THE EPIDEMIOLOGY OF FOODBORNE PATHOGENS AND POTENTIAL MECHANISMS OF DISEASE

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THE EPIDEMIOLOGY OF FOODBORNE PATHOGENS AND POTENTIAL MECHANISMS OF DISEASE

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The purpose of this study was to contribute to the understanding of the presence of foodborne pathogens at different levels in the food supply chain of animal origin and the potential factors associated with their risk, and possible mechanisms of disease in humans. Given the broad spectrum of the issue, this study focuses on bacterial pathogens among different aspects of the food chain in the diverse State of Qatar, including animal, human and retail samples, and among conventional and organic dairies in New York State.

Using a combination of bacterial enrichments, biochemical and agglutination tests, and molecular detection, the presence of pathogens was tested for among samples taken from farms, slaughterhouses, retail stores and restaurants and from fecal samples obtained from humans admitted to hospitals with cases of gastroenteritis in Qatar, and milk and milk filter samples from conventional and organic dairies in New York.

Other shiga toxin-producing serotypes are becoming nearly as much of a concern as the more commonly known *E. coli* O157:H7. The presence of the cytolethal distending toxin (CDT) in isolates recovered from gastroenteritis cases suggests a role in the pathogenesis of the condition. Furthermore, the detection of CDT among food animal isolates along the food supply chain highlights the potential zoonotic risk. Being exposed to foodborne pathogens can increase the risk of chronic gastroenteritis sequelae, including Inflammatory Bowel Disease.

BIOGRAPHICAL SKETCH

Kenlyn E. Peters was born March 17, 1990 in Fort Wayne, Indiana, USA. She is the daughter of Susan and Martin Peters and sister of Brennan. Growing up on a farm around horses, dogs and cats, she developed an early love and fascination of animals and nature. After graduating from Canterbury High School in 2008, she attended Michigan State University and transferred to Cornell University where she received a Bachelor of Science degree in Animal Science. Working in an insect pathology lab and studying vector borne diseases she developed an interest in epidemiology and joined the epidemiology division at the Cornell University College of Veterinary Medicine to pursue a Ph.D.

This is dedicated to my family and friends, especially my grandfather, Frederick A. Hellmer, without whose unwavering support this work would not have been possible.

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CHAPTER 1

INTRODUCTION

Foodborne pathogens are a major health burden worldwide. In the U.S. alone, an estimated 48 million people are affected by foodborne pathogens each year and 128,000 of those cases result in hospitalization, and 3,000 in death (1). Worldwide it is estimated that there are 2.2 million deaths due to food and waterborne diseases (2).

Among the top bacterial foodborne pathogens are *E. coli*, *Salmonella* and *Campylobacter*. Other top pathogens include *Listeria*, Norovirus, *Clostridium* and *Toxoplasma* (3). *E. coli* is a bacillus, gram negative, facultative anaerobe. Most strains are harmless and occur naturally in the intestines of ruminants. There are six pathotypes associated with causing diarrhea, Shiga toxin-producing *E. coli* (STEC) is the group that is most commonly associated with foodborne illness, it is sometimes also referred to as verocytotoxin-producing (VTEC) or enterohemorrhagic (EHEC) *E. coli* (4). Cattle are the main source of human illness, due to ingestion of raw milk, meat or vegetables contaminated with manure. Contact with feces of infected humans is also a major source of illness. There are 265,000 cases estimated annually in the U.S. (4).

Salmonella is a bacillus, gram negative, facultative anaerobe which contains two species, *S. enterica* and *S. bongori*. There are six subspecies of *S. enterica* and more than 2,500 serotypes. Important pathogenic serotypes are *S.* enteritidis and typhimurium. The main sources of *Salmonella* infection are poultry, cattle and swine and it is also common in pets such as reptiles and birds. The major source of human illness is improper handling and preparation of food that has come into contact with manure such as beef, poultry, eggs and produce. Contact with infected people is also a cause of illness. There are 1.2 million estimated cases annually in the U.S. (5).

Campylobacter is a spiral, gram negative, microaerophilic anaerobe. Important pathogenic species are *C. coli*, *C. jejuni*, *C. lari* and *C. fetus*. Poultry and cattle are the main sources, as the bacteria naturally occur in their gastrointestinal tracts. Again, the main source of illness is improper handling and preparation of food contaminated with feces. There are 1.3 million estimated cases annually in the U.S. (6).

Gastroenteritis is an inflammation of the gastrointestinal tract caused by viruses, bacteria or other microorganisms. Symptoms include abdominal pain, vomiting and diarrhea. In addition to the immediate gastrointestinal symptoms associated with infection of these pathogens, there is the risk of chronic sequelae such as Inflammatory Bowel Disease (IBD) (7,8,9). The mechanisms by which these pathogens predispose hosts to these sequelae are poorly understood. Several studies hypothesize the role of the cytolethal distending toxin (CDT) in the pathogenesis (10,11,12). However the data remains scarce.

CDT is a bacterial toxin that initiates cell cycle arrest prior to mitosis in eukaryotic cells. It consists of three protein subunits, CdtA, CdtB, and CdtC. CdtB is the active subunit, the other two subunits bind and deliver CdtB into cells. CdtB's DNase I-like activity results in DNA double-strand breaks which can cause cellular distention and arrest of the G1 and G2 phases of the cell cycle with eventual cell death by apoptosis (10,11). Due to these occurrences, foodborne pathogens that carry the CDT genes have been implicated in the risk of gastroenteritis as an important virulence factor.

The following chapters are a series of complementary epidemiological studies to address and contribute to the body of knowledge on the occurrence of these pathogens in different human and animal populations. The epidemiology of foodborne pathogens in the food supply chain and

among humans is complex, this approach aims to contribute to gaps in knowledge regarding the issue. These studies were designed to address the following objectives:

1) Investigate the presence of CDT in major foodborne pathogens isolated from animals in the food supply chain and from gastroenteritis cases in a highly dynamic and diverse population.

2) Determine the prevalence of pathogens in retail samples to potentially identify sources and assess the potential risk of different types of retail foods.

3) Determine the prevalence of the same pathogens in fecal samples from human gastroenteritis cases and assess potential risk factors for each pathogen.

4) Assess the potential risk of foodborne pathogens at conventional and organic dairy farms.

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CHAPTER 2*

PREVALENCE OF CYTOLETHAL DISTENDING TOXIN IN *SALMONELLA* AND *CAMPYLOBACTER* SPP. ISOLATED FROM FOOD ANIMALS AND HUMAN GASTROENTERITIS CASES IN QATAR

*This chapter has been prepared in the format for submission to the Journal of Infection and Public Health for publication

Abstract

Background. *Campylobacter* and *Salmonella* are two of the major foodborne pathogens that contribute to the burden of disease. The cytolethal distending toxin (CDT) has been identified as one of the virulence factors that may contribute to pathogenesis and gastroenteritis. The CDT is a trimeric subunit toxin produced by gram-negative bacteria that initiates cell-cycle arrest and causes affected cells to die by apoptosis. This study investigated the occurrence of CDT among *Salmonella* and *Campylobacter* spp. isolates recovered from animals along the food supply chain and from gastroenteritis cases in Qatar.

Methods. Samples were screened for the presence of the two pathogens using a combination of bacterial enrichment and molecular detection and positive samples were examined for the presence of CDT using a polymerase chain reaction (PCR) approach.

Results. *C. jejuni* and *C. coli* occurred at similar rates among non-human sources, whereas *C. jejuni* occurred at a higher rate compared to *C. coli* in human cases. Both *cdtB* and *cdtC* were detected at a higher rate among *C. jejuni* than *C. coli* recovered from human cases. Only *cdtB* was detected in *Salmonella* spp. isolates from animals and at a much lower rate.

Conclusions. The presence of CDT in isolates recovered from gastroenteritis cases suggests a role in the pathogenesis of the condition. Furthermore, the detection of CDT among food animal isolates along the food supply chain highlights the potential zoonotic risk.

1. Introduction

Foodborne illnesses pose major health burdens worldwide. In the U.S. alone, it is estimated that 48 million people become ill due to foodborne diseases, 128,000 of those cases being hospitalized and 3,000 resulting in death [1]. The World Health Organization (WHO) estimates that 2.2 million people worldwide die per year of diarrheal food and waterborne diseases alone [2]. Although data on individual countries is available, information on the global burden of foodborne diseases is lacking, but estimated cost per individual nation is high [3,4]. Foodborne Diseases Burden Epidemiology Reference Group (FERG) along with WHO are currently undertaking the estimation of the worldwide burden of foodborne disease, listing *Campylobacter* and *Salmonella* spp. among the top challenges [2].

The risk of foodborne pathogen transmission is exacerbated by the ease of travel and the globalization of trade [5]. This is especially true in such international areas as Qatar, where the ratio of expatriates to natives is 9:1. Areas of such cultural diversity are key to studying foodborne illnesses. *Campylobacter* and *Salmonella* are two of the major foodborne pathogens that contribute to the burden of disease [6] especially in very international regions. Current detailed data on the pathogenicity of foodborne pathogens is required to mitigate the risks of transmission.

Food animals are known to be reservoirs for foodborne pathogens, therefore food products from these animals could be considered a threat to the safety of the food supply chain, putting humans at risk of contracting salmonellosis [7,8,9,10]. Perpetuation of pathogens in the environment may exacerbate the risk of salmonellosis as well from direct exposure through occupational practices [11,12]. Furthermore, the risk of pathogenesis in the population has been

attributed to cross-contamination between meat handlers and carcasses in processing plants around the world [13,14]. Knowledge of the occurrence and distribution of these pathogens along the food supply chain of animal origin is needed in order to devise cost-effective strategies to mitigate associated risks.

In addition to the immediate gastrointestinal symptoms associated with infection of these pathogens, there is the risk of chronic sequelae. The mechanisms by which these pathogens predispose hosts to these sequelae are poorly understood. Several studies hypothesize the role of CDT (cytolethal distending toxin) in the pathogenesis [15,16,17]. However the data remains scarce.

CDT is a bacterial toxin that initiates cell cycle arrest prior to mitosis in eukaryotic cells. It consists of three protein subunits, CdtA, CdtB, and CdtC. CdtB is the active subunit, the other two subunits bind and deliver CdtB into cells. CdtB's DNase I-like activity results in DNA double-strand breaks which can cause cellular distention and arrest of the G1 and G2 phases of the cell cycle with eventual cell death by apoptosis [15,16]. Due to these occurrences, foodborne pathogens that carry the CDT genes have been implicated in the risk of gastroenteritis as an important virulence factor. Our objectives were: 1) to investigate the presence of this gene in major foodborne pathogens isolated along the food supply chain and from gastroenteritis cases in a highly dynamic and diverse population; and 2) examine the potential correlation between the presence of the CDT among different supply chain samples and human cases.

2. Methods

2.1. Target and Study Populations

We carried out a cross-sectional study to address the stated objective. *Campylobacter* and *Salmonella* spp. were recovered from the target populations. Two populations were involved in the sampling, human and non-human. Human subjects were selected from individuals admitted to Hamad Medical Corporation hospitals in Qatar with complaints of gastroenteritis. Fecal samples were collected from the patients and tested bacteriologically for the presence of *Salmonella* and *Campylobacter* spp. These samples were collected during routine patient care. Ethical approval was granted from the Institutional Review Board for the use of these samples and patient data. The patients' backgrounds were diverse, including differing ethnicity, nationality, gender, age and diagnosis.

Non-human samples were collected along the food supply chain of animal origin. The animal sources included cattle, camels, chickens and sheep, either at dairy operations, or at abattoirs being processed for human consumption. The animal operations were located throughout the country, and the abattoirs were located in the capitol and processed all the meat supply to the market. Farms were selected randomly and sampled during both the hot (April-October) and cold (November-March) seasons. The abattoirs were also sampled during both seasons to capture potential seasonal variation and animals were selected randomly within the abattoirs. Farmers and managers were sent letters of solicitation outlining the objectives of the study and participation was voluntary.

