply: incidence, outbreaks, isolation and detection, p. 101-63. Dans : Ketley JM, Konkel ME (ed), *Campylobacter, molecular and cellular biology.* Horizon Bioscience. Norfolk, U.K.

Tenkate TD, Stafford RJ (2001) Risk factors for *Campylobacter* infection in infants and young children: a matched case-control study. *Epidemiol Infect* 127(3):399-404.

Tenover FC, Arbeit RD, Goering RV (1997) How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Molecular Typing Working Group of the Society for Healthcare Epidemiology of America. *Infect Control Hosp Epidemiol* 18(6):426-39.

Teunis P, Van den Brandhof W, Nauta M, Wagenaar J, Van den Kerkhof H, Van Pelt W (2005) A reconsideration of the *Campylobacter* dose-response relation. *Epidemiol Infect* 133(4):583-92.

Thomas MK, Majowicz SE, Sockett PN, Fazil A, Pollari F, Doré K, Flint JA, Edge VL (2006) Estimated Numbers of Community Cases of Illness Due to *Salmonella*, *Campylobacter* and Verotoxigenic *Escherichia Coli*: Pathogen-specific Community Rates. *Can J Infect Dis Med Microbiol* 17(4):229-34.

Thompson JS, Cahoon FE, Hodge DS (1986) Rate of Campylobacter spp. isolation in three regions of Ontario, Canada, from 1978 to 1985. *J Clin Microbiol* 24(5):876-8.

Trieber CA, Taylor DE (2000) Mechanisms of antibiotic resistance in *Campylobacter*, p.441-54. Dans : Nachamkin I, Blaser MJ (ed), *Campylobacter*. ASM Press. 2nd ed. Washington, D. C.

Uhlmann S, Galanis E, Takaro T, Mak S, Gustafson L, Embree G, Bellack N, Corbett K, Isaac-Renton J (2009) Where's the pump? Associating sporadic enteric disease with drinking water using a geographic information system, in British Columbia, Canada, 1996-2005. *J Water Health* 7(4):692-8.

Unicomb L, Ferguson J, Riley TV, Collignon P (2003) Fluoroquinolone resistance in *Campylobacter* absent from isolates, Australia. *Emerg Infect Dis* 9(11):1482-3.

Vacher S, Menard A, Bernard E, Santos A, Megraud F (2005) Detection of mutations associated with macrolide resistance in thermophilic *Campylobacter spp.* by real-time PCR. *Microb Drug Resist* 11(1):40-7.

van Bergen MA, Dingle KE, Maiden MC, Newell DG, van der Graaf-Van Bloois L, van Putten JP, Wagenaar JA (2005) Clonal nature of *Campylobacter fetus* as defined by multilocus sequence typing. *J Clin Microbiol* 43(12):5888-98.

Vandamme P (2000) Taxonomy of the family *Campylobacteraceae*, p. 3-26. Dans : Nachamkin I, Blaser MJ (ed), *Campylobacter*. ASM Press. 2nd ed. Washington, D. C.

Vellinga A, Van Loock F (2002) The dioxin crisis as experiment to determine poultry-related *Campylobacter* enteritis. *Emerg Infect Dis* 8(1):19-22.

Wahlström H, Tysén E, Olsson Engvall E, Brändström B, Eriksson E, Mörner T, Vågsholm I (2003) Survey of *Campylobacter* species, VTEC O157 and *Salmonella* species in Swedish wildlife. *Vet Rec* 153(3):74-80.

Wassenaar TM, Newell DG (2000) Genotyping of *Campylobacter spp. Appl Environ Microbiol* 66(1):1-9.

Whelan CD, Monaghan P, Girdwood RW, Fricker CR (1988) The significance of wild birds (*Larus sp.*) in the epidemiology of *Campylobacter* infections in humans. *Epidemiol Infect* 101(2):259-67.

Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA, Diggle PJ (2008) Tracing the source of campylobacteriosis. *PLoS Genet* 4(9):e1000203.

Wood RC, MacDonald KL, Osterholm MT (1992) *Campylobacter* enteritis outbreaks associated with drinking raw milk during youth activities. A 10-year review of outbreaks in the United States. *JAMA* 268(22):3228-30.

Young I, Rajić A, Letellier A, Cox B, Leslie M, Sanei B, McEwen SA (2010) Knowledge and attitudes toward food safety and use of good production practices among Canadian broiler chicken producers. *J Food Prot* 73(7):1278-87.

