Risk Profile:
Campylobacter jejuni/coli in Raw Milk

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RISK PROFILE:
CAMPYLOBACTER JEJUNI/COLI
IN RAW MILK

Client report FW13050

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April 2014

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Prepared for the Ministry for Primary Industries
under project MRP/13/01 - Microbiological Risk Profiles,
as part of an overall contract for scientific services

Client report no. FW13050

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GOVERNMENT AGENCY MERGERS AFFECTING THIS DOCUMENT

On 1 July 2010, the New Zealand Food Safety Authority (NZFSA) and the Ministry of Agriculture and Forestry (MAF) were amalgamated. On 30 April 2012, MAF was renamed as the Ministry for Primary Industries (MPI).

This Risk Profile still uses the names NZFSA and MAF for documents produced during the existence of these organisations.

ACKNOWLEDGMENTS

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GLOSSARY AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ANS	The 2009 Adult Nutrition Survey
a_w	Measure of water activity (max = 1.000 = pure distilled water)
ACMSF	Advisory Committee on Microbiological Safety of Foods
CFU	Colony forming unit
CI	Confidence Interval
CNS	The 2002 National Children's Nutrition Survey
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agricultural Organisation of the United Nations
FSANZ	Food Standards Australia New Zealand
GBS	Guillain-Barré Syndrome
Isolate	Bacteria derived from a single colony on an agar plate from a sample (e.g. clinical specimen, food or water)
MLST	Multi-locus sequence typing
MPI	Ministry for Primary Industries
MPN	Most Probable Number
NNS	The 1997 National Nutrition Survey
NZFSA	New Zealand Food Safety Authority (now MPI)
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
pH	Measure of acidity (min = 0 = most acidic; max = 14)
RMP	Risk Management Programme (under the *Animal Products Act* 1999)
STEC	Shiga toxin-producing *Escherichia coli*
Strain	A subtype of a species as assessed by one of several typing schemes
SVR	Short Variable Region (typing)
USA	United States of America

SUMMARY

This Risk Profile considers *Campylobacter jejuni* and *Campylobacter coli* in raw milk from cows, sheep, goats and buffaloes. Infection by *C. jejuni* or *C. coli* in humans usually results in a self-limiting gastroenteritis which may or may not involve bloody diarrhoea. In a small proportion of cases infection may progress to more serious autoimmune disorders or chronic gastrointestinal diseases. On rare occasions infection may result in death.

The purpose of this Risk Profile is to critically review information to answer the following risk management question: What is the public health risk from *C. jejuni/coli* in raw milk consumed in New Zealand? The Ministry for Primary Industries (MPI) completed an assessment of the microbiological risks associated with raw milk in June 2013. This quantitative risk assessment was based on data up until February 2013 and concluded that the risk of *Campylobacter* spp. infection through consumption of raw milk was high. This Risk Profile also includes relevant information since February 2013, particularly updated human health surveillance data.

Campylobacter spp. have been detected in two recent surveys of raw cows' milk from bulk tanks on farms in New Zealand at prevalences of 0.3% and 0.6%. The concentrations in the three positive samples were <1 cell/ml (two samples were positive for *C. jejuni*, the species was not reported in the third sample). *Campylobacter* spp. were detected in one sample of a survey of goats' milk in New Zealand (n=56, 1.8%).

If *C. jejuni/coli* is present in raw milk it will not grow if refrigeration temperatures are maintained. The numbers of *C. jejuni/coli* will decline during refrigerated storage but the rate of reduction is highly variable depending on the strain.

Surveys of the prevalence of *Campylobacter* spp. in the faeces of New Zealand dairy cows show that *C. jejuni* occurs frequently (>50% of samples) while *C. coli* is less prevalent (≤20%). Available data suggest seasonal variation with a higher prevalence and faecal concentration of *Campylobacter* spp. during spring and autumn. *C. jejuni* and *C. coli* have been detected in faeces from a single herd of dairy goats in New Zealand. No data for the prevalence of *Campylobacter* spp. among New Zealand sheep or buffaloes kept for milking were available, but *Campylobacter* spp. have been detected in faeces from non-dairy sheep.

The number of people consuming raw milk in New Zealand is still uncertain. Recent estimates suggest the proportion of the population consuming raw milk is low (1% adults, 0.5% children). People living or working on dairy farms are more likely to consume raw milk. There are no data on consumption patterns (e.g. serving sizes) for raw milk, although consumption patterns for cold milk could serve as a proxy. The frequency of consumption is likely to depend on how easily consumers can access raw milk supplies.

The currently available evidence suggests that the risk of *C. jejuni/coli* infection for consumers of raw milk in New Zealand is high. The risk will be greatest for milk obtained and consumed closest to the point of milking, as *Campylobacter* numbers will decline during storage.

The strongest evidence supporting this evaluation is human health surveillance data from New Zealand. Drinking raw milk has been reported as a risk factor in up to a third of foodborne campylobacteriosis outbreaks each year since 2006. There is strong evidence from two campylobacteriosis outbreak investigations to link illness with drinking raw cows' milk.

Consumption of raw milk has also been the cause of some sporadic cases of campylobacteriosis. While the proportion of sporadic cases of campylobacteriosis reporting consumption of raw milk (amongst other risk factors) in New Zealand is small, this information is not routinely collected, and is almost certainly under reported.

A case control study conducted in New Zealand during 1994/95 found there was a significant risk of campylobacteriosis from consumption of any unpasteurised milk in the previous 10 days. Recent intensive surveillance of campylobacteriosis cases in the Manawatu region of New Zealand identified a cluster of sporadic cases that comprised an outbreak caused by drinking raw milk. This surveillance also found an association ($p<0.0001$) between drinking raw milk and infection with a cattle-associated strain of *Campylobacter* spp. for cases that did not have contact with farm animals. Although this analysis cannot exclude other pathways of infection by these cattle-associated strains, this result does support raw milk being the source of infection for these cases.