2.2. Sampling Procedures

Three types of samples were collected from animals on the dairy and camel farms, including udder swabs, milk and fecal samples. Sterile gauze pads (4x4in) were used to swab the teats on the udders of individual animals and placed in sterile vials. Composite milk samples

(approx. 25mL from each teat) were also collected in sterile vials. Approximately 100g of feces were collected per rectum using artificial insemination gloves and sterile lube and placed in plastic sterile collection containers. In addition to samples collected from the animals, environmental samples such as bedding, and water and feed trough swabs were collected. Approximately 100g of bedding was collected and placed in sterile bags. Water and feed troughs were also swabbed with sterile gauze pads (4x4in) and placed in sterile vials.

At the abattoirs, approximately 50g of feces was collected from the inside of the large intestines of slaughtered sheep and placed in sterile containers. Carcasses of sheep, cattle and camels were swabbed with sterile gauze pads in four different areas after being dressed, washed and inspected by authorities. Areas targeted were the neck, thorax, flank, brisket and rump. Sterile gloves were worn and changed between each sample so as to prevent cross-contamination. Four swabs were taken from chicken carcasses, two from the inside, and two from the outside, targeting the neck, breast, underwing, thigh and visceral cavity. All samples were transported to Weill Cornell Medical College of Qatar in ice boxes for processing.

2.3. Pathogen Isolation

2.3.1. *Human Samples*

Pathogens were isolated from human samples using standard bacteriological procedure. 1g of the collected stool samples was diluted with 10ml of phosphate buffered saline (PBS, pH 7.2; Sigma, St. Louis, MO, USA) and 500µl of this dilution was added to 5ml of Selenite broth (Oxoid, Basingstoke, Hampshire, UK) for enrichment and was incubated at 37°C for 24-48hr.

2.3.1.1. *Salmonella* spp.- The enriched samples were subcultured onto MacConkey agar and incubated at 37°C for 24hr. Colonies were screened using biochemical tests such as Kilger's

iron agar, motility indole-urea agar, Lysin iron agar and o-nitrophenyl-β-D-galactopyranoside. Colonies from these screenings were identified with confirmatory biochemical tests using API 20E (bioMereux, Marcy I'Etoile, France) or VITEK (bioMereux).

2.3.1.2. *Campylobacter* spp.- Samples were incubated on CAMP agar at 42°C in microaerophilic conditions for the isolation of *Campylobacter* spp. Identification of presumptive pathogens was performed using biochemical tests and serum agglutination reactions according to standard methods.

2.3.2. *Non-human Samples*

2.3.2.1. *Salmonella* spp.— Non-human *Salmonella* samples were enriched in Tetrathionate broth (Becton, Dickinson and Company; Sparks, MD, USA) and were then spread on XLT4 agar plates (MOLTOX®, Boone, NC, USA). Positive colonies of the primary enrichment media were then transferred to 500µl of BHI broth (secondary enrichment) and incubated for 24hr at 37°C.

2.3.2.2. *Campylobacter* spp.– The primary enrichment consisted of BHI supplemented with Cefoperazone (6mg/L), Vancomycin (6mg/L), and Amphotericin B (2mg/L). The primary enrichment was inoculated with the samples and incubated at 37°C for 24hr. The secondary enrichment also was BHI and was also incubated at 37°C for 24hr.

2.4. *PCR Detection*

PCR detection was performed to determine pathogen presence using the BAX® Automated System (Dupont, USA). A 5µl aliquot of the respective secondary enrichment (BHI or MEC broth) was added to 200µl of the buffer (proteinase-containing lysis buffer) provided by

the manufacturer. Samples were then heated in the lysis reagent solution to rupture the bacterial cell wall and release the DNA. PCR tablets, which contain all the reagents necessary for PCR plus fluorescent dye, were hydrated with the lysed sample and processed in the cycler/detector provided by the manufacturer. Within a few hours, the polymerase chain reaction (PCR) amplified a DNA fragment specific to the target. The amplified DNA generates a fluorescent signal, which the BAX® system application uses to analyze the findings. Results are displayed on a monitor screen as simple positive or negative symbols.

2.5. *CDT Detection*

Genomic DNA was then extracted from the pure cultures following the MasterPure DNA purification kit (Epicenter Biotechnologies, Madison, WI, USA). Then PCR amplification and gel electrophoresis were utilized to determine the presence of the genes for CDT subunits among the samples. CdtB was the primary subunit tested for due to it being the active subunit (*15, 16*). The CDT genes were detected in *Campylobacter* samples using the Takara Campylobacter Detection Kit (Takara Bio Inc., Japan). Primers used for *cdtB* detection in *Salmonella* samples were; Forward: 5'-tgcagctatatttcttttgcctgcg-3' and Reverse: 5'-acagcttcgtgccaaaaaggc-3'.

2.6. Statistical Analysis

The population specific prevalence of these pathogens was computed as the proportion that tested positive out of all samples that were tested within each population. The prevalence of CDT within each pathogen and among different populations was computed as the proportion that tested positive for *cdt* genes out of all the samples that tested positive for the particular pathogens. The significance of differences in the prevalence of the pathogens between humans and non-human, and the prevalence of CDT among isolates from the two populations was

evaluated using the student-test. The odds of a particular pathogen within each population was evaluated using logistic regression and quantified using the odds ratio (OR). The significance of association of both pathogens in a particular sample from non-human sources was evaluated using the McNemar chi-square test. All statistical tests were performed using the SPSS v.23 (IBM-statistical software, White Plains, NY) and P-values were evaluated at $P < 0.05$.

3. Results

Salmonella spp. was present in 159 out of 1128 non-human samples (14%) and in 402 out of 776 human samples (52%). There was a significant difference in the prevalence of *Salmonella* spp. between the two populations. The odds were about 7 times greater to isolate *Salmonella* spp. from humans in comparison to non-humans (Table 2.1). *Campylobacter* was present in 155 out of 1128 non-human samples (14%) and 177 out of 776 human samples (23%). The odds were about two times greater to detect *Campylobacter* spp. among gastroenteritis cases in comparison to non-human sources (Table 2.1).

Within the non-human samples there was no significant difference in the prevalence of *Salmonella* in comparison to *Campylobacter* (both 14%). We also evaluated the odds of pathogens, *Campylobacter* spp. and *Salmonella* spp. occurring in samples collected from non-human sources using the McNemar chi-square test. A total of 30 samples had both pathogens and there was no significant association which indicates that the occurrence of both pathogens was random.

C. jejuni was more common among the human isolates in comparison to *C. coli* (77 vs. 24%) (Fig. 2.1). *C. lari* was not detected among the samples collected from humans. The odds

Table 2.1. The significance of association of the distribution of the pathogens in the study between human and non-human sources.

Source	Positive	Negative	Odds ratio and (95%Confidence Interval)
Salmonella spp.			
Human	402	374	
Non-human	159	969	6.6 (5.3, 8.2)
Campylobacter spp.			
Human	177	599	
Non-human	155	973	1.9 (1.5, 2.4)

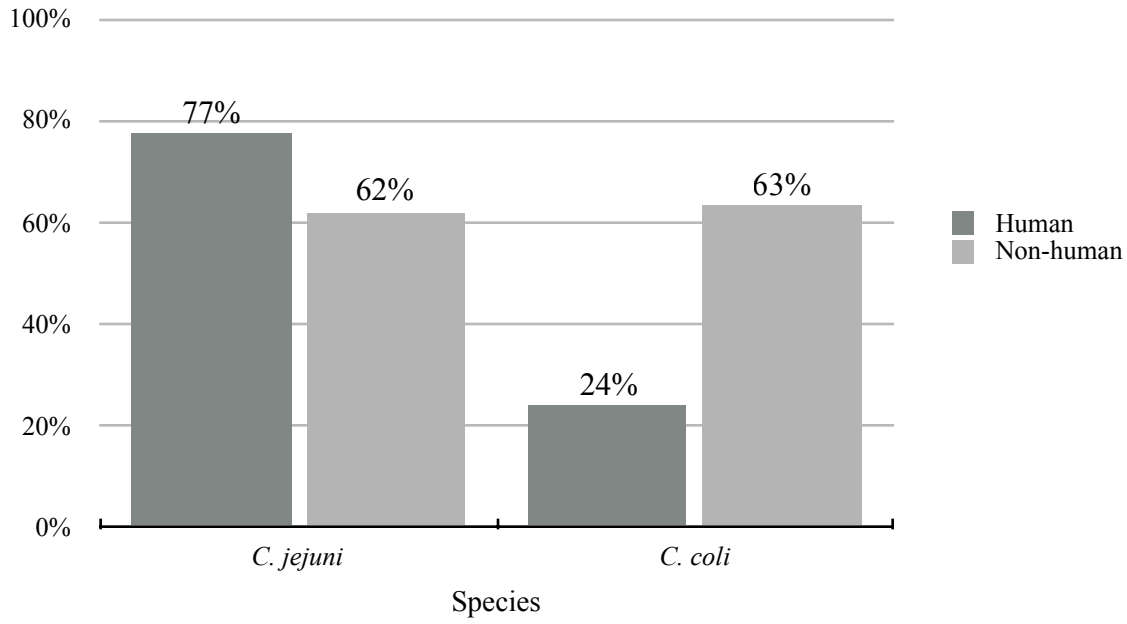

Figure 2.1. Prevalence of *Campylobacter* spp. in samples along the food supply chain of animal origin and in cases of gastroenteritis in the target populations.

were 4 times greater to isolate *C. jejuni* from gastroenteritis cases compared to *C. coli* (OR = 4.0) (Table 2.2). Both *C. jejuni* and *C. coli* occurred at an equal proportion among non-human samples (62 and 63%) (Fig. 2.1). The odds of *C. jejuni* occurrence was two times greater in samples collected from gastroenteritis cases compared to samples from non-human sources (OR = 2.1) (Table 2.3). On the other hand, the odds were about six times greater to detect *C. coli* in samples from non-human sources in comparison to gastroenteritis cases (OR = 5.5) (Table 2.3). Neither of the pathogens were isolated from the same case of gastroenteritis.

Among the *Campylobacter* spp. isolates from non-human sources, 96 were *C. jejuni* and 98 were *C. coli*. The *cdtB* gene was more common among the *C. coli* isolates in comparison to *C. jejuni* isolates (54 vs. 29%), occurrence of the *cdtC* gene was similar (51 vs. 34%) (Fig 2.2). Table 2.4 shows the significance of the concurrent occurrence of *cdtB* and *cdtC* in *Campylobacter* spp. isolated from each source. The kappa statistics demonstrate they are likely pathogenic due to the occurrence of both genes (Table 2.4).

The distribution of *cdtB* and *cdtC* gene among the human isolates of *C. jejuni* and *C. coli* are shown in Figure 2.3. These two genes are about three times more common among the *C. jejuni* than *C. coli* isolates and were detected at a relatively similar proportion within each species (Fig 2.3). The majority of *C. jejuni* from humans had both genes and there was significant agreement in their occurrence beyond chance (*kappa* = 0.6) (Table 2.4). Although the two genes were detected at a lower rate among the *C. coli* isolates in comparison to *C. jejuni*, there was significant agreement in the occurrence of the *cdtB* and *cdtC* among the *C. coli* isolates (*kappa* = 0.8) (Table 2.4).

Table 2.2. The odds of *Campylobacter* spp. within each source (human and non-human) as computed using the McNemar's chi-square test.

Source		*C. coli*		Odds ratio and 95%CI	*Kappa* statistics
		Positive	Negative		
Human					
C. jejuni	Positive	10	127		
	Negative	32	8	4.0 (2.7, 6.0)	0
Non-human					
C. jejuni	Positive	40	58		
	Negative	56	1	1.0 (0.7, 1.5)	0

Table 2.3. The significance of association of the distribution of *Campylobacter* spp. in the study between human and non-human sources.