ANNEXE

Liste des publications

Lévesque S, St-Pierre K, Frost E, Arbeit RD, Michaud S (2012) Use of amplified-fragment length polymorphism to study the ecology of *Campylobacter jejuni* in environmental water and to predict multilocus sequence typing clonal complexes. *Appl Environ Microbiol* 78(7):2470-3.

Lévesque S, St-Pierre K, Frost E, Arbeit RD, Michaud S (2011) Determination of the optimal culture conditions for detecting thermophilic campylobacters in environmental water. *J Microbiol Methods* 86(1):82-8.

Lévesque S, Michaud S, Arbeit RD, Frost EH (2011) High-resolution melting system to perform multilocus sequence typing of *Campylobacter jejuni*. *PLoS One* 6(1):e16167.

St-Pierre K, Lévesque S, Frost E, Carrier N, Arbeit RD, Michaud S (2009) Thermotolerant coliforms are not a good surrogate for *Campylobacter spp* in environmental water. *Appl Environ Microbiol* 75(21):6736-44.

Lévesque S, Frost E, Arbeit RD, 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(10):3404-11.

Lévesque S, Frost E, Michaud S (2007) Comparison of antimicrobial resistance of *Campylobacter jejuni* isolated from humans, chickens, raw milk, and environmental water in Québec. *J Food Prot* 70(3):729-35.

Gibreel A, Kos VN, Keelan M, Trieber CA, Levesque S, Michaud S, Taylor DE (2005) Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*: molecular mechanism and stability of the resistance phenotype. *Antimicrob Agents Chemother* 49(7):2753-9.

Liste des communications (présentations orales)

Lévesque S, Frost E, Arbeit RD, Michaud S. Comparison of Pulsed Field Gel Electrophoresis and Fla typing for predicting Multilocus Sequence Typing clonal complexes of Campylobacter jejuni. Canadian Campylobacter Conference, septembre 2008. Montréal, Québec, Canada.

Bonfils D, Lavoie A, Lévesque S, Frost E, St-Pierre K, Desbiens R, Carrier N, Bénié GB, Michaud S. Spatial analysis and modeling of interactions between environment and Campylobacter jejuni in Eastern Township surface water. Canadian Campylobacter Conference, septembre 2008. Montréal, Québec, Canada.

St-Pierre K, Lévesque S, Frost E, Michaud S. Les coliformes fécaux ne sont pas un bon indicateur de la présence du Campylobacter dans l'eau environnementale. 32è Congrès annuel de l'Association des Médecins Microbiologistes-Infectiologues du Québec, juin 2007. St-Sauveur, Québec, Canada.

St-Pierre K, Lévesque S, Frost E, Michaud S. Les coliformes fécaux ne sont pas un bon indicateur de la présence du Campylobacter dans l'eau environnementale. ACFAS, mai 2007. Université du Québec à Trois-Rivières.

St-Pierre K, Lévesque S, Frost E, Michaud S. Les coliformes fécaux ne sont pas un bon indicateur de la présence du Campylobacter dans l'eau environnementale. Journée de la recherche, février 2007. Université de Sherbrooke.

Lévesque S, Frost E, Michaud S. Multilocus Sequence Typing for Comparison of Water, Chicken, Raw Milk and Human Isolates of Campylobacter jejuni in Quebec. Canadian Campylobacter Conference, November 2006. Vancouver, British Columbia, Canada.

St-Pierre K, Lévesque S, Frost E, Michaud S. Molecular Approach for Species-Level Identification of Campylobacter jejuni (CJ), C. coli (CC), C. lari (CL), C. upsialensis (CU) and C. fetus (CF) Based on PCR of the Hippuricase Gene and PCR-RFLP Using AluI. Canadian Campylobacter Conference, November 2006. Vancouver, British Columbia, Canada.

St-Pierre K, Lévesque S, Frost E, Michaud S. Prevalence and Quantification of Campylobacter in Environmental Water Eastern Townships, Québec. Canadian Campylobacter Conference, November 2006. Vancouver, British Columbia, Canada.

Chammat A, Lévesque S, Frost E, Michaud S. Incidence of human campylobacteriosis and prevalence of Campylobacter in fresh whole retail chikens, Eastern Townships, Québec. Canadian Campylobacter Conference, November 2006. Vancouver, British Columbia, Canada.

St-Pierre K, Lévesque S, Frost E, Michaud S. Développement et validation d'un PCR-RFLP pour l'identification de 5 espèces de Campylobacter d'intérêt médical. 31è Congrès annuel de l'Association des Médecins Microbiologistes-Infectiologues du Québec, juin 2006. Rivière du Loup, Québec, Canada.