Data on the carriage of *C. jejuni* and *C. coli* by New Zealand dairy cows indicates substantial potential for bacteria to be present in raw milk through faecal or environmental contamination, even though prevalences found in raw milk surveys were <1%. Because these pathogens die off in raw milk held under refrigeration, the time between milking and testing raw milk samples for the presence of *Campylobacter* spp. will reduce prevalence values. Much higher prevalence estimates (92% for at least one *Campylobacter* cell in bulk milk tank) have been derived from models.

The limited data on raw milk and faeces from goats in New Zealand shows that there is a risk of exposure to *Campylobacter* spp. from raw goats' milk. There are insufficient data to evaluate the risk from raw milk from sheep and buffaloes in New Zealand.

Campylobacteriosis outbreaks caused by drinking raw milk have been reported in many developed countries overseas.

1   INTRODUCTION

This Risk Profile considers *Campylobacter jejuni* and *Campylobacter coli* in raw milk from cows, sheep, goats and buffaloes. This Risk Profile only considers these two *Campylobacter* species and uses the shortened term *C. jejuni/coli* to refer to them both. This Risk Profile does not consider products made from raw milk such as cheese or yoghurt.

The purpose of this Risk Profile is to review critically information to answer the following risk management question:

- What is the public health risk from *C. jejuni/coli* in raw milk consumed in New Zealand?

Risk Profiles provide scientific information relevant to a food/hazard combination for risk managers and describe potential risk management options (NZFSA, 2010).[1]

MPI completed an assessment of the microbiological risks associated with raw milk in June 2013 (MPI, 2013a). This quantitative risk assessment was based on data up until February 2013. This Risk Profile also includes relevant information since February 2013, particularly updated human health surveillance data.

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[1] Risk Profiles commissioned by MPI and its predecessors can be viewed at: http://www.foodsafety.govt.nz.

2   HAZARD AND FOOD

2.1   The Pathogens: *C. jejuni* and *C. coli*

KEY FINDINGS

> Most cases of campylobacteriosis are caused by the two species *C. jejuni* and *C. coli*, with *C. jejuni* being the predominant pathogen.
>
> There is still uncertainty over the mechanisms of pathogenicity so all *C. jejuni/coli* are considered pathogenic to humans.

Appendix 1 contains additional information on Campylobacter.

There are many species of *Campylobacter* but the evidence in New Zealand suggests that two species, *C. jejuni* and *C. coli,* are of major significance to public health.

Other species, such as *C. upsaliensis, C. fetus, C. hyointestinalis*, *C. lari* and *C. ureolyticus* have occasionally been reported as causing human illness (Yan *et al.*, 2005). However, their significance to human health in New Zealand is unknown as different methods are required for their isolation and these are not routinely used in diagnostic laboratories (Nicol *et al.*, 2010).

The terms thermophilic or thermotolerant *Campylobacter* are often encountered in the literature, and include the species *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*.

Genotyping methods are important for identifying the sources of *C. jejuni/coli* infections and the most commonly applied methods in New Zealand have been pulsed field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). Alternative methods are amplified fragment length polymorphism (AFLP) or short variable region (SVR) sequencing, based on the flagellin (*fla*) genes. See Appendix 1 for further details.

Information on the sources and transmission routes of *C. jejuni/coli* can be found in a recent review (Whiley *et al.*, 2013) and summarised in a microbiological data sheet.[2]

2.1.1   Pathogenicity

A recent review of the pathogenicity factors of *C. jejuni* concluded that this bacterium only has a small number of virulence factors but little is known about their specific function in mediating disease (Dasti *et al.*, 2010). Factors involved in the pathogenesis of *C. jejuni* infection include flagellar motility, adherence and invasion through mucosal cells, and the cytotoxic effects of cytolethal distending toxin (Bereswill and Kist, 2003).

An alternative view of the pathogenic mechanism of *Campylobacter* spp. infection is that symptoms of enteritis may result from a local over-reaction of the intestinal innate immune system (Wassenaar, 2011). When invasive and motile *Campylobacter* spp. cells penetrate the intestinal mucus layer, they are engulfed by the intestinal cells, a process which ultimately leads to the release of cytokines, part of the immune response. The cytokines are mostly

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[2] http://www.foodsafety.govt.nz/elibrary/industry/Campylobacter-Organism_Causes.pdf (accessed 12 February 2014).

responsible for the symptoms of diarrhoea (van Putten *et al.*, 2009). This mechanism would imply that pathogenicity does not depend on the presence of specific factors, and all *Campylobacter* strains are able to cause disease.

The differences observed in the prevalence of specific *Campylobacter* strains isolated from human cases may be due to the superior ability of some strains to survive in the environment and have an opportunity to cause infection, rather than the presence of specific virulence factors (On *et al.*, 2006).

Until new evidence becomes available, all *C. jejuni/coli* strains are considered pathogenic to humans.

2.2 The Food: Raw milk

KEY FINDINGS

MPI defines raw milk as: "milk (secreted by mammals and used as food by human beings) that has not been subjected to any processing intended to alter the quality or composition characteristics of the milk." (MPI, 2013a).

Milk supports the growth of microorganisms. It is impossible to produce sterile raw milk and if pathogenic bacteria are among the microorganisms in the milk, there is a risk of illness for people who consume the milk.

The volume of cows' milk produced in New Zealand is increasing. While the exact quantity of cows' milk consumed as raw is not known, some evidence suggests that availability of raw milk to domestic consumers is increasing.

The quantity of raw drinking milk from sheep, goats and buffaloes that is available to domestic consumers is also unknown, but is likely to be lower than cows' milk.

The farm gate is the only point at which raw milk sales are allowed in New Zealand. Raw milk vending machines are now being installed on dairy farms in New Zealand.