Source	*C. jejuni* positive	*C. jejuni* positive	Odds ratio and 95%CI	*C. coli* positive	*C. coli* positive	Odds ratio and 95%CI
Human	137	40		42	135	
Non-human	96	59		98	37	
			2.1 (1.3, 3.4)			Inverse = 5.5 (3.4, 8.9)

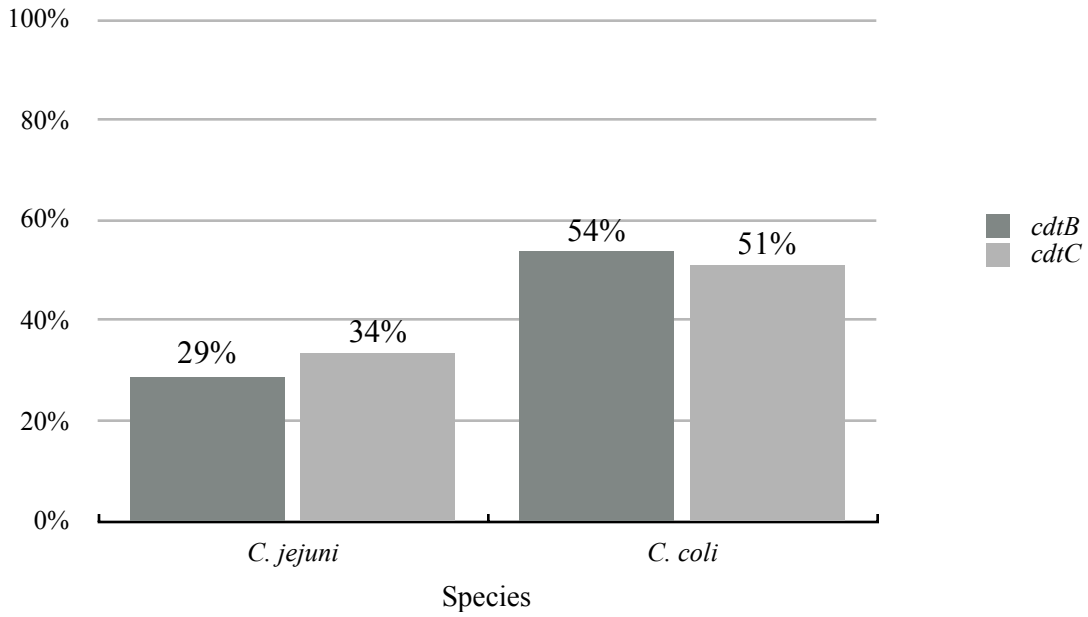

Fig 2.2. The proportion of samples with *cdtB* and *cdtC* among *C. jejuni* and *C. coli* isolates recovered from the food supply chain (non-human) in the target population.

Table 2.4. The association between *cdtB* and *cdtC* in *Campylobacter* spp. isolated from each source as computed using the McNemar's chi-square test.

Source		*cdtC*		Odds ratio and 95%CI	*Kappa* statistics
		Positive	Negative		
Human *C. jejuni*					
cdtB	Positive	99	19		
	Negative	12	47	1.6 (0.7, 3.6)	0.6
Human *C. coli*					
cdtB	Positive	31	8		
	Negative	3	135	2.7 (0.6, 15.6)	0.8
Non-human *C. jejuni*					
cdtB	Positive	41	11		
	Negative	4	99	2.8 (0.8, 11.8)	0.8
Non-human *C. coli*					
cdtB	Positive	66	17		
	Negative	13	61	1.3 (0.6, 2.9)	0.6

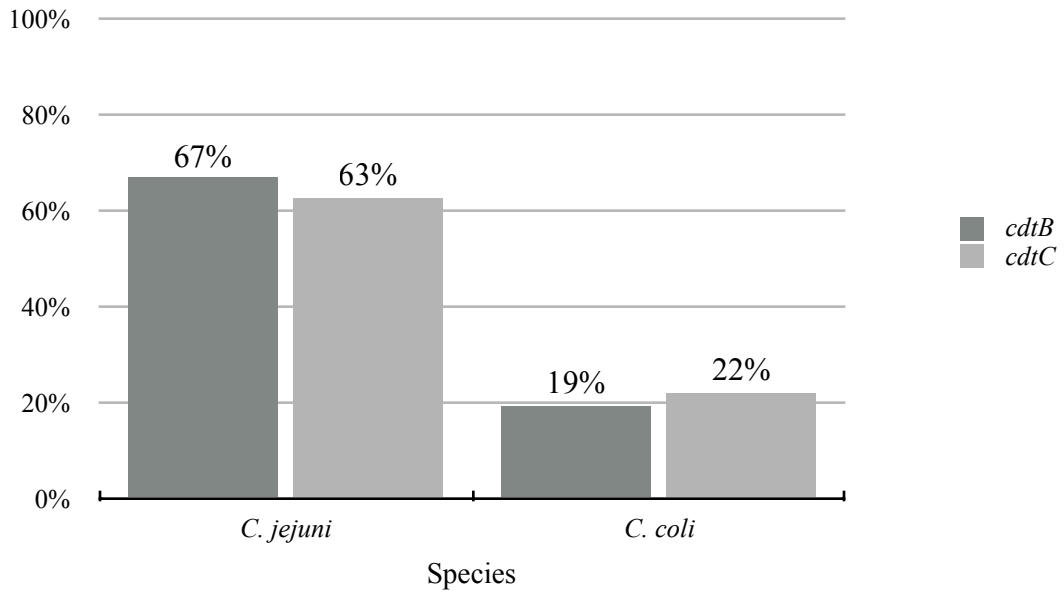

Figure 2.3 The proportion of samples with *cdtB* and *cdtC* among *C. jejuni* and *C. coli* isolates recovered from cases of gastroenteritis (human) in the target population.

Only *cdtB* was detected in *Salmonella* spp. animal samples and at a much lower proportion (3%) in comparison to its proportion among the *Campylobacter* isolates. Similarly, the *cdtB* gene was detected at a lower proportion among the *Salmonella* spp isolates from human samples (5%).

4. Discussion

The overall objectives of the current study were to shed light on the mechanisms by which foodborne pathogens predispose people to the risk of illness by identifying possible pathogenic agents. The study focused on two major foodborne pathogens, *Campylobacter* and *Salmonella* spp. and the possible role of CDT in pathogenesis. Foodborne pathogens have been incriminated in the risk of gastroenteritis and the mechanism of disease is currently not fully understood [6]. The two foodborne pathogens investigated in this study are among the common pathogens that pose significant burden of disease around the world [2,3,4]. Gastroenteritis infections caused by these two pathogens are mostly self-limiting and admissions to hospitals are not common. In a few cases, around 6-7%, patients with gastrointestinal illness may develop sequelae with serious consequences [4]. Many factors could play roles in exacerbating the risk of gastroenteritis including CDT [15,17].

We used a multidisciplinary epidemiological approach to investigate the occurrence of these foodborne pathogens in diverse populations (non-humans and humans) and determined the presence of the CDT among the pathogens in order to shed light on one of the putative mechanisms of pathogenicity [15,17]. Qatar was chosen due to its diverse social and cultural population which includes diverse food and methods of food preparation. Because of this diversity, we would expect to see diverse pathogens and mechanisms of disease. The more

knowledge gained on mechanisms of pathogenicity would greatly aid in mitigating the risk of these pathogens.

We examined the occurrence of these two foodborne pathogens among two populations, humans and animals. To our knowledge this is the first study that attempted to investigate these pathogens in human and animal populations in the same geographic area [11]. Other studies examine the risk of transmission of these pathogens between animals and humans among certain sectors, food handlers or processors [12,13]. Our study showed that both pathogens occur at relatively high proportions in samples collected from gastroenteritis cases and from the food supply chain of animal origin. The data also hinted at the potential of transmission of the two pathogens through the food supply chain to human hosts. The investigation was a cross-sectional study where samples were collected from the sources at one point of time and it is difficult to extrapolate the directionality of the transmission. The rationale for the apparent higher occurrence of the pathogens among humans in this study could be explained by the fact that all the samples were collected from patients admitted to the hospital with the complaint of gastroenteritis, a condition associated with *Campylobacter* and *Salmonella* spp. infections.

Although CDT is known as one of the virulence factors that plays a role in the pathogenicity of *Campylobacter* spp., the exact mechanism by which gastroenteritis develops is poorly understood. Most of the published research has focused on the method in which CDT affects cells and not proportion of infection. It has been suggested that differences in the protein subunits *cdtA* and *cdtC* could influence which types of cells CDT affects, so while CDT is not specific to different types of bacteria, it may be affected by cell specificity which could explain differing pathogenicity among hosts [15,16]. In this study, the association between the presence

of both *cdtB* and *cdtC* among gastroenteritis cases was investigated and we found that both subunits were detected in 84% of *C. jejuni* and 79% of *C. coli* cases with CDT genes. In comparison to Mortensen's et al. study, we were able to detect both subunits of the CDT in a lesser, but not significantly different, proportion [18]. However, the role of the CDT in the pathogenesis of gastroenteritis is not fully understood. By virtue of its toxicity, CDT has been incriminated in the risk of chronic gastroenteritis sequelae, including Inflammatory Bowel Disease as shown in studies on rats [17]. CDT has the ability to attack the cells of intestinal villi, which allows normal bacterial biota to enter and cause infection.

One of our objectives was to shed light on the correlation in the occurrence of these foodborne pathogens and the CDT genes in the food supply chain of animal origin and in cases of gastroenteritis in humans. The intent was to evaluate this correlation at the population level and examine whether the rate of occurrence of the CDT gene for these pathogens was similar among isolates from the two populations. The rationale was to explore whether the food supply chain of animal origin has the potential to predispose humans to the risk of gastroenteritis and whether we could identify points of intervention along the chain. We are not familiar with studies that have attempted to address a similar objective. One study examined the occurrence of the CDT genes in *Campylobacter* spp. from animal and human sources, however it was not clear if the isolates were collected at the same time [19]. Acik et al. examined the prevalence of the *cdtB* and *cdtC* in samples of *C. jejuni* and *C. coli* recovered from sheep and reported a similar proportional occurrence of this virulence factor as observed in our study [20]. Other studies observed convenient samples of isolates or single species [21,22,23] and some specifically looked at strains known to carry CDT genes [24, 25]. Reports of *Campylobacter* prevalence are

similar to our findings, *C. jejuni* prevalence was much higher than *C. coli* [21,23], though these studies were solely in regards to chickens. We also sampled multiple species of animals, some not previously reported. Work has been done on the different variations of CDT production by different strains of *E. coli* [24, 26] and extensive work has been done on *Campylobacter* [21,23,25,27], but little data is present on *Salmonella*.

5. Conclusion

The high prevalence of the *cdt* genes among *Campylobacter* isolates, especially *C. jejuni* in human gastroenteritis cases (*cdtB*: 67%, *cdtC*: 63%) points to a critical role as a virulence factor in the pathogenesis of the condition. The concurrent occurrence of this virulence factor (*cdtB* and *cdtC*) among isolates from the food supply chain of animal origin indicates that these food sources pose a risk to humans in terms of gastroenteritis and its sequelae. In spite of the fact that the *Campylobacter* spp. isolates had a similar rate of occurrence of the genes as gastroenteritis cases, none of the live animals showed any clinical signs of disease. By virtue of its toxicity, CDT has been incriminated in the risk of chronic gastroenteritis sequelae, including Inflammatory Bowel Disease and Inflammatory Bowel Syndrome as shown in studies on rats [17]. Knowledge gained on the occurrence of these foodborne pathogens in the food supply system and the presence of the virulence factor could be used to introduce intervention strategies to mitigate their associated risk.

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CHAPTER 3

RISK OF FOODBORNE PATHOGENS ASSOCIATED WITH RETAIL PRODUCTS IN QATAR

*This chapter has been prepared in the format for submission to the Journal of Food Safety for publication

Abstract

Foodborne illness has been determined to be one of the major limitations to the advancement of world health. Bacterial pathogens among the leading causes of foodborne illness are *E. coli*, *Campylobacter*, *Salmonella*, and *Listeria*. In an effort to understand the risk of these foodborne pathogens in the food supply chain in Qatar, this study investigated the presence of these pathogens among retail products. Using a combination of bacterial enrichments and molecular detection, swabs and food samples collected from retail items were screened for the presence of these foodborne pathogens. E. coli O157:H7 was detected at a proportion of 4.2%. Other *E. coli* serogroups were detected at a variant proportions: O26 (5.9%), O111 (3.5%), O121 (1.4%), O45 (20.2%), O103 (2.1%) and O145 (2.1%). The occurrences of the other pathogens varied among the samples: *Salmonella* (13.6%), *Listeria* (5.2%), and *Campylobacter jejuni* (1%), *coli* (8%) and *lari* (0%).