Lévesque S, Frost E, Michaud S. Multilocus sequence typing for comparison of water, chicken, raw mild and human isolates of Campylobacter jejuni in Quebec. 13th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2005. Gold Coast, Queensland, Australia.

Lévesque S, Frost E, Michaud S. Comparaison des profils de résistance aux antibiotiques du Campylobacter jejuni isolé chez l'humain, le poulet, le lait cru et l'eau au Québec. 30è Congrès annuel de l'Association des Médecins Microbiologistes-Infectiologues du Québec, juin 2005. Bromont, Québec, Canada.

Lévesque S, Frost E, Michaud S. Analyse des populations génétiques du Campylobacter jejuni de source humaine, alimentaire et hydrique par Multilocus Sequence Typing (MLST). 30è Congrès annuel de l'Association des Médecins Microbiologistes-Infectiologues du Québec, juin 2005. Bromont, Québec, Canada.

Lévesque S, Michaud S, Martel J, Frost E. Développement d'un protocole de détection du Campylobacter dans l'eau. 29è Congrès annuel de l'Association des Médecins Microbiologistes-Infectiologues du Québec, juin 2004. Québec, Québec, Canada.

Lévesque S, Michaud S, Martel J, Frost E. Développement d'un protocole de détection du Campylobacter dans l'eau. 33è édition, Journée scientifique, mai 2004. Faculté de Médecine, Université de Sherbrooke.
Gagnant du premier prix pour la meilleure présentation toute catégorie.

Lévesque S, Michaud S. Comparaison des profils de résistances des souches de C. jejuni isolées chez l'humain et le poulet. 28è Congrès annuel de l'Association des Médecins Microbiologistes-Infectiologues du Québec, juin 2003. St-Hyacinthe, Québec, Canada.

Lévesque S, Michaud S. Comparaison des profils de résistances des souches de C. jejuni isolées chez l'humain et le poulet. 32è édition, Journée scientifique, mai 2003. Faculté de Médecine, Université de Sherbrooke.

Liste des communications (présentations par affiche)

Lévesque S, St-Pierre K, Frost E, Michaud S. Molecular typing of Campylobacter isolates from environmental water using Amplified Fragment-Length Polymorphism (AFLP). Canadian Campylobacter Conference, September 2008. Montréal, Québec, Canada

Frost E, St-Pierre K, Lévesque S, Michaud S. Quantitative Real Time PCR for Campylobacter jejuni, C. coli and C. lari in Environmental and Untreated Drinking Water. Canadian Campylobacter Conference, September 2008. Montréal, Québec, Canada

Lavoie A, Michaud S, Frost E, Bénié GB, Lévesque S, St-Pierre K, Bonfils D. Role of rainfall and physicochemical parameters of water on the distribution of Campylobacter in the Eastern Townships. Canadian Campylobacter Conference, September 2008. Montréal, Québec, Canada.

Lévesque S, Chammat A, Frost E, Michaud S. Incidence of human campylobacteriosis and prevalence of Campylobacter in fresh whole retail chikens, Eastern Townships, Québec. 14th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2007. Rotterdam, The Netherlands.

St-Pierre K, Lévesque S, Frost E, Michaud S. Prevalence and Quantification of Campylobacter in Environmental Water Eastern Townships, Québec. 14th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2007. Rotterdam, The Netherlands.

St-Pierre K, Lévesque S, Frost E, Michaud S. Identification of 5 Campylobacter species based on PCR of the Hippuricase gene and PCR-RFLP. 14th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2007. Rotterdam, The Netherlands.

Lévesque S, Michaud S, Frost E, Bénié G, Pomerleau M, St-Pierre K, Chammat A, Desbiens R, Bonfils D, Thomas O, Bonn F, Quessy S, Ménard S, Milord F, Désilets J, Arbeit RD. The CAMPYLOGIS project : putting Campylobacter on the map. 14th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2007. Rotterdam, The Netherlands.

Lévesque S, Michaud S, Frost E, Bénié G, Pomerleau M, St-Pierre K, Chammat A, Desbiens R, Bonfils D, Thomas O, Bonn F, Quessy S, Ménard S, Milord F, Désilets J, Arbeit RD. Le projet CAMPYLOGIS, pour mettre le Campylobacter sur la « map ». 32è Congrès annuel de l'Association des Médecins Microbiologistes-Infectiologues du Québec, juin 2007. St-Sauveur, Québec, Canada.