Milk is made up of water, protein, fat, lactose, vitamins and minerals, with the types and proportions of each varying with animal breed, feed, age and phase of lactation (Amigo and Fontecha, 2011; Fox, 2011; Ramos and Juarez, 2011; Sindhu and Arora, 2011). Raw milk has a high water activity (a_w = 0.99) and an almost neutral pH (Roos, 2011). Milk is an excellent substrate for the growth of microorganisms (ICMSF, 2005).

2.2.1 Milk production in New Zealand

The volume of cows' milk processed by New Zealand dairy companies has increased almost every season for over 30 seasons since 1982/83, to approximately 19 million litres in 2012/13 (LIC, 2013). While the exact quantity of cows' milk consumed as raw is not known, some evidence suggests that availability of raw milk to domestic consumers is increasing.

The farm gate is the only point at which raw milk sales are allowed in New Zealand. Raw milk vending machines are now being installed on dairy farms in New Zealand.[3] Based on news reports about raw milk vending machines, supply for these outlets is provided by small

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[3] http://www.villagemilk.co.nz/get-village-milk/ (accessed 13 January 2014).

herds (<50 cows). There is also anecdotal evidence for informal distribution networks of raw milk.

There are a few buffalo herds in New Zealand, but the milk from these animals is usually used for producing yoghurt or cheese, because of the higher solids and fat content compared to cows' milk (Han *et al.*, 2012; Sindhu and Arora, 2011).

Dairy goat farms in New Zealand produce milk that is used for making cheese or for processing into infant formula.[4] The availability of raw goats' milk directly to consumers is unknown.

There are a few milking sheep herds in New Zealand, but the milk from these animals is usually used for producing cheese, ice cream or powdered milk.[5]

2.3   Behaviour of *C. jejuni/coli* in Raw Milk

KEY FINDINGS

Raw milk can become contaminated with *C. jejuni/coli* via a number of routes, primarily originating from environmental contamination by animal faeces. These organisms can also be shed directly into the milk as a result of mastitis in the milking animals or biofilms in the milking equipment.

C. jejuni/coli will not grow in raw milk during storage and distribution provided refrigeration temperatures are maintained. The concentration of *C. jejuni/coli* will decrease during storage at 4°C but the rate of reduction will be highly variable depending on the strain. Values ranging from 1.25 to >14 days have been reported for the time for *C. jejuni/coli* to reduce by 1 $\log_{10}$ CFU/ml in raw cows' held at 4°C. Because of the lack of data, it is not possible to say how the rate of decline might differ at other temperatures.

Most studies of *C. jejuni/coli* behaviour in raw milk used raw cows' milk. The single study in raw goats' milk showed that *C. jejuni* also reduced in concentration over time.

2.3.1   Contamination of raw milk by *Campylobacter*

It is impossible to produce sterile raw milk. Raw milk can become contaminated with *C. jejuni/coli* through:

- Contaminated udders or teat canals;

- Mastitic animals (which may or may not be symptomatic); or

- Contaminated milking equipment, cleaning water, workers, and the environment (Leedom, 2006).

C. jejuni/coli are frequently present in the gut microflora of dairy animals (without causing disease). They can be shed in faeces and subsequently spread throughout the animals' environment. The udders and teats of milking animals may be contaminated with

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[4] The Dairy Goat Cooperative receives an annual supply of 20 million litres of goat milk from 30,000 milking goats to produce infant formula (http://www.dgc.co.nz; accessed 21 May 2013).
[5] As ascertained from the websites of various New Zealand sheep milk producers.

microorganisms when they come into contact with faeces, urine, feed (e.g. silage), soil, contaminated water and other animals. The udders of housed cows can also become contaminated from contact with bedding materials (e.g. hay, sawdust).

Mastitis caused by *C. jejuni* appears to be rare, but occurrences have been documented:

- 1983, UK, campylobacteriosis outbreak (75 cases, 50 confirmed): Outbreak traced to two cows with asymptomatic mastitis caused by *C. jejuni* infection (Hutchinson *et al.*, 1985). *C. jejuni* was isolated from milk collected from these cows, samples of bulk milk at the farm and pooled samples of retail milk. No counts were done but the concentration of *C. jejuni* in the milk from two individual cows was sufficiently high that it could be detected even when diluted by milk from other cows in the herd.

- 1984, UK, campylobacteriosis outbreak (18 cases): Caused by one cow with *C. jejuni* mastitis (Morgan *et al.*, 1985). Monitoring of the cow found a single quarter was infected by *C. jejuni* for at least 12 weeks and the highest concentration measured was 10^4 CFU/ml. For most of the 12 week period the cow was asymptomatic.

- 1992, UK, campylobacteriosis outbreak (2 cases): *C. jejuni* was initially detected in two samples of raw milk from a producer who managed a herd of 31 cows producing around 460 l/day (Orr *et al.*, 1995). A foremilk sample of one cow and a bulk milk sample taken on same day were both positive for *C. jejuni*. Typing data revealed two cases of campylobacteriosis who had consumed the milk were infected with the same strain of *C. jejuni*.

- 2010-12, Italy, detection during survey: *C. jejuni* was isolated from the single quarter of an asymptomatic cow from a herd of 165 cows, and the same strain was detected in bulk milk from this herd (Bianchini *et al.*, 2014). Similar observations have been reported in an earlier Italian study of herds (270 and 180 cows) on two separate farms. On each farm, *C. jejuni* detected in bulk milk was traced to infection in one quarter of a single cow in the herd (Luini *et al.*, 2009).

C. jejuni is capable of forming biofilms and this capability is enhanced under aerobic conditions (Brown *et al.*, 2013; Reuter *et al.*, 2010). A continuous, passive sloughing off of cells from a *C. jejuni* biofilm on a glass surface under laboratory broth has been observed (Reuter *et al.*, 2010). While no information was located on *Campylobacter* spp. forming biofilms on surfaces under milk, the presence of *Campylobacter* biofilms in milking equipment could lead to continued contamination of the milk flowing over the biofilm.