Introduction

Foodborne illness is a major health burden worldwide. In the U.S alone it is estimated by the Centers for Disease Control and Prevention (CDC) that 48 million people become ill due to foodborne diseases, 128,000 of those being hospitalized and 3,000 resulting in death (Centers for Disease Control and Prevention 2015). The World Health Organization (WHO) estimates that 2.2 million people per year worldwide die of diarrheal food and waterborne diseases alone (World Health Organization 2015). Foodborne Diseases Burden Epidemiology Reference Group (FERG) along with WHO are currently undertaking the estimation of the worldwide burden of foodborne disease, but when estimated by individual nations, the cost per episode is high (Havelaar et al. 2009; Hoffmann et al. 2012).

The risk of foodborne pathogen transmission is increased by the ease of travel and the globalization of trade (Käferstein et al. 1997). This is especially true in international areas such as Qatar, where the ratio of expatriates to natives is 9:1. Areas of such cultural diversity are key to studying foodborne illnesses. *Campylobacter* and *Salmonella* are two of the major foodborne pathogens that contribute to the burden of disease (Hird et al. 2009) especially in very international regions. The risk of consumers contracting foodborne illnesses is increased by food consumption in public places due to potential post retail contamination. Current detailed data on the pathogenicity of foodborne pathogens is required to mitigate the risks of transmission.

Several modes of transmission have been proposed in the retail world. Recontamination is a major concern in factories, stores, restaurants and the home (den Aantrekker et al. 2003; Reij et al. 2004; Kusumaningrum et al. 2003). A few studies have investigated bacterial survival on surfaces such as stainless steel, aprons, gloves and hands (Jackson et al. 2007; Lues and Van

Tonder 2007). Another potential cause is the presence of biofilm in factories and production facilities (den Aantrekker et al. 2003). Risk assessments are in place in order analyze food safety but it has been called into question whether some of these methods are an accurate measurement (Havelaar et al. 2010; Newell et al. 2010). Our objective was to assess the prevalence of major foodborne pathogens among retail samples in a highly dynamic and diverse population to shed further light on the risk associated with different types of retail food.

Methods

Sample Collection

The Municipality of Doha was contacted and permission was acquired for sampling. Their officers recommended five major retail stores and five large restaurants to sample and accompanied our research team on sampling trips. At the retail stores, samples were taken from different types of meat, packages, displays and the processing area. At restaurants, samples were taken from the processing area before storage, the storage area and the food prep area. Sterile gauze pads (4x4in) were used to swab the surfaces and utensils and the swab was immediately placed into a sterile vial. Cuts of meat samples, cheese, and samples of ready-to-eat (RTE) food were collected aseptically and placed directly into the sterile tubes. All samples were transported to Weill Cornell Medical College of Qatar in ice boxes for processing. Various types of foods were sampled, including beef, chicken, lamb, goat, camel, seafood (fish, shrimp, crab, cuttlefish, squid), cheese and salads. Ready to eat (RTE) foods included deli meats, cheeses and salads. Surfaces included tables, cutting boards, knives, containers, refrigerators, serving plates, gloves, balances and mincing machines. At the laboratory, the initial enrichment media was added aseptically directly to the sampling tubes.

Sample Processing and Pathogen Detection

Samples were screened for *E. coli* O157:H7, non-O157 shiga toxin-producing *E. coli*, *Salmonella*, *Campylobacter jejuni*, *coli* and *lari,* and *Listeria monocytogenes* using the BAX® System (Dupont, USA). The BAX® system is a real-time polymerase chain reaction (PCR) process which consists of bacterial enrichment and molecular detection. The bacterial enrichment included two steps: primary (bacterial repair) and secondary (bacterial growth).

E. coli O157:H7—In the primary enrichment all samples were inoculated into Modified *E. coli* broth (MEC broth) supplemented with novobiocin (16 mg/L) at a ratio of 1:10. The inoculum was incubated for 24hr at 37^{o}C. A total of 20µl of the incubated enriched inoculum was transferred into 1 ml of the secondary enrichment medium (Brain Heart Infusion (BHI) without antibiotics) and incubated for three hours at 37^{o}C before processed for the real-time PCR.

***Non-O157 Shiga Toxin-Producing E. coli*—**The test was performed on the bacterial lysate prepared after the secondary enrichment described above. The samples were initially screened using the STEC Suite kit which targets the *stx1*, *stx2* and *eae* genes. Positive samples were further screened for the food adulterant serotypes of *E. coli* which include O26, O45, O103, O 111, O121, and O145.

Salmonella spp.— Samples were enriched in Tetrathionate broth (Becton, Dickinson and Company; Sparks, MD, USA) and were then spread on XLT4 agar plates (MOLTOX®, Boone, NC, USA). Positive colonies of the primary enrichment media were then transferred to 500µl of BHI broth (secondary enrichment) and incubated for 24hr at 37^{o}C.

Campylobacter jejuni*, *coli* and *lari- The primary enrichment consisted of BHI supplemented with Cefoperazone (6mg/L), Vancomycin (6mg/L), and Amphotericin B (2mg/L). The primary

enrichment was inoculated with the samples and incubated at 37°C for 24hr. The secondary enrichment also was BHI and was also incubated at 37°C for 24hr.

***L. monocytogenes*—** The samples were pre-enriched with Demi-Fraser broth (Oxoid) and incubated for 22-26hr at 30°C. The MOPS Buffered Listeria Enrichment Broth - BBL Listeria enrichment broth, MOPS free acid and Mops sodium salt (Fisher Scientific) was used as a selective media in which the samples were again incubated at 35°C for 18-24hr.

***PCR Detection*—**The PCR detection was performed using the BAX® Automated System. A 5µl aliquot of the respective secondary enrichment (BHI or mEC broth) was added to 200µl of the buffer (proteinase-containing lysis) provided by the manufacturer. Samples were then heated in a lysis reagent solution to rupture the bacterial cell wall and release the DNA. PCR tablets, which contain all the reagents necessary for PCR plus fluorescent dye, were hydrated with lysed sample and processed in the cycler/detector provided by the manufacturer. Within a few hours, the polymerase chain reaction (PCR) amplified a DNA fragment specific to the target. The amplified DNA generates a fluorescent signal, which the BAX® system application uses to analyze the findings. Results are displayed on a monitor screen as simple positive or negative symbols.

Statistical Analysis

The prevalence of a particular pathogens or serogroup in each sample was computed as the proportion of the samples that tested positive out of all samples tested from that particular food. The odds of a particular pathogen within each category was evaluated using logistic regression and quantified using the odds ratio (OR). All statistical tests were performed using the SPSS v.23 (IBM-statistical software, White Plains, NY) and p-values were evaluated at $P < 0.05$.

Results

E. coli O157:H7 was detected among all the samples at a proportion of 4.2%. *E. coli* genes *stx* and *eae,* which are linked to Shiga toxin-producing *E. coli* (STEC), were detected at a proportion of 26.8% and 26.5% respectively. Samples that were positive for both the *stx* and *eae* gene were considered positive for STEC, and 16.7% of the samples were positive (Figure 3.1). Samples were also tested for other specific serotypes of non-O157 STEC that are known as food adulterants. 5.9% of the samples were positive for *E. coli* O26, 3.5% for O111, 1.4% for O121, 20.2% for O45, 2.1% for O103 and 2.1% for O145 (Figure 3.1). *Salmonella* spp. were detected in 13.6% of the samples and *Listeria monocytogenes* was detected in 5.2 %. None of the samples were positive for *Campylobacter lari* but 1% were positive for *jejuni* and 8% were positive for *coli* (Figure 3.1).

Table 3.1 shows the distribution of *E. coli* serogroups by food type. *E. coli* O157:H7 had the highest detection proportion in beef samples (beef chops and minced beef), it was not detected in chicken samples. None of the serogroups were detected in cheese samples. Serogroup O45 had the highest occurrence, especially among seafood, RTE, and chicken samples. The seafood and chicken samples were fresh samples. The RTE samples consisted of seasoned chicken, seasoned beef, and chicken cooked with rice. The serogroups O103, O111, O121, and O145 were not common among the samples (Table 3.1).

The detection of *Salmonella* spp., *L. monocytogenes*, *C. jejuni*, and *C. coli* by type of food is shown in Table 3.2. *Salmonella* spp. were common among beef, chicken, mutton, and surface samples. There was no significant difference in the odds of detection of *Salmonella* spp. from most sample types when compared to beef, but it was 2.5 (inverse of odds ratio) times

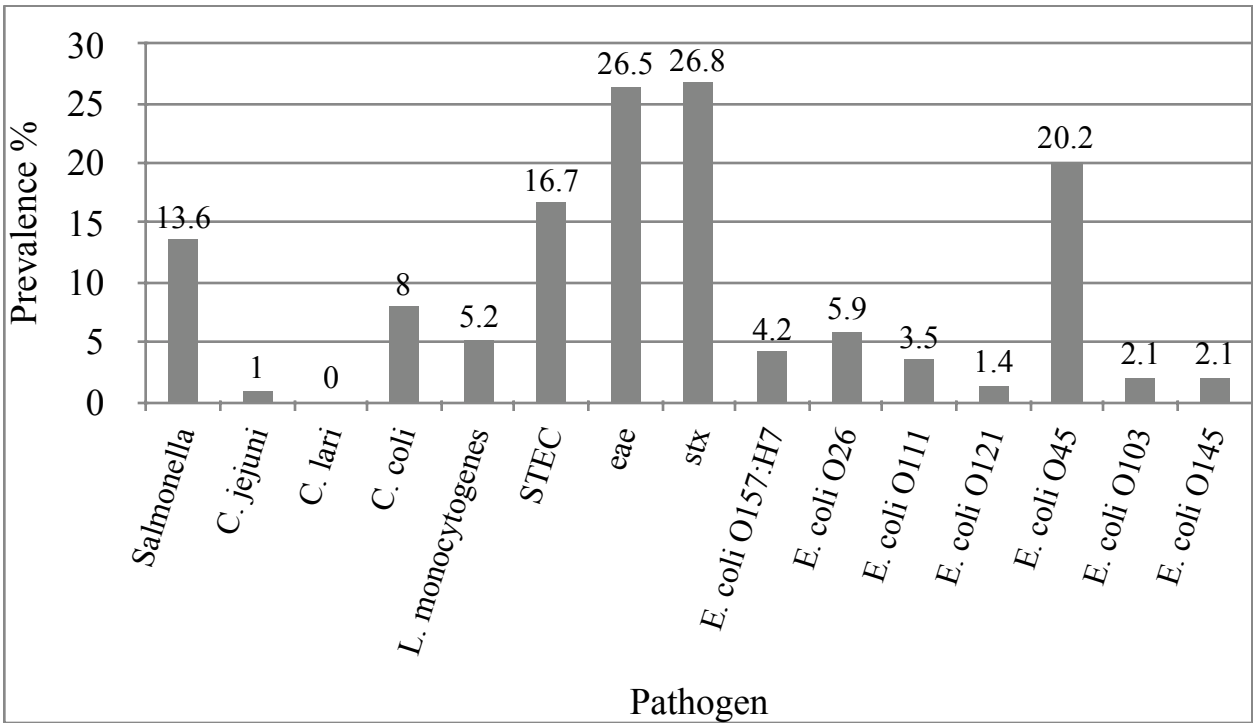

Figure 3.1. Overall pathogen prevalence.

Table 3.1. The occurrence of E. coli O157:H7 and the other non-O157 food adulterants among the different retail products and surfaces surveyed in the study.

Food type	*E. coli* serogroup						
	O157:H7	026	045	0103	0111	0121	0145
Beef [40][a]	4 (10%)[b]	4 (10%)	7 (17.5%)	1 (2.5%)	0	2 (5.0%)	1 (2.5%)
Chicken [21]	0	2 (9.5%)	7 (33.3%)	3 (14.3%)	2 (9.5%)	0	0
Mutton [30]	2 (6.7%)	4 (13.3%)	4 (13.3%)	0	0	0	2 (6.7%)
Seafood [62]	1 (1.6%)	0	14 (22.6%)	0	4 (6.5%)	0	1 (1.6%)
Cheese [11]	0	0	0	0	0	0	0
RTE [55]	3 (5.5%)	3 (5.5%)	13 (23.6%)	1 (1.8%)	3 (5.5%)	2 (3.6%)	1 (1.8%)
Surface [68]	2 (2.9%)	4 (5.9%)	13 (19.1%)	1 (1.5%)	1 (1.5%)	0	1 (1.5%)
Total [287]	12 (4.2%)	17 (5.9%)	58 (20.2%)	6 (2.1%)	10 (3.5%)	4 (1.4%)	6 (2.1%)

[a]: Number of samples tested

[b]: Proportion tested positive

Table 3.2. The occurrence of *Salmonella* spp., *Listeria monocytogenes*, *C. jejuni*, and *C. coli* among the different retail products and surfaces.