Chammat A, Lévesque S, Frost E, Michaud S. Incidence de la campylobactériose humaine et prévalence du Campylobacter dans le poulet frais entier de l'Estrie. 32è Congrès annuel de l'Association des Médecins Microbiologistes-Infectiologues du Québec, juin 2007. St-Sauveur, Québec, Canada.

St-Pierre K, Lévesque S, Frost E, Michaud S. Les coliformes fécaux ne sont pas un bon indicateur de la présence du Campylobacter dans l'eau environnementale. 36è édition, Journée scientifique, mars 2007. Faculté de Médecine, Université de Sherbrooke.

Chammat A, Lévesque S, Frost E, Michaud S. Incidence de la campylobactériose humaine et prévalence du Campylobacter dans le poulet frais entier de l'Estrie. Journée de la recherche, février 2007. Université de Sherbrooke

Michaud S, Frost E, Lévesque S, Bénié G, Pomerleau M, St-Pierre K, Chammat A, Desbiens R, Bonfils D, Thomas O, Bonn F, Quessy S, Ménard S, Milord F, Désilets J, Arbeit RD. The CAMPYLOGIS project: putting Campylobacter on the map. Canadian Campylobacter Conference, November 2006. Vancouver, British Columbia, Canada.

Lévesque S, Frost E, Michaud S. Analyse des populations génétiques du Campylobacter jejuni de sources humaines, alimentaires et hydriques par Multilocus sequence typing (MLST). 35è édition, Journée scientifique, mars 2006. Faculté de Médecine, Université de Sherbrooke.
Gagnant du premier prix pour la meilleure présentation par affiche.

Lévesque S, Frost E, Michaud S. Multilocus Sequence Typing for Comparison of Water, Chicken, Raw Milk and Human Isolates of Campylobacter jejuni in Quebec 2006 Annual Conference of CACMID and AMMI, March 2006. Victoria, British Columbia, Canada.

Lévesque S, Frost E, Michaud S. Analyse des populations génétiques du Campylobacter jejuni de sources humaines, alimentaires et hydriques par Multilocus sequence typing (MLST). Journée de la recherche, février 2006. Université de Sherbrooke.

Lévesque S, Frost E, Michaud S. Multilocus sequence typing for comparison of water, chicken, raw mild and human isolates of Campylobacter jejuni in Quebec. Water Network Researcher Retreat, November 2005. Kananaskis, Alberta, Canada.

Lévesque S, Martel J, Frost E, Michaud S. Prevalence of Campylobacter in river and tributary water in the Eastern Townships, Quebec, Canada. 13th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2005. Gold Coast, Queensland, Australia.

Lévesque S, Bureau ME, Gagnon A, St-Pierre K, Frost E, Michaud S. Performance and reproducibility of disc diffusion compared with agar dilution for antibiotic susceptibility testing of Campylobacter. 13th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2005. Gold Coast, Queensland, Australia.

Lévesque S, St-Pierre K, Frost E, Michaud S. Determination of the optimal culture conditions for detecting Campylobacter spp. in environmental water. 13th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2005. Gold Coast, Queensland, Australia.

Lévesque S, Frost E, Michaud S. Comparison of antibiotic susceptibility profiles of Campylobacter jejuni cultured from humans, chickens, raw milk and environmental water in Quebec. 13th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2005. Gold Coast, Queensland, Australia.

Gibreel A, Kos VN, Keelan M, Trieber CA, Levesque S, Michaud S, Taylor DE. Macrolide resistance in Campylobacter jejuni and Campylobacter coli: molecular mechanism and stability of the resistance phenotype. 13th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2005. Gold Coast, Queensland, Australia.

Lévesque S, Frost E, Michaud S. Development of a Detection Protocol for Campylobacter in Water. 72nd Conjoint Meeting on Infectious Diseases (CACMID and AMMI), November 2004. Regina, Saskatchewan, Canada.

Lévesque S, Frost E, Michaud S. Performance and Reproducibility of Disc Diffusion Compared with Agar Dilution for Antibiotic Susceptibility Testing for Campylobacter. 72nd Conjoint Meeting on Infectious Diseases (CACMID and AMMI), November 2004. Regina, Saskatchewan, Canada.

Lévesque S, Michaud S. Comparison of antimicrobial resistance profiles of C. jejuni strains isolated from humans and chickens. 71st Conjoint Meeting of CACMID and AMMI, November 2003. Montréal, Québec, Canada.

Lévesque S, Michaud S. Comparison of antimicrobial resistance profiles of C. jejuni strains isolated from humans and chickens. 12th Internationnal Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO), September 2003. Aarhus, Denmark.