2.3.2 Behaviour of *C. jejuni/coli* in raw milk

The generally accepted minimum growth temperature of *C. jejuni* and *C. coli* is 32°C (ICMSF, 1996). This means that there is no likelihood of growth occurring during the storage and distribution of raw milk where refrigeration temperatures are maintained. If the organism is not growing it will be declining in concentration. The rate of decline is likely to depend on the temperature of the milk.

The concentration of *C. jejuni/coli* in raw milk has been shown to decline at refrigeration temperatures. Most of the literature has focused on the behaviour of the organism in raw cows' milk at 4°C. Changes in concentration of *C. jejuni* based on data from a study published in 1982 are shown in Figure 1 (Doyle and Roman, 1982). Of note is the variability in the survival of individual isolates. Strains FRI-CF8 and FR-CF3 declined rapidly (D time

1.25 days) in a log-linear manner.[6] In most other cases the rate of decline in concentration reduced over time, shown most notably by strain FRI-CF147B. In this case there was only a 1.5 $\log_{10}$ reduction in concentration over 10 days of incubation.

Figure 1:   Survival of eight isolates of *C. jejuni* in raw milk at 4°C.
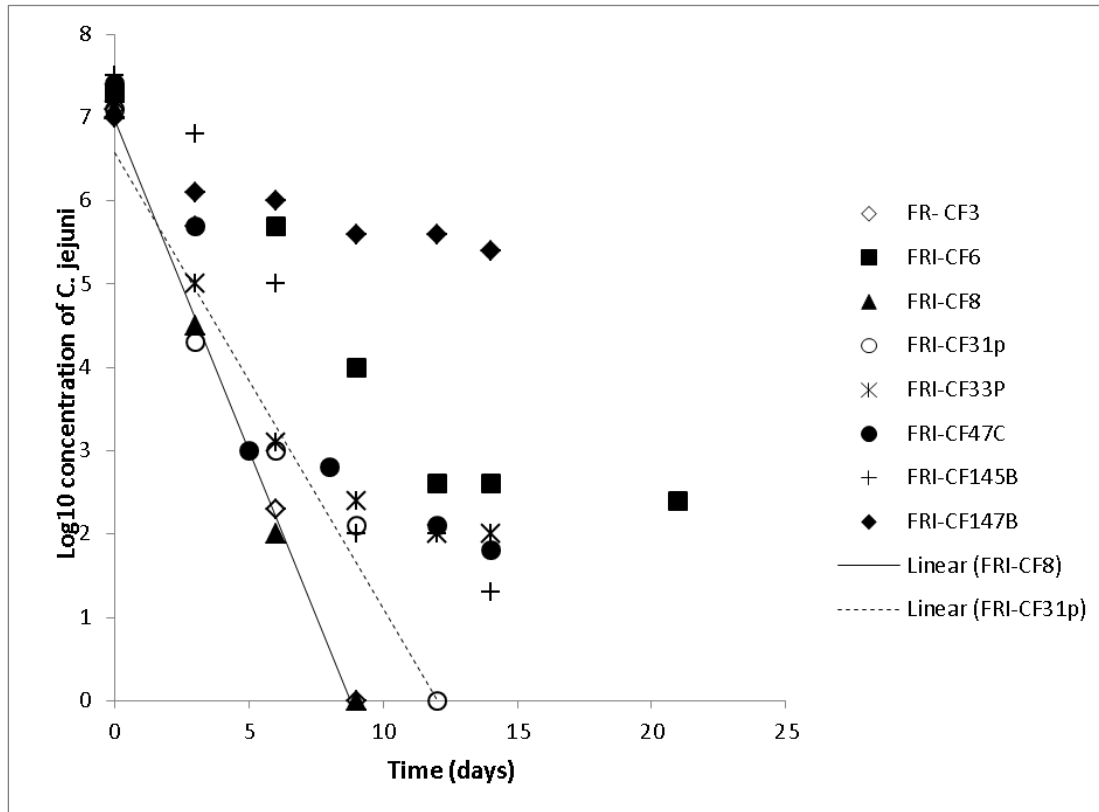
Note to Figure 1: Data are from Doyle and Roman (1982).

D times at 4°C reported in the literature are highly variable, ranging from >14 days (Xiong, 2009) to 1.25 days (Doyle and Roman, 1982). Variability in the survival of a single isolate in different milk batches has been shown in (Xiong, 2009). When inoculated into five batches of raw milk the isolate had a mean D time of 4.7 days with a standard deviation of 1.3 days. However, this variability was less than that observed between different isolates.

Some data suggest that survival in static conditions is longer than when the milk is agitated and therefore oxygenated. For two *C. jejuni* isolates, D times at 4°C were reported to be 1.5 and 2.1 days when the milk was shaken at 100 rpm, but when samples were incubated under static conditions the rate of decline was slower, with distinct non-log-linear inactivation curves (although the data were not for the same isolates) (Koidis and Doyle, 1984).

Data for the inactivation of *C. coli* showed similar results to those obtained for *C. jejuni* (Xiong, 2009). There was a considerable variation in the D time (from 3.2 to >14 days) among six tested isolates. The concentrations of the two most persistent isolates reduced by only 0.4 and 0.9 $\log_{10}$ CFU/ml over 14 days.

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[6] The D time is the time taken for the concentration of a bacterium to decrease by 90% (1 $\log_{10}$) at a defined temperature.

An inactivation model for *Campylobacter* in raw cows' milk at 4°C based on the data in Figure 1 has been published (FSANZ, 2009a). To accommodate variability among isolates a non-linear mixed model equation was used.

Because of the lack of data, it is not possible to say how the rate of decline might differ at other temperatures in cows' milk.

Information on survival of *Campylobacter* spp. in raw milk from species other than cows is limited. Some of the data from a study of *C. jejuni* in raw goats' milk allowed the estimation of a D time at 5°C of 0.45 days and at 10°C of 0.43 days (Simms and Mac Rae, 1989).