Food type	Foodborne pathogen			
	Salmonella spp	*Listeria monocytogenes*	*C. jejuni*	*C.coli*
Beef [40][a]	8 (20%)[b]	5 (12.5%)	0	0
Chicken [21]	5 (23.8%)	0	1 (4.8%)	1 (4.8%)
Mutton [30]	6 (20%)	2 (6.7%)	0	3 (10%)
Seafood [62]	0	4 (6.5%)	1 (1.6%)	6 (9.7%)
Cheese [11]	0	0	0	1 (9.1%)
RTE [55]	5 (9.1%)	3 (5.5%)	1 (1.8%)	5 (9.1%)
Surface [68]	15 (22.1%)	1 (1.5%)	0	7 (10.3%)
Total [287]	39 (13.6%)	15 (5.2%)	3 (1.0%)	23 (8%)

[a]: Number of samples tested

[b]: Proportion tested positive

Table 3.3. The odds of STEC and *Salmonella* spp. among the different retail products and surfaces.

	STEC			***Salmonella***		
Food Type	Regression coefficient	Standard error	Odds ratio and 95% CI	Regression coefficient	Standard error	Odds ratio and 95% CI
Beef	0		1.0	0		1.0
Chicken	-2.46	1.08	0.08 (0.01, 0.7)	0.22	0.65	1.3 (0.4, 4.4)
Mutton	-0.50	0.53	0.6 (0.2, 1.7)	0	0.60	1.0
Seafood	-2.89	0.79	0.07 (0.01, 0.3)	NS		1.0
Cheese	NS		1.0	NS		1.0
RTE	-1.26	0.50	0.3 (0.1, 0.8)	-0.92	0.61	0.4 (0.1, 1.3)
Surface	-0.84	0.44	0.4 (0.2, 1.0)	0.12	0.49	1.1 (0.4, 3.0)
Constant	-0.51	0.33		-1.39	0.40	

greater to isolate it from beef compared to RTE food (Table 3.3). *L. monocytogenes* was detected in beef, mutton, seafood, RTE, and surfaces and there was no significant difference in the detection probability of this pathogen among the different food types (Table 3.2). *C. jejuni* was detected in chicken, seafood, and RTE samples at a low probability. However, *C. coli* was detected at a higher proportion in mutton, seafood, Cheese, RTE, and surface samples (Table 3.2).

Table 3.3 shows the odds of detection of the STEC among the different food types. There was a significant association between the odds of these groups and the food type. Beef samples had the highest odds of harboring STEC pathogens. The odds were about 12 and 14 times greater to isolate STEC from beef samples compared to chicken and seafood samples, more than twice greater compared to surfaces, and three times greater compared to RTE foods (inverse of odds ratios, Table 3.3).

We then combined the samples into four categories of fresh meat (beef, mutton, chicken), RTE (RTE, cheese), seafood, and surface samples to further evaluate of the odds of these pathogens (Table 3.4). The odds were 3.2 (inverse of odds ratio) times greater to detect *Salmonella* spp. in meat samples in comparison to RTE. There was no significant difference in the detection of *Salmonella* between meat and other types of samples. It appeared that the odds of detecting *Campylobacter* spp. was about two times more in other samples compared to meat, however, the difference was not statistically significant (Table 3.4). The odds of detecting the STEC group in RTE and seafood samples was less in comparison to meat (inverse of the OR = 2.6, 11, respectively) (Table 3.4).

Table 3.4. The odds of the foodborne pathogens among meat, RTE, seafood and surface categories.

Salmonella					
Category	Regression coefficient	Standard error	p-value	Odds ratio	95% CI
Meat	0		0	1	
RTE	-1.17	0.53	0.03	0.31	(0.11, 0.88)
Seafood	-19.87	5104.51	1	0	0
Surface	0.07	0.39	0.86	1.07	(0.50, 2.30)
Constant	-1.33	0.26	0	0.26	
Campylobacter					
Category	Regression coefficient	Standard error	p-value	Odds ratio	95% CI
Meat	0		0.57	1	
RTE	0.71	0.61	0.24	2.04	(0.62, 6.74)
Seafood	0.78	0.61	0.20	2.19	(0.66, 7.24)
Surface	0.68	0.61	0.26	1.97	(0.60, 6.51)
Constant	-2.85	0.46	0	0.06	
STEC					
Category	Regression coefficient	Standard error	p-value	Odds ratio	95% CI
Meat	0		0.006	1	
RTE	-0.95	0.45	0.03	0.39	(0.16, 0.92)
Seafood	-2.38	0.76	0.002	0.09	(0.02, 0.41)
Surface	-0.32	0.38	0.40	0.72	(0.34, 1.53)
Constant	-1.03	0.24	0	0.36	

Discussion

Our results differed from similar studies in that other studies showed a higher prevalence of *Listeria*, *Campylobacter* and *Salmonella*. In a Canadian study on raw poultry and meat products, only one beef sample was positive for STEC, 30% of raw chicken legs were positive for *Salmonella* and 52% of raw ground beef and 34% of raw chicken legs were positive for *Listeria monocytogenes* (Bohaychuk et al. 2006). In a study of retail meats in the Washington D.C. area, 70.7% of chicken samples were positive for *Campylobacter*, 38.7% for *E. coli* and 9% for *Salmonella*. Only one sample of beef was positive for *Campylobacter*, 21.7% were positive for *E. coli* and 3% for *Salmonella* (Zhao et al. 2001). In a study on the prevalence of pathogens in retail food in Japan, including raw meats, fruits, vegetables and seafood, *Salmonella* was found in 33.5% of ground chicken samples and 12.7% in raw chicken samples, *E. coli* was found in 57.5% of ground beef, 75.6% of ground chicken, 18.2% of raw beef and 22.8% of raw chicken. *C. jejuni* and *coli* were found in 20.9% of ground chicken and 13.3% of raw chicken samples. None of the studies specifically tested for different serotypes of *E. coli* other than O157:H7 or general STEC genes. Significance in our study could be truly non-significant or one of the factors could be the low sample size.

Many species of bacteria including *Salmonella* and *E. coli* can survive on surfaces for an extended period of time, some for hours and some up to days (Lues and Van Tonder 2007). This increases the risk of food becoming contaminated if proper cleaning and sterilization methods are not utilized. Bacteria can survive on many surfaces, including hands, utensils, aprons, sponges even stainless steel surfaces (Kusumaningrum et al. 2003). Storage areas such as refrigerators are also of concern because some bacteria can still survive at low temperatures

(Jackson et al. 2007). One possibility for our higher rate of STEC is that minced beef is a very popular dish so there could be an increased chance for cross-contamination from raw beef on surfaces.

Outbreaks due to foodborne pathogens are often caused by recontamination. 75% of outbreaks are typically not traced back to the source, 25% are traced back to recontamination (Reij et al. 2004). Recontamination can either be caused by direct or indirect contact with surfaces, environmental vectors or air. Ready-to-eat meals are often contaminated by manual contact, poultry and dairy products by surface contact, and ice cream and powders by air. In factories, biofilms are cause for concern as they are resistant to disinfection, and air contamination is a concern because often power washing is utilized and this can aerosolize bacteria (den Aantrekker et al. 2003). Among processed foods recontamination is usually caused by insufficient hygiene, improper storage and contaminated equipment or personnel. Soiled packaging is also a main cause (Reij et al. 2004).

Public perception must also be taken into account. There are ways to manage transmission of foodborne pathogens such as irradiation, but it is expensive and there is public stigma against it. People accept the risk that poultry products are sent to market still contaminated with Salmonella because they can cook it themselves and lower the risk of infection, but then consumer responsibility is expected. Responsibility is needed at all levels of the food chain. Changing consumer perception with educational practices has been shown to be difficult (Havelaar et al. 2010).

Conclusion

While the *E. coli* O157:H7 is usually the focus of tests for foodborne pathogen contamination, our study shows that other food adulterant serotypes, such as *E. coli* O45, are playing a role in infection. Although the prevalence for many pathogens is low, the higher prevalence of STEC genes and STEC serotype O45 is cause for concern. Other shiga toxin-producing serotypes are becoming as much of a concern as the more commonly known O157:H7. Being exposed to foodborne pathogens can increase the risk of chronic gastroenteritis sequelae, including Inflammatory Bowel Disease, so it is important to lower the risk of infection through proper food handling, preparation and education.

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CHAPTER 4*

RISK OF FOODBORNE PATHOGEN INFECTION AMONG GASTROENTERITIS CASES IN QATAR

*This chapter has been prepared in the format for submission to Archives of Medical Research for publication

Abstract

Foodborne illness has been determined to be one of the major limitations to the advancement of world health. With the ease of travel around the world and the increase in trade of food and animal products, the risk has escalated in recent years. Bacterial pathogens among the leading causes of foodborne illness are *E. coli*, *Campylobacter*, *Salmonella*, and *Listeria*. Fecal samples from patients admitted to the hospital with complaints of gastroenteritis were screened for the presence of the pathogens using a combination of bacterial enrichments, biochemical and agglutination tests, and molecular detection. Among the study population, *Salmonella* was the most common pathogen (42.9%), followed by *E. coli* (35.3%), and *Campylobacter* (21.0%). EPEC 4 was the most common pathotype lineage of the *E. coli* samples (31.4%), followed by EPEC 3 (25%). *C. jejuni* was the most common species of *Campylobacter* (71.4%). Most *Salmonella* samples belonged to Group D (35.5%) and Group B (31.1%). Age was a statistically significant risk factor for all pathogens.

1. Introduction

Foodborne illnesses pose major health burdens worldwide. In the U.S. alone, it is estimated that 48 million people become ill due to foodborne diseases, 128,000 of those cases being hospitalized and 3,000 resulting in death.[1] The World Health Organization (WHO) estimates that 2.2 million people worldwide die per year of diarrheal food and waterborne diseases alone.[2] Although data on individual countries is available, information on the global burden of foodborne diseases is lacking, but estimated cost per individual nation is high.[3,4] Foodborne Diseases Burden Epidemiology Reference Group (FERG) along with WHO are currently undertaking the estimation of the worldwide burden of foodborne disease, listing *E. coli*, *Campylobacter* and *Salmonella* spp. among the top challenges.[2]

The risk of foodborne pathogen transmission is exacerbated by the ease of travel and the globalization of trade.[5] This is especially true in such international areas as Qatar, where the ratio of expatriates to natives is 9:1. Areas of such cultural diversity are key to studying foodborne illnesses. *E. coli*, *Campylobacter* and *Salmonella* are three of the major foodborne pathogens that contribute to the burden of disease,[6] especially in very international regions.

We have been carrying out complementary studies on the occurrence of foodborne pathogens at different levels of the food chain, focusing on examining the presence of these pathogens at the production level among food animals, followed by products as they move through processing plants and as final products in retail store and restaurants.[7,8] This study complements our effort in tracing the pathogens and assessing the presence of these pathogens among human gastroenteritis cases.

Gastroenteritis is an inflammation of the gastrointestinal tract caused by viruses, bacteria or other microorganisms. Symptoms include abdominal pain, vomiting and diarrhea. In addition to the immediate gastrointestinal symptoms associated with infection of these pathogens, there is the risk of chronic sequelae such as Inflammatory Bowel Disease (IBD).[9,10,11] In this study we assessed the prevalence and risk factors of foodborne pathogens, including *E. coli*, *Campylobacter*, *Salmonella* and *Listeria* among gastroenteritis cases in the diverse population of Qatar in hopes of shedding light on the roles of these pathogens.

2. Methods

2.1. Target and Study Populations

We carried out a cross-sectional study to address the stated objective. *E. coli*, *Campylobacter, Salmonella* spp. and *Listeria monocytogenes* were recovered from the target populations. Subjects were a subset of patients selected from individuals admitted to Hamad Medical Corporation hospitals in Qatar with complaints of gastroenteritis during the period of August 2011 to May 2014. Fecal samples were collected from the patients and tested bacteriologically for the presence of these pathogens. These samples were collected during routine patient care. Ethical approval was granted from the Institutional Review Board for the use of these samples and patient data. The patients' backgrounds were diverse, including differing ethnicity, nationality, gender, age and diagnosis.