2.4   Exposure Assessment

KEY FINDINGS

Campylobacter spp. have been detected in two recent surveys of raw cows' milk in farm vats in New Zealand at prevalences of 0.3% and 0.6%. The concentrations in the three positive samples were <1 cell/ml (two samples were positive for *C. jejuni*, the species was not reported in the third sample). *Campylobacter* spp. were detected in one sample during a survey of goats' milk in New Zealand (n=56, 1.8%).

Surveys of the prevalence of *Campylobacter* spp. in the faeces of New Zealand dairy cows show that *C. jejuni* occurs frequently (>50% of samples) while *C. coli* is less prevalent (≤20%). Available data suggest seasonal variation with a higher prevalence and concentration of *Campylobacter* spp. in cows' faeces during spring and autumn. A study of a single herd of dairy goats in New Zealand detected *C. jejuni* and *C. coli* in faeces at 20% and 7% respectively, over a nine month period. *Campylobacter* spp. have been detected in faeces from non-milking sheep.

The number of people consuming raw milk in New Zealand is still uncertain. Recent estimates suggest the proportion of the population consuming raw milk is low (1% adults, 0.5% children). People living or working on dairy farms are more likely to consume raw milk. There are no data on consumption patterns (e.g. serving sizes) for raw milk, although consumption patterns for cold milk could serve as a proxy. The frequency of consumption is likely to depend on how easily consumers can access raw milk supplies.

2.4.1   New Zealand prevalence studies

2.4.1.1   *Prevalence of Campylobacter spp. in raw milk*

C. jejuni was not detected in 71 samples (25 ml) of raw cows' milk collected from tankers in 1986/87 (Stone, 1987). *Campylobacter* spp. were detected in 1 (0.9%) of 111 samples (10 ml) of raw cows' milk from farms in 1996/97 (Hudson *et al.*, 1999). Since 2000, two microbiological surveys of raw cows' milk in farm vats have been published that included testing for *Campylobacter* spp. (Table 1).

It should be noted that the milk sampled during both of these studies was destined for pasteurisation and/or processing into dairy products and was not necessarily also sold by the farmers as raw milk for direct human consumption.

Table 1: **Prevalence of *Campylobacter* spp. in two New Zealand surveys of raw cows' milk**

Raw milk survey	Survey period	Sample source	Prevalence of *Campylobacter* spp.*	Reference
Fonterra study	April 2007-May 2008	Farm vats, 290 dairy farms	1/296 (0.3%) (95% CI 0.01-1.87%)	(Hill *et al.*, 2012)
MPI study	November 2011-August 2012	Farm vats, 80 dairy farms	2/342 (0.6%) (95% CI 0.07 – 2.10%)	(MPI, 2013a; Soboleva *et al.*, 2013)

* Limit of detection 0.04 MPN/ml.

The two positive samples in the MPI study came from different farms indicating a between farms prevalence of 2/80, or 2.5%.

In the 2007/08 survey by Fonterra (Hill *et al.*, 2012) the concentration of *Campylobacter* spp. in the single positive sample was estimated as 0.05 MPN/ml. The concentrations in the two positive samples in the MPI survey (2013a) were 0.072 and 0.13 MPN/ml (both *C. jejuni*).

The prevalences of *Campylobacter* spp. detected in the MPI and Fonterra surveys will be reduced by the time period between sampling and commencement of testing in the laboratory, due to the decline in the number of cells during refrigerated storage. For the MPI survey this period has been estimated as 29-70 hours (Paul Jamieson, MilkTestNZ, pers. comm., March 2014).

In an additional study, one raw cows' milk sample (25 ml) was taken from each of six farms associated with human campylobacteriosis cases, but *Campylobacter* spp. were not detected in these samples (Gilpin *et al.*, 2008a).

Modelling work has estimated that straight after milking 92% of bulk milk tanks in New Zealand are likely to contain at least one *C. jejuni* cell, although the model predicts that *C. jejuni* would be detected in only approximately 6% of bulk milk tanks if the detection limit was set at one cell per 25 ml sample (MPI, 2013a). Models were based on data on total bacterial counts in raw milk, faecal concentration, and within and between herd prevalence. The models did not take into account any decline in *Campylobacter* numbers during the period between sampling and analysis.

A survey of raw goat milk samples was undertaken by MPI in three rounds from August 2012 to August 2013. A total of 56 samples from 20 farms (representing approximately 25% of all dairy goat farms in New Zealand) were tested for *Campylobacter* spp., and the organism was found in one sample (1.8%) (Tanya Soboleva (MPI), pers. comm., January 2014). To the authors' knowledge there are no surveys of *Campylobacter* spp. in milk produced from sheep or buffaloes in New Zealand.

2.4.1.2 Prevalence of Campylobacter spp. among New Zealand dairy animals

Five studies provide *C. jejuni/coli* prevalence data among New Zealand dairy cows (Table 2; also see note 3 to this table). The in-herd prevalence of *C. jejuni* was consistently higher than *C. coli*, but both species were present among dairy cows.

Table 2: **Prevalence of *Campylobacter* spp. among dairy cows in New Zealand**

Study period	Sample source	Number of samples	Prevalence: No. positive (% positive)		Reference
			C. jejuni	*C. coli*	
2000/01	Rectal samples, 36 farms (Matamata-Piako District)	225 cow 185 calf	114 (51)[1] 121 (65)	24 (11) 37 (20)	(Gilpin *et al.*, 2008b)
2002	Rectal samples, 1 farm (Manawatu)[3]	52	28 (54)	NR	(Adhikari *et al.*, 2004)
2003	Fresh cow pats, 7 farms (Waikato, Canterbury)	83	53 (64)[4]	2 (2)[4]	(Gilpin *et al.*, 2008a)
2005/06	Fresh cow pats, 4 farms (Waikato, Manawatu, Canterbury, Southland)	155	95 (61)[5]	10 (6)[5]	(Moriarty *et al.*, 2008)

NR, Not Reported

[1] *C. jejuni* was isolated from all 36 farms.