2.2. Pathogen Isolation

Pathogens were isolated from human samples using standard bacteriological procedure. 1g of the collected stool samples was diluted with 10ml of phosphate buffered saline (PBS, pH

7.2; Sigma, St. Louis, MO, USA) and 500µl of this dilution was added to 5ml of Selenite broth (Oxoid, Basingstoke, Hampshire, UK) for enrichment and was incubated at 37°C for 24-48hr.

2.2.1. *E. coli*

Samples were inoculated onto sorbitol_MacConkey agar (SMAC). From SMAC at least five non-sorbitol-fermenting (NSF) colonies, if any, were picked. All *E. coli* isolates were tested using the slide agglutination test using polyvalent and appropriate monovalent EPEC O-specific antiserum (Bio-Rad Laboratories, Inc., UK).

2.2.2. *Campylobacter*

Samples were incubated on CAMP agar at 42°C in microaerophilic conditions for the isolation of *Campylobacter* spp. Identification of presumptive pathogens was performed using biochemical tests and serum agglutination reactions according to standard methods.

2.2.3. *Salmonella*

The enriched samples were subcultured onto MacConkey agar and incubated at 37°C for 24hr. Colonies were screened using biochemical tests such as Kilger's iron agar, motility indole-urea agar, Lysin iron agar and o-nitrophenyl-β-D-galactopyranoside. Colonies from these screenings were identified with confirmatory biochemical tests using API 20E (bioMereux, Marcy I'Etoile, France) or VITEK (bioMereux).

2.2.4 *Listeria monocytogenes*

The samples were pre-enriched with Demi-Fraser broth (Oxoid, Hampshire, England) and incubated for 22–26 h at 30°C. The morpholinepropanesulfonic (MOPS) acid-buffered buffered listeria enrichment broth (BBL) listeria enrichment broth, MOPS free acid and MOPS

sodium salt (Fisher Scientific, Pittsburgh, PA, USA) was used as a selective medium in which the samples were incubated at 35°C for 18–24hr.

2.3 PCR Detection

Samples were then sent to Cornell University for further testing. PCR detection was performed on a subset of samples to determine pathogen presence using the BAX® Automated System (Dupont, USA). A 5µl aliquot of the respective secondary enrichment (BHI - *Campylobacter*, *Salmonella*; MEC broth - *E. coli*) was added to 200µl of the buffer (proteinase-containing lysis buffer) provided by the manufacturer. Samples were then heated in the lysis reagent solution to rupture the bacterial cell wall and release the DNA. PCR tablets, which contain all the reagents necessary for PCR plus fluorescent dye, were hydrated with the lysed sample and processed in the cycler/detector provided by the manufacturer. Within a few hours, the polymerase chain reaction (PCR) amplified a DNA fragment specific to the target. The amplified DNA generates a fluorescent signal, which the BAX® system application uses to analyze the findings. Results are displayed on a monitor screen as simple positive or negative symbols.

2.4 Data Collection

Data on putative risk factors associated with the presence of these pathogens were extracted from the medical records. This data included age, sex and nationality, which was used as a proxy for food preparation. The significance of association of the presence of a particular pathogen was compared to other pathogens combined using the logistic regression analysis. Furthermore, the significance of association of age, sex and nationality with the odds of a particular pathogen was evaluated using logistic regression. Factors that were significant in the

initial association we considered further in a multivariate logistic regression analysis to assess the significance of each factor while simultaneously controlling for the association with other factors.

3. Results

The 1110 patients sampled were admitted with the complaint of gastroenteritis and had stools obtained and examined for the targeted pathogens. Patients admitted with the complaint of gastroenteritis but had no stool samples collected were excluded from the study. Among patients that met the inclusion criteria, 476 tested positive for *Salmonella* (42.9%), 392 for *E. coli* (35.3%), 233 for *Campylobacter* (21.0%) and 9 for *Listeria* (0.8%) (Figure 4.1). *Salmonella* spp. was the most common pathogen detected in these cases. The odds were about two times greater to detect *Salmonella* spp. from patients among our sampling in comparison to *Campylobacter* spp.

E. coli samples were tested for pathotype lineages and 123 were EPEC 4 (31.4%), 98 were EPEC 3 (25%), 57 were EPEC 2 (14.5%), 39 were not specified and were undefined EPEC (9.9%) and 75 were unknown (19.1%) (Table 4.1). Pathotype 4 was more common among cases with *E. coli* compared to 2 or 3.

Campylobacter samples were further tested for species. More than two thirds of the isolates were genotyped as *C. jejuni* (71.4%), 44 were *C. coli* (20%), 2 had both *C. jejuni* and *coli* (0.9%), only one sample was *C. upsaliensis* and 33 samples were undetermined (15%) (Table 4.2).

Salmonella was further tested for groups, most samples belonged to Group D (35.5%) and Group B (31.1%). The other subgroups (A, C, C1, C2, G, and G1) were detected among the

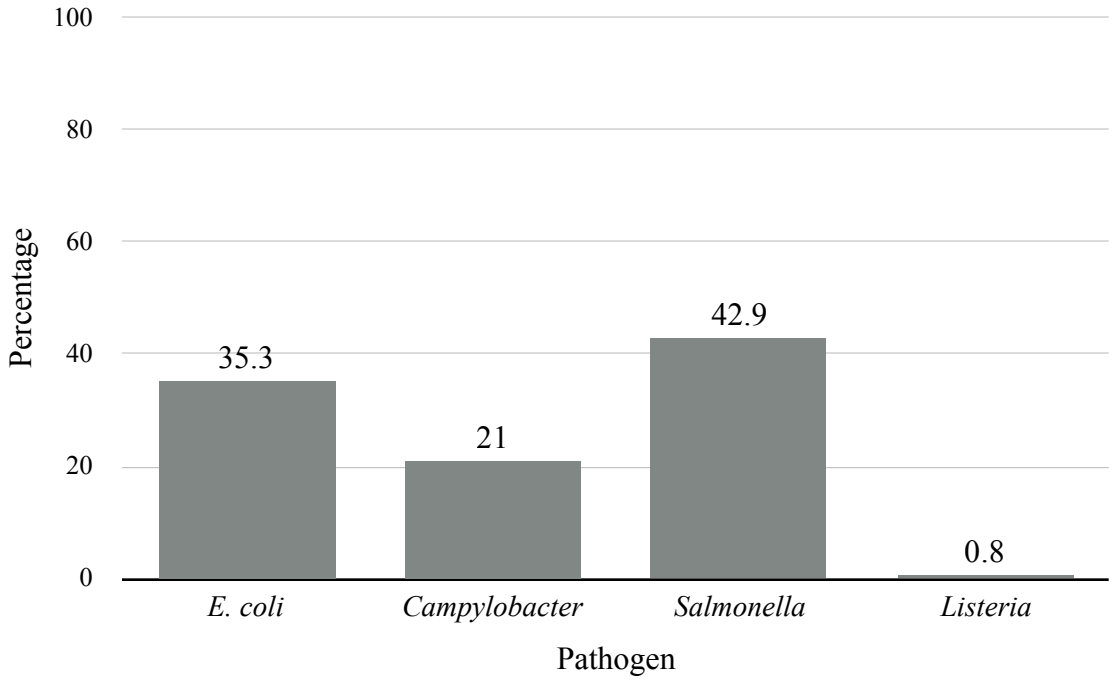

Figure 4.1. Overall pathogen prevalence.

Table 4.1. *E coli.* pathotype prevalence.

E. coli		
Pathotype	**Total**	**Prevalence (%)**
EPEC 2	57	14.5%
EPEC 3	98	25.0%
EPEC 4	123	31.4%
Undefined EPEC	39	9.9%
Uknown	75	19.1%

Table 4.2. *Campylobacter* species prevalence.

Campylobacter		
Species	**Total**	**Prevalence (%)**
C. coli	44	20.0%
C. jejuni	157	71.4%
Both *C. coli* & *jejuni*	2	0.9%
C. upsaliensis	1	0.5%
Unkown	33	15.0%

Table 4.3. *Salmonella* group prevalence.

Salmonella		
Group	**Total**	**Prevalence (%)**
A	3	0.6%
B	148	31.1%
C	11	2.3%
C1	29	6.1%
C2	12	2.5%
D	169	35.5%
E	15	3.2%
G	1	0.2%
G1	3	0.6%
Unknown	83	17.4%

study population but at significantly lower proportions (Table 4.3).

Subsets of the *E. coli* samples were also further tested for the virulence genes *stx* and *eae*, as well as STEC serotypes. A total of 311 isolates were tested for *stx* and *eae* and STEC, 23.2% had the *eae* gene and 5.8% had the *stx* gene, only 3.2% were positive for STEC. In addition, 96 samples were tested for the F17 and F41 antigen, 78.1% were positive for F41 and only 1% was positive for F17. Out of 266 tested for O157:H7, only 7 were positive (2.6%). Samples that were positive for virulence genes were further tested for the six main non-O157 STEC serotypes O26, O45, O103, O111, O121 and O145. 9% were positive for O111, and 1.3% were positive for O26 and O45 (Table 4.4).

We also analyzed patient data such as age, sex, the region they were from and whether they were admitted during the hot (April-Nov.) or cold (Dec.-March) season. The average age of patients admitted with *E. coli* was 1.2 years (oldest was 49, youngest was 2 days), for *Campylobacter* it was 7.7 years (oldest: 64, youngest: 2 months) and for *Salmonella* it was 10.3 years (oldest: 86, youngest: 1 month and 10 days). Over half of the patients admitted with *E. coli* and *Campylobacter* were men (51.4%; 57.3%) but of those admitted with *Salmonella*, 60.7% were women. The majority of patients with *E. coli* (68.6%) and *Campylobacter* (60.3%) were admitted during the cold season, whereas the majority with *Salmonella* (67.2%) were admitted during the hot season (Table 4.5).

Patients were from a total of 33 countries which we then divided into regional groups (Table 4.6). The majority of patients were understandably from the Middle East, the next largest group was Asia. Prevalences among pathogens were similar, though the number of patients with *Salmonella* from Asia was notably higher than *E. coli* or *Campylobacter*, patients from the

Table 4.4. *E. coli* serotype prevalence.

***E. coli* Prevalence**			
	Total Positive	Total Samples Tested	Prevalence (%)
O157:H7	7	266	2.6%
stx	18	311	5.8%
eae	72	311	23.2%
STEC	10	311	3.2%
F17	1	96	1.0%
F41	75	96	78.1%
O26	1	78	1.3%
O111	7	78	9.0%
O121	0	78	0.0%
O45	1	78	1.3%
O103	0	78	0
O145	0	78	0

Table 4.5. Demographic data.

Demographics			
	E. coli	*Campylobacter*	*Salmonella*
Average Age	1.2	7.7	10.3
Female	48.6%	42.7%	60.7%
Male	51.4%	57.3%	39.3%
Cold Season	68.6%	60.3%	32.8%
Hot Season	31.4%	39.7%	67.2%

Table 4.6. Complete list of patients' countries of origin divided by regions.

Middle East	Asia	Africa	Other
Egypt	Bangladesh	Eritrea	Canada
Iran	Philippines	Morocco	Estonia
Iraq	India	Nigeria	France
Jordan	Indonesia	Somalia	Russia
Lebanon	Malaysia	Sudan	United Kingdom
Oman	Nepal	Tunisia	United States
Pakistan	Sri Lanka		
Palestine			
Qatar			
Saudi Arabia			
Syria			
Turkey			
United Arab Emirates			
Yemen			

Middle East had a lower prevalence of *Salmonella* than the other two pathogens, and the patients from Africa had a lower prevalence of *E. coli* than the other two pathogens (Table 4.7).

Dividing patients into age groups, 54.7% of patients with *E. coli* were under 1 year old compared to 25.3% for *Campylobacter* and 24.3% for *Salmonella*. 98.9% of patients with *E. coli* were under 5 years of age, whereas patients were more distributed among other age groups for *Campylobacter* and *Salmonella*. Notably 16.4% of patients with *Salmonella* were between 20 and 50 years of age, larger than the other two pathogens, and 3.6% were over fifty, similar to *Campylobacter* (3.2%) (Table 4.8).

The odds of the risk of age, region and sex was evaluated using logistic regression and quantified using the odds ratio (OR). All statistical tests were performed using the SPSS v.23 (IBM-statistical software, White Plains, NY) and p-values were evaluated at $P < 0.05$. The only statistically significant factor was age (Table 4.9).

4. Discussion

We used a multidisciplinary epidemiological approach to investigate the occurrence of these foodborne pathogens among cases of gastroenteritis. Qatar was chosen due to its diverse social and cultural population which includes diverse food and methods of food preparation. Because of this diversity, we would expect to see diverse pathogens. The more knowledge gained on mechanisms of pathogenicity would greatly aid in mitigating the risk of these pathogens. Estimates of foodborne illness are difficult to make due to the number of potential incriminated pathogens and the fact that not all cases are tested for specific pathogens. A survey in the US from 2000-2008 determined that the number one cause for hospitalization was *Salmonella* (35% of cases) followed by norovirus (26%) and *Campylobacter* (15%).[12] Our findings that

Table 4.7. Prevalence of pathogens by regions of patient origin.

	E. coli		***Campylobacter***		***Salmonella***	
Middle East	210	81.1%	74	77.9%	92	71.9%
Asia	31	12.0%	11	11.6%	23	18.0%
Africa	12	4.6%	6	6.3%	9	7.0%
Americas	4	1.5%	3	3.2%	2	1.6%
Europe	2	0.8%	1	1.1%	1	0.8%
Russia	0	0.0%	0	0.0%	1	0.8%
Totals	259		95		128	

Table 4.8. Prevalence of pathogens among different age groups.

E. coli		
Age Group	**Total**	**Prevalence (%)**
Under 1	141	54.7%
1-5	114	44.2%
5-10	1	0.4%
10-20	1	0.4%
20-50	1	0.4%
Over 50	0	0.0%
Total	258	

Campylobacter		
Age Group	**Total**	**Prevalence (%)**
Under 1	24	25.3%
1-5	50	52.6%
5-10	5	5.3%
10-20	5	5.3%
20-50	8	8.4%
Over 50	3	3.2%
Total	95	

Salmonella		
Age Group	**Total**	**Prevalence (%)**
Under 1	34	24.3%
1-5	60	42.9%
5-10	12	8.6%
10-20	6	4.3%
20-50	23	16.4%
Over 50	5	3.6%
Total	140	

Table 4.9. The odds of age as a risk for infection.

Age					
Category	Regression coefficient	Standard error	p-value	Odds ratio	95% CI
E. coli	-0.32	0.07	0	0.73	(0.64, 0.83)
E. coli Constant	0.87	0.14	0	2.38	
Campylobacter	0.03	0.01	0.004	1.03	(1.01, 1.04)
Campylobacter Constant	-1.70	0.14	0	0.18	
Salmonella	0.04	0.01	0	1.04	(1.02, 1.06)
Salmonella Constant	-1.18	0.12	0	0.31	

Salmonella was most common in hospitalized patients was similar, but we did not screen for viruses.

Our study focused on three major foodborne pathogens, *E. coli*, *Campylobacter* and *Salmonella* spp. Foodborne pathogens have been incriminated in the risk of gastroenteritis and the mechanism of disease is currently not fully understood.[6] The three foodborne pathogens investigated in this study are among the common pathogens that pose significant burden of disease around the world.[2,3,4] Gastroenteritis infections caused by these pathogens are mostly self-limiting and admissions to hospitals are not common. In a few cases, around 6-7%, patients with gastrointestinal illness may develop sequelae with serious consequences.[4]

For instance, Inflammatory Bowel Disease. A study drawing from medical records of 2,000 general practitioners in the UK looked at the occurrence of IBD (including Crohn's, ulcerative colitis and indeterminate colitis) in patients admitted with acute gastroenteritis. The estimated incidence rate of IBD for patients admitted with gastroenteritis was 68.4 per 100,000 person-years vs 29.7 per 100,000 person-years in the control group.[9] Another study looking at the short and long-term risk of IBD in patients specifically after Salmonella and Campylobacter gastroenteritis in Denmark following patients over a 15 year period found the greatest risk of IBD was in the first year after infection.[10]

Another study performed in Qatar tested for viruses and bacterial pathogens among acute gastroenteritis cases. Out of the 288 patients enrolled, most patients were under 10 years old (39.1%) and between 11 and 20 years old (26%) and 53.1% were male and 44.8% were female. Their focus was on viruses, finding that 28.5% of patients were positive for norovirus and 6.25%

were positive for adenovirus. They also tested for *Salmonella*, *Campylobacter* and *E. coli* but they were found at lower rates, *Salmonella* was detected in 8% of patients, *Campylobacter* in 1% and *E. coli* in 2.1%. They did state that antibiotics may have been administered so this would have affected the results.[13]

E. coli is known to mostly affect younger children, which is concurrent with our findings that age is a statistically significant risk factor. In our study the majority of all patients were under 10 years of age, but there were more older patients with *Campylobacter* and *Salmonella*. Our finding that being from different regions was not statistically significant could imply that different cultural food preferences or preparations of food are not affecting the mode infection, which means the foodborne pathogens could possibly be post preparation contamination. It should be noted that we only tested samples from patients with gastroenteritis, no one without symptoms was tested.

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CHAPTER 5*

POTENTIAL RISK OF FOODBORNE PATHOGENS ASSOCIATED WITH CONVENTIONAL AND ORGANIC DAIRY FARMS IN NEW YORK STATE

*This chapter has been prepared in the format for submission to the Journal of Dairy Science and Technology for publication

Abstract

Purpose - While most milk is pasteurized, foodborne pathogens are cause for concern in the raw milk market and to farmers who drink milk directly from their farms. We carried out a study to examine the prevalence of three main foodborne pathogens, *E. coli*, *Campylobacter* and *Salmonella*, amongst both conventional and organic dairy farms in New York State.

Methods - Using a combination of bacterial enrichments and PCR detection we tested for the presence of these pathogens on milk filters and in samples of bulk tank milk from these two subpopulations.

Results - *E. coli* O157:H7, O145, *C. jejuni* and *C. coli* were detected at higher proportions in samples from conventional farms and 28.6% of the organic filter samples were positive for *E. coli* O121, whereas 16.7% of the conventional samples were positive. The prevalence of *Salmonella* spp. was detected at a higher proportion among samples collected from conventional dairy farms in comparison to samples from organic dairies (20 vs. 4.8%).

Conclusions - There was no significant difference in the prevalences of these pathogens between these subpopulations, except for the above mentioned pathogens. Other food adulterant non-O157:H7 STEC serotypes are proving to be more common. Raw, and even pasteurized milk, should be tested in order to prevent transmission of foodborne pathogens.

1. Introduction

Foodborne illness is a major health burden worldwide. In the U.S alone, it is estimated by the Centers for Disease Control and Prevention (CDC) that 48 million people become ill due to foodborne diseases, 128,000 of those being hospitalized and 3,000 resulting in death (CDC 2015). The World Health Organization (WHO) estimates that 2.2 million people per year worldwide die of diarrheal food and waterborne diseases alone (WHO 2015). Foodborne Diseases Burden Epidemiology Reference Group (FERG), along with WHO, are currently undertaking the estimation of the worldwide burden of foodborne disease, but when estimated by individual nations, the cost per episode is high (Havelaar et al. 2009; Hoffmann et al. 2005).

Dairy farms are known sources of foodborne pathogens. Foodborne disease outbreaks have been traced back to raw milk and even pasteurized milk. The ruminant intestinal tract is a natural reservoir for foodborne pathogens such as *Campylobacter*, *Salmonella* and *E. coli*. Cattle most likely become infected through the consumption of feed or water contaminated with feces and are usually asymptomatic (Oliver et al. 2005).

Pasteurization has reduced the risk of contracting a foodborne illness from milk, but there is still a part of the population that consumes raw milk. Some people also believe that raw milk is more nutritionally beneficial than pasteurized milk (Jayarao and Henning 2001), though no research has demonstrated this (CDC 2015). Cheeses made from raw milk could also pose a risk (Oliver et al. 2005). In Italy it has even been allowed to sell raw milk from vending machines since 2004 (Giacometti et al. 2012).

It is common practice for dairy farm owners and workers to consume raw milk. For example, in a survey of farmers in Pennsylvania (Jayarao et al. 2006) out of 248 dairy producers

interviewed, 42.3% said they consumed raw milk. Bulk tank milk from the same farms were sampled and 2.4% were positive for *C. jejuni*, 2.4% for STEC, 6% for *Salmonella* and 2.8% for *Listeria monocytogenes*. Our long term objective is to assess the risk of foodborne pathogens from organic and conventional dairy farms. In this study we tested milk filters, which the milk travels through before entering the bulk tank, from 30 conventional dairy farms and 21 organic farms in New York. We also tested bulk tank samples from all 30 conventional dairies, but only received 8 bulk tank samples from the organic dairies.

2. Materials and Methods

2.1 Target Populations - The target population for our study was dairy farms in New York State (NYS) which were divided into two subpopulations, organic and conventional dairies. We contacted the Dairy Extension staff at Cornell Cooperative Extension (CCE) to help recruit organic dairy operations. A list of organic dairy operations in NYS was collated and a letter of solicitation was sent to all operations through Extension personnel. Letters were also followed up by personal phone calls. In addition, we also participated in the regional meeting for organic dairy operations to encourage participation in the study. Recruitment of conventional dairy operations was done through staff at the Quality Milk Production Services (QMPS) at the Cornell Animal Health and Diagnostic Laboratory. A letter of solicitation was also sent to potential participants.

2.2 Sampling Procedures - With the help of CCE and QMPS, letters of solicitation were given to New York State dairy farmers explaining that the study was voluntary and anonymous and milk filters were collected from bulk tank lines at 30 conventional and 21 organic dairies in New York State. Bulk tank milk samples were also taken in addition to milk filters at the 30

conventional farms and we were able to acquire 8 milk samples from organic dairies. Samples were then transported in coolers to the Cornell College of Veterinary Medicine and processed in the lab.

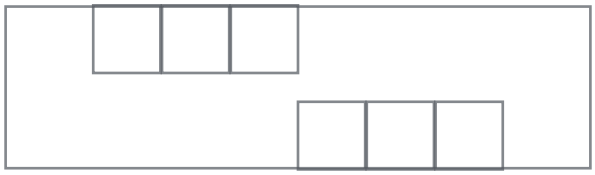
2.3 - Pathogen Isolation - Depending on the size and shape of the milk filters collected, three 1.5x1.5in squares were cut from two different locations on the milk filter, two samples for two repetitions of each bacterial enrichment (*E. coli*, *Campylobacter* and *Salmonella*) (Figure 5.1) and incubated in 100ml of the appropriate bacterial enrichment, if one of the repetitions was positive the sample was counted as positive. Milk samples were incubated at a ratio of 1:10, 10ml of milk per 100ml of bacterial enrichment.

2.3.1 *E. coli* - For the primary enrichment all samples were inoculated into Modified *E. coli* broth (MEC broth) supplemented with novobiocin (16 mg/L). The inoculum was incubated for 24hr at 37^{o}C. A total of 20µl of the incubated enriched inoculum was transferred into 1 ml of the secondary enrichment medium (Brain Heart Infusion (BHI) without antibiotics) and incubated for three hours at 37^{o}C before processed for the real-time PCR.

2.3.2 *Campylobacter* - The primary enrichment consisted of BHI supplemented with Cefoperazone (6mg/L), Vancomycin (6mg/L), and Amphotericin B (2mg/L). The primary enrichment was inoculated with the samples and incubated at 37^{o}C for 24hr. 1ml of the secondary enrichment media (non-supplemented BHI) was inoculated with 20µl of the primary enrichment and also incubated at 37^{o}C for 24hr.

2.3.3 *Salmonella* - Samples were added to Buffered Peptone Water supplemented with Novobiocin and were incubated for 24hr at 37^{o}C. 20µl of the primary enrichment was transferred

A.



B.

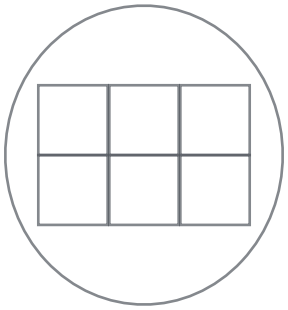


Figure 5.1. Example of milk filter sampling, two repetitions for each pathogen. A.) flattened tubular filter B.) circular filter.

into 1ml of non-supplemented BHI and also incubated for 24hr at 37°C for the secondary enrichment.