[2] Each sample was a composite of five cow pats.

[3] *Campylobacter* spp. have also been isolated from faeces from cows on this farm in earlier studies (Ahmed, 1999; Fakir, 1986; Meanger and Marshall, 1989; Wu, 2001).

[4] The *C. jejuni/coli* prevalence for each farm ranged 44-100%.

[5] 88 were positive for *C. jejuni*, 3 for *C. coli* and 7 for a mixture of *C. jejuni* and *C. coli*. Detection limit was 30 CFU/g.

Campylobacter spp. were enumerated in two longitudinal studies. In fresh cow pat samples, the median concentration of *Campylobacter* spp. was 4.3×10^2 CFU/g (95th percentile 3.9×10^5 CFU/g) and the maximum was above 1.8×10^7 CFU/g (the highest value that could be measured by the method chosen) (Moriarty *et al.*, 2008). In a study of faecal grabs from 35 cows on two farms the concentration of *C. jejuni* ranged from not detected (<0.3 MPN/g) to $6.0 \log_{10}$ MPN/g (Rapp *et al.*, 2012). This study found that most cows shed the organism intermittently, but 17% of animals shed the organism frequently (≥80% samples positive) and at concentrations of $3.3\text{-}3.6 \log_{10}$ CFU/g.

Seasonal patterns in *Campylobacter* spp. shedding were shown in three studies:

- The prevalence of *C. jejuni* among dairy cows was higher in autumn (18%) than in summer and winter (8%, 7% respectively), and the prevalence of *C. coli* was about the same in summer (14%) and autumn (13%) while the prevalence in winter was 4%. Considering both pathogens together, the prevalence of *C. jejuni* and/or *C. coli* among dairy cows was 31% in autumn, 24% in summer, and 12% in winter (Fakir, 1986; Meanger and Marshall, 1989).

- The prevalence of *C. jejuni* among dairy cows was higher in spring (61%) than in summer (44%), as was *C. coli* (20% spring, 4% summer) (Gilpin *et al.*, 2008b).

- Higher concentrations of *Campylobacter* spp. in dairy cow faeces were recorded in spring (median 2.0×10^4 MPN/g) compared with summer (median 68 MPN/g), autumn (median 2.3×10^2 MPN/g) and winter (median 15 MPN/g) (Moriarty *et al.*, 2008).

These data suggest that the prevalence and concentration of *Campylobacter* spp. are higher in dairy cow faeces during spring and autumn, but it is difficult to discern any distinct patterns from these studies as only one covered all four seasons.

Of 249 dairy goat faecal samples collected at five sampling points over nine months from the barn floor of a single herd, the prevalences for *C. jejuni* and *C. coli* were 20% and 7%, respectively (Rapp and Ross, 2012). Prevalence values across the five samplings ranged 4-43% for *C. jejuni* and from not detected to 20% for *C. coli*.

Similar data for Zealand sheep or buffaloes kept for milking were not available. *C. jejuni/coli* have been isolated from faeces from non-dairy sheep in New Zealand (Devane *et al.*, 2005; French and the Molecular Epidemiology and Veterinary Public Health Group, 2008).

2.4.2   Food consumption: Raw milk

ESR has analysed data from three New Zealand nutrition surveys to estimate raw milk consumption. The three data sets analysed were:

- The 1997 National Nutrition Survey (NNS; 4,636 people aged 15+ years) (Russell *et al.*, 1999);

- The 2002 National Children's Nutrition Survey (CNS; 3,275 people aged 5-15 years) (Ministry of Health, 2003); and

- The 2009 Adult Nutrition Survey (ANS; 4,721 people aged 15+ years) (University of Otago and Ministry of Health, 2011).

2.4.2.1   Number of people consuming raw milk in New Zealand

People were not specifically asked about consumption of raw milk. The following estimates are made from the available data:

- NNS: 1.0% (95% CI 0.8-1.4%) of the adult population consumed "fresh cows' milk" as one of the categories included under "other" type of milk.

- CNS: 0.5% (95% CI 0.3-0.8%) of the child population consumed "vat milk", "farm milk", "real milk" and "cows' milk".

- ANS: An upper bound of 1.1% of the adult population "mostly" using raw milk.

Another recent estimate was provided by a national case-control study of Shiga toxin-producing *Escherichia coli* infection carried out from 2011/12 (Jaros *et al.*, 2013). It was found that 16/506 controls (3.2%; 95% CI 1.8-5.1%) reported raw milk or raw milk product consumption, which is higher than the estimates from nutrition surveys. The difference might be real and reflect an increase in raw milk consumption since the 2009 ANS, or it may be because the question asked in the case-control study also captured people who consume raw milk products.

People who live or work on dairy farms are more likely to consume raw milk, as shown by a Massey University survey in 2011 which found that 64% (858/1,337) of dairy farmers reported consuming raw milk (McFadden *et al.*, 2011).

There is also anecdotal evidence that raw milk availability is increasing. Raw cow and goat milk is advertised on auction and other websites, and raw milk vending machines are now operating in some areas.

2.4.2.2   *Raw milk servings*

The ANS and CNS data were analysed to extract consumption patterns for all milk, and then this was partitioned into servings considered to be cold milk only, by removing servings where the milk was thermally treated in some way, e.g. added to hot beverages, used to prepare porridge or added to cooking. A summary of the results is presented in Table 3. These data may be used as a proxy for raw milk servings (size and frequency of consumption), in the absence of data specific for raw milk.

Table 3:   Consumption of cold milk by New Zealanders (national nutrition surveys)

Statistic	Adult (2009 ANS)	Child (2002 CNS)
Number of respondents	4,721	3,275
Number of servings	1,902	2,425
Number of consumers (percentage of total respondents)	1,653 (35.0%)	1,778 (54.3%)
Servings/consumer/day (average)	1.1	1.4
Consumer mean (g/person/day)	231.9	273.4
Mean serving size (g)	201.5	200.5
Median serving size (g)	169.6	194.0
95th percentile serving size (g)	424.0	387.0

2.4.3   Potential for growth of *Campylobacter* spp. along the raw milk food chain

If *C. jejuni/coli* is present in raw milk it will not grow if refrigeration temperatures are maintained. The numbers of *C. jejuni/coli* will decline during refrigerated storage but the rate of reduction is highly variable depending on the strain.

2.5   **Data on *Campylobacter* spp. and Raw Milk from Other Countries**

KEY FINDINGS

The reported prevalences of *C. jejuni* in surveys of raw cows' milk overseas are similar to data from New Zealand i.e. generally <1.5%. *Campylobacter* spp. are detected less frequently in raw milk from sheep or goats but fewer studies are available on these types of milk. Prevalence data for *Campylobacter* spp. in buffaloes' milk were not found, nor were any recent data on the concentration of *Campylobacter* spp. in raw milk.

The prevalence of *C. jejuni/coli* in faecal samples from dairy cows in other countries is similar to that found for New Zealand. The prevalence of *C. jejuni* ranged 19-50% and the prevalence of *C. coli* ranged 2-15%. Data for goats, sheep and buffaloes used for milking are scarce or absent.

Estimates of raw milk consumption in developed countries (up to 3% of the population, with people living or working on dairy farms being more likely to consume raw milk) are similar to estimates for New Zealand.

Appendix 1 contains the detailed data which are summarised in this section.

2.5.1   Prevalence and frequency studies in other countries

While data collected in other countries are useful for comparative purposes and to augment New Zealand data, it is important to note that dairy farming methods in New Zealand are different to those used in other countries. For example, dairy herds in New Zealand are much larger than those generally seen in the European Union (EU), and larger volumes of milk are processed. New Zealand dairy herds are generally not housed and are predominantly fed on pasture (Hill *et al.*, 2012). Factors such as housing conditions and feed supply can affect the prevalence of pathogenic microorganisms among dairy animals.

2.5.1.1   *Campylobacter spp. in raw milk*

Five surveys published since 2007 detected *C. jejuni* in raw cows' milk at prevalence values ranging 0.4-5%, and a prevalence of 12% was reported from an Italian survey (Bianchi *et al.*, 2013; Bianchini *et al.*, 2014; Giacometti *et al.*, 2012b; Løvseth *et al.*, 2007; Messelhäusser *et al.*, 2008; Wysok *et al.*, 2011).   All isolates were *C. jejuni*; *C. coli* was not isolated. *Campylobacter* spp. were not detected in another three surveys (FSANZ, 2009a; Hakkinen and Hänninen, 2009; Ruusunen *et al.*, 2013). A recent paper has reported a prevalence range of 0-6% for *C. jejuni* and *C. coli* in European raw cows' milk (Claeys *et al.*, 2013).

There are few studies of *Campylobacter* spp. prevalence in milk from other animal species. Available data show that *Campylobacter* spp. was often not found in milk from these species. When *Campylobacter* spp. was found in sheep or goats' milk, the reported prevalences were 5% (goats' milk) and 1% (mixed sheep and goats' milk) (FSANZ, 2009b; Schoder *et al.*, 2010).

There are no recent studies that measured the concentration of *Campylobacter* spp. in raw milk produced overseas.

2.5.1.2   *Campylobacter spp. among dairy animals*

The prevalence of *C. jejuni/coli* in faecal samples from dairy cows is much higher than in milk. According to six studies published since 2007, the prevalence of *C. jejuni* among individual adult dairy cows ranged 19-50%, and the prevalence of *C. coli* was lower (range Not Detected-15%) (Bianchini *et al.*, 2014; Fernández and Hitschfeld, 2009; Grove-White *et al.*, 2010; Hakkinen and Hänninen, 2009; Oporto *et al.*, 2007; Ramonaitė *et al.*, 2013; Sanad *et al.*, 2013). There may be a seasonal pattern as many studies found peaks during warmer months, but this finding is not consistent (Horrocks *et al.*, 2009). The prevalence was higher when animals were confined to houses or feedlots (Besser *et al.*, 2005). The concentration of *Campylobacter* spp. in faeces from dairy cows was 2-3 $\log_{10}$ CFU/g.

There were no data for the prevalence of *Campylobacter* spp. among sheep or buffaloes used for producing milk, and *Campylobacter* spp. were not isolated in a study of dairy goats in Spain (Cortés *et al.*, 2006). *C. jejuni* and *C. coli* have been isolated from the faeces of non-dairy sheep.

2.5.2   Raw milk consumption in other countries

Estimates for the proportions of the populations drinking raw milk in other developed countries are low (up to 3%), irrespective of the legal status of raw milk sales (Buzby *et al.*,

2013; Giacometti *et al.*, 2012a). The proportion of people living or working on dairy farms and consuming raw milk is higher in most surveys (up to 60% has been reported), which is similar to the situation in New Zealand (Oliver *et al.*, 2009; Nesbitt *et al.*, 2009).

3   EVALUATION OF ADVERSE HEALTH EFFECTS

3.1   Disease Characteristics

KEY FINDINGS

Infection by *C. jejuni* or *C. coli* in humans usually results in a self-limiting gastroenteritis which may or may not involve bloody diarrhoea. In a small proportion of cases infection may progress to more serious autoimmune disorders or chronic gastrointestinal diseases. On rare occasions infection may result in death.