2.4 PCR Detection - The PCR detection was performed using the BAX® Automated System. A 5µl aliquot of the respective secondary enrichment was added to 200µl of the buffer (proteinase-containing lysis) provided by the manufacturer. Samples were then heated in a lysis reagent solution to rupture the bacterial cell wall and release the DNA. PCR tablets, which contain all the reagents necessary for PCR plus fluorescent dye, were hydrated with lysed sample and processed in the cycler/detector provided by the manufacturer. Within a few hours, the polymerase chain reaction (PCR) amplified a DNA fragment specific to the target. The amplified DNA generates a fluorescent signal, which the BAX® system application uses to analyze the findings. Results are displayed on a monitor screen as simple positive or negative symbols.

3. Results

E. coli O157:H7, O145, *C. jejuni* and *C. coli* were only found in samples collected from conventional dairy operations. *E. coli* O111 and *C. lari* were not found at any of the farms. Other prevalences between samples from conventional and organic farms were similar (Figure 5.2). Notable differences were *E.coli* O121 and *Salmonella*. Of the organic filter samples, 28.6% were positive for *E. coli* O121, whereas 16.7% of the conventional samples were positive, and 4.8% of the organic samples were positive for *Salmonella* vs. 20% of the conventional (Table 5.1). Of the bulk tank milk samples, one conventional farm milk filter was positive for the *stx* gene, one was positive for the *eae* gene, one was positive for *C. coli* and another was positive for the *eae* gene, *E. coli* O26, O121, O45 and *Salmonella*. Of the eight organic milk samples one

farm's filter was positive for both *stx* and *eae* genes and another was positive for *C. coli* (Table 5.2).

We were also able to collect information about the conventional farms, such as the number of cows, feed source, housing type, bedding type and whether or not they were closed herds. We then looked at similarities between the groups whose milk filters were negative for all pathogens, the groups for each pathogen, and those that were positive for 3 or more pathogens. Out of the 13 farms whose filters were negative, the main similarity was herd size. 10 had herds under 100 cows, 2 were between 100-200 and one was over 500. There were five farms that had 3 or more pathogens, 3 had over 200 cows, 1 had under 100 and one had between 100-200. Out of all of the farms, 5 had sand bedding and three of those 5 farms' filters had 3 or more pathogens. The farm whose bulk tank sample tested positive for E. coli O26, O45, O121 and *Salmonella*, had by far the largest herd at 1,300 cows and had free-stall housing and sand bedding. Though the next largest herd at 780 had a negative milk filter and the third largest herd at 500 had a milk filter positive for *C. coli*.

4. Discussion

Our finding that milk filters were positive for certain pathogens but the corresponding bulk tank milk was negative for the same pathogens may be due to the large volume of milk, the bacteria could be so diluted that it was undetectable in the samples we tested. So there is still potential that the milk could contain the pathogen. Testing multiple samples from the bulk tank would be more accurate. There was one instance where the milk sample was positive for *C. coli* but the milk filter was negative. This could be due to the presence of the pathogen from previous

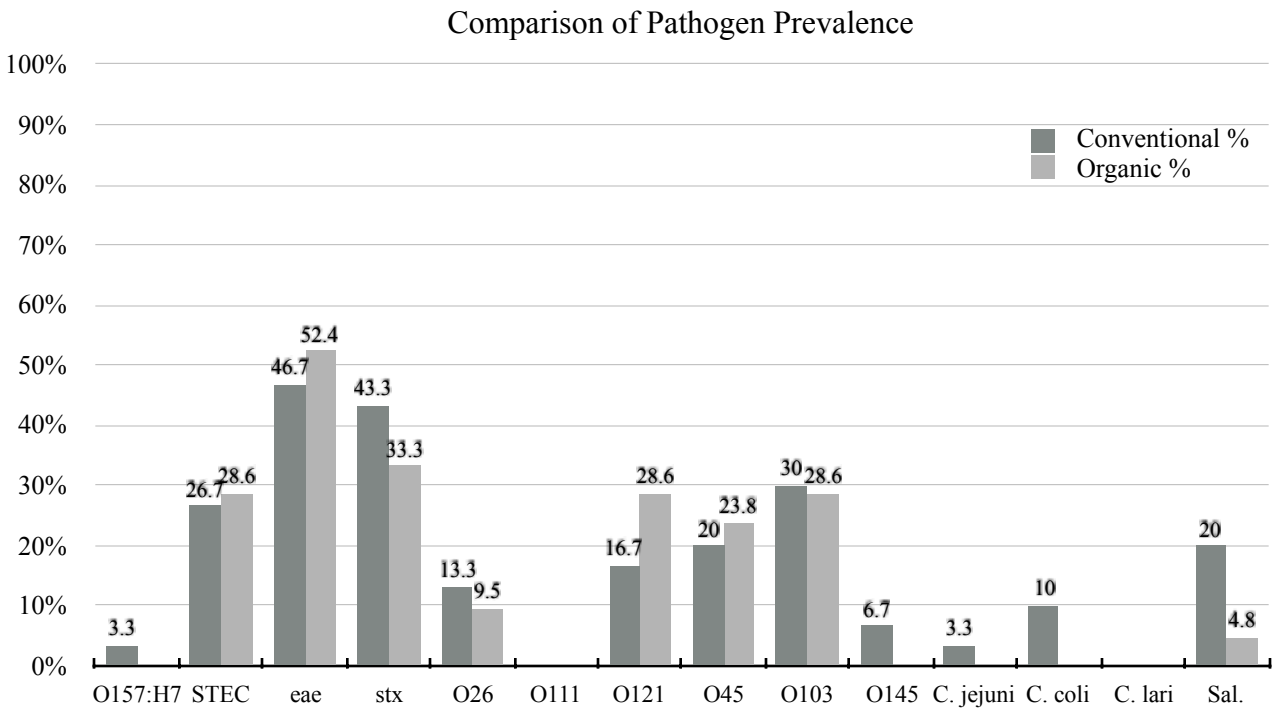


Figure 5.2. Comparison of pathogen prevalence between conventional and organic farms.

Table 5.1. Prevalence of pathogens.

	Conventional - 30 farms	**Conventional Prevalence**	**Organic - 21 farms**	**Organic Prevalence**
O157:H7	1	3.3%	0	0.0%
STEC	8	26.7%	6	28.6%
eae	14	46.7%	11	52.4%
stx	13	43.3%	7	33.3%
O26	4	13.3%	2	9.5%
O111	0	0.0%	0	0.0%
O121	5	16.7%	6	28.6%
O45	6	20.0%	5	23.8%
O103	9	30.0%	6	28.6%
O145	2	6.7%	0	0.0%
C. jejuni	1	3.3%	0	0.0%
C. coli	3	10.0%	0	0.0%
Salmonella	6	20.0%	1	4.8%

Table 5.2. Occurrence of pathogens in bulk tank milk samples.

	Conventional - 30 farms	**Organic - 8 farms**
O157:H7	0	0
STEC	0	1
eae	2	1
stx	1	1
O26	1	0
O111	0	0
O121	1	0
O45	1	0
O103	0	0
O145	0	0
C. jejuni	0	0
C. coli	1	1
C. lari	0	0
Salmonella	1	0

milkings subsisting in the tank. Oliver et al. also had similar findings on the dilution effect (Oliver et al. 2005).

A similar study by Van Kessel et al. across 17 states (including New York) found using PCR that 24.7% of farms' filters tested positive for *Salmonella* and 10.8% of farms' bulk tank milk tested positive for *Salmonella*. Of those, 5.9% had both positive milk and positive filter samples, 18.5% had positive filters and negative milk samples, and 5.1% had positive milk but negative filter samples. They also tested for *E. coli* virulence genes *stx* and *eaeA*, finding 15.2% of their samples to be positive for *stx*, 16.1% for *eaeA*, and 5.1% to be positive for both (Van Kessel et al. 2011). We found much higher prevalences with 46.7% of conventional and 52.4% of organic filters testing positive for *eae* and 43.3% of conventional and 33.3% of organic filters for *stx*, and 26.7% of conventional and 28.6% of organic having both genes. They had a much larger sample size at 538 dairies, so our results may have been more similar with further testing and increased sample size. In addition to the Pennsylvania study mentioned earlier, Jayarao et al. also conducted a study in South Dakota and Minnesota looking at bulk tank milk samples from 131 dairies where they found 9.2% to be positive for *C. jejuni*, 3.8% for STEC, and 6.1% for *Salmonella* where again, our STEC prevalences were much higher, *Salmonella* was similar in the organic farms (4.8%) but much higher in the conventional (20%) (Jayarao and Henning 2001).

A previous cross-sectional study by Hassan et al. sampling 400 farms in New York State found a 1.5% prevalence for *Salmonella* (Hassan et al. 2000). A much earlier study by McEwen et al. sampled milk filters from 22 dairy farms in Ontario and found a 2.9% prevalence of *Salmonella* (McEwen et al. 1988). Both were lower than our 4.8% for organic and much lower than our 20% for conventional.

Another study also in Ontario by Rahn et al. testing the persistence of E. coli O157:H7 and VTEC (Verocytotoxin-producing, same as STEC) on dairy farms sampled eight dairy farms that had previously tested positive for O157:H7. They took rectal swabs from cows and calves and environmental samples including milk filters. Out of the 241 environmental samples tested, 14.1% were VTEC positive, 48.7% of calves and 16.8% of cows were VTEC positive. Of those, 16.5% of calves, 8.9% of cows, and 1.3% of environmental samples were non-O157 serotypes (Rahn et al. 1997). Internationally, a recent study from looking at milk filters from 27 dairies authorized to sell raw milk in Northern Italy found that out of 378 filters (14 filters per farm), 8.4% were positive for VTEC, 6.4% for *Campylobacter* and no *Salmonella* was found. Non-O157 serotypes that were found were O103, O145, and O157 (Giacometti et al. 2012).

At the National Mastitis Council Annual Meeting Proceedings in 2005, Oliver et al. presented a communication review of multiple published studies of the prevalence of *Campylobacter*, STEC, *Salmonella* and *Listeria monocytogenes* in milk and dairy environments. Over seven studies C. *jejuni* isolation rate from bulk tank milk ranged from 0.5 to 12.3%, averaging at 3.7%. STEC was isolated at 0.8, 0.9 and 3.8% from three different studies and between eight studies, *Salmonella* ranged from 0.2 to 8.9%, averaging at 3.6% (Oliver et al. 2005), all are much lower then our findings except *C. jejuni* which was similar in conventional farms. We also found *C. coli* in 10% of the conventional farms.

Less is known about STEC. As shown above, general STEC are usually tested for but only a few studies have tested for specific serotypes. Non-O157:H7 serotypes are not regularly checked (Oliver et al. 2005). E. coli O157:H7 is the serotype primarily tested for in milk. Murinda et al. found it to be present on 8 of 30 farms (26.7%) tested, but it was only found in 2

out of 268 milk samples (0.75%) (Murinda et al. 2002). We only found one milk filter to be positive for O157:H7. In another study they did look at other STEC and found 16.35% to be positive for non-O157:H7 STEC, 1.92, 3.85 and 0.96% for O26, O111, and O103 (Murinda et al. 2004). We found a higher prevalence of O26 at 13.3% for conventional and for 9.5% organic and a much higher prevalence for O103 at for 30% conventional and 28.6% for organic but no O111 positive samples. More data on specific non-O157:H7 serotypes is needed.

5. Conclusion

Our study shows that other food adulterant non-O157:H7 STEC serotypes are more common, more specific serotype testing would be beneficial for farmers and consumers. The only notable difference between the conventional and organic prevalences were O121 (Conventional:16.7%, Organic: 28.6%) and *Salmonella* (Conventional: 20%, Organic: 4.8%) and *E. coli* O157:H7, O145, *C. jejuni* and *C. coli* were only found at the conventional farms sampled. Raw, and even pasteurized milk, should be tested in order to prevent transmission of foodborne pathogens. Being exposed to foodborne pathogens can increase the risk of chronic gastroenteritis sequelae so it is important to lower the risk of infection.

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