C. jejuni and *C. coli* can colonise the lower intestinal tract of humans without causing any symptoms. When infection does occur, disease is typically characterised by 1-3 days of prodromal symptoms (fever, vomiting and headaches) followed by 3-7 days of watery or bloody diarrhoea with abdominal pain (Dasti *et al.*, 2010). The incubation period is usually 2-5 days (range 1-10 days) (Ministry of Health, 2012). Campylobacteriosis is self-limiting and treatment other than rehydration is rarely required, but antibiotics such as ciprofloxacin may be used in severe cases. The use of the antacid drug omeprazole predisposes people to campylobacteriosis (Neal *et al.*, 1996) and immunocompromised patients appear predisposed to chronic or recurrent *C. jejuni* enterocolitis (Peterson, 1994).

A small proportion of cases of campylobacteriosis can develop into more serious sequelae such as reactive arthritis and Guillain-Barré Syndrome (GBS). Evidence is emerging that a *Campylobacter* spp. infection can predispose people to chronic diseases such as irritable bowel syndrome, inflammatory bowel disease, coeliac disease and functional dyspepsia (Riddle *et al.*, 2012). *C. jejuni* may cause septicaemia (Dhawan *et al.*, 1986), abortions, stillbirths and neonatal deaths in humans but these outcomes are rare (Smith, 2002). On rare occasions infection may result in death, usually amongst elderly patients or those with another serious disease (Blaser and Engberg, 2008). Further information on GBS and reactive arthritis is included in Appendix 2, and further information on *Campylobacter* spp. infection is summarised in a microbiological data sheet.[7]

3.2   Dose Response

KEY FINDINGS

The dose response relationship for *Campylobacter* spp. makes a distinction between the dose required to cause infection and the probability of symptomatic illness once infected. Based on available data, approximately 800 cells gives a probability of infection of 50%. The probability of illness once infected is not well defined, but has been estimated to be between 20% and 50%. Regular exposure to *Campylobacter* spp. may confer some immunity to repeated illness.

Appendix 2 contains detail on dose response.

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[7] http://www.foodsafety.govt.nz/elibrary/industry/Campylobacter-Organism_Causes.pdf (accessed 12 February 2014).

The dose response is the relationship between the probability of infection or disease and the number of cells consumed. In the case of *Campylobacter* spp. the situation is a little more complicated as infection (multiplication in the intestine) can occur in the absence of illness. Hence there is a probability of disease conditional on a probability of infection.

Data from a human feeding trial have been evaluated and reported by an expert group assembled by the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) (FAO/WHO, 2002). Infection, where the microorganism is reproducing in the body, was modelled separately from illness, which is less frequent. The likelihood of infection increased from approximately 50% at 800 cells to about 100% at $1x10^8$ cells.

The FAO/WHO hazard characterisation (FAO/WHO, 2002) also explored the idea that there is a conditional probability of disease in humans resulting from infection. This model predicts that in the vast majority of cases where people become infected there is >20% and <50% chance of the person subsequently becoming sick, with the maximum probability at 33%.

There is some evidence to show that previous exposure to *Campylobacter* spp. can lead to acquired immunity (Havelaar *et al.*, 2009; Tribble *et al.*, 2010).

3.3   New Zealand Human Health Surveillance

KEY FINDINGS

Evidence to link campylobacteriosis in New Zealand with raw milk consumption is provided by a number of human health surveillance and epidemiological sources.

Each year since 2008, foodborne campylobacteriosis outbreaks have represented approximately 10% of the over 100 annual reported foodborne outbreaks of enteric disease, with raw milk reported as a risk factor in up to 29% of the foodborne campylobacteriosis outbreaks. There is strong evidence from two campylobacteriosis outbreak investigations to link illness with drinking raw cows' milk.

Consumption of raw milk has also caused some sporadic cases of campylobacteriosis. While, the proportion of sporadic cases of campylobacteriosis reporting consumption of raw milk (amongst other risk factors) in New Zealand is small, this information is not routinely collected, and is almost certainly under reported.

A case control study conducted in New Zealand during 1994/95 found there was a significant risk of campylobacteriosis from consumption of any unpasteurised milk in the previous 10 days. Recent intensive surveillance of campylobacteriosis cases in the Manawatu region of New Zealand identified a cluster of cases that comprised an outbreak caused by drinking raw milk. In addition, data from cases in the Manawatu have been analysed to show a link between raw milk consumption and infection with a cattle-associated strain of *Campylobacter* spp. The link was shown to be stronger when cases with contact with farm animals as an additional risk factor were excluded. Although this analysis cannot exclude other pathways of infection with ruminant associated subtypes, this result does support raw milk being the source of infection for these cases.

3.3.1   Raw milk consumption as a risk factor for *Campylobacter* infection

3.3.1.1   Sporadic cases

For the ten years from 1 January 2004 to 31 December 2013 there were 476 reported campylobacteriosis cases where raw milk was reported as being consumed by the cases (approximately 0.4% of all reported campylobacteriosis cases).[8] The vehicle for infection was not confirmed in any of these cases, but conclusive evidence for transmission vehicles is rarely obtained from sporadic cases and small outbreaks, mostly because obtaining samples of food consumed by cases is difficult. The enteric infection case report form used for campylobacteriosis cases does not specifically ask about consumption of raw milk so this information is not routinely collected.

3.3.1.2   Outbreaks

The earliest report of an outbreak of campylobacteriosis linked to raw milk in New Zealand was in 1984 (Brieseman, 1984). Two different camp sites in Christchurch were involved, with 88 children becoming ill. *Campylobacter* spp. were isolated from faecal samples from 50 of these cases. Raw milk was obtained from a local farm. Although no *Campylobacter* spp. were isolated from milk samples, this source was inferred from epidemiological evidence, i.e. the intermittent nature of the problem (which was considered to make the water supply unlikely as a source), and the fact that sickness occurred amongst children in 5 of 7 camping events where raw milk was consumed.

Between January 2006 and February 2014 there were 16 outbreaks of campylobacteriosis reported in EpiSurv where raw milk consumption was recorded as a risk factor (one outbreak involved two cases, one of which was reported as a mixed infection of *Campylobacter* spp. and shiga toxin-producing *Escherichia coli* (STEC)). An additional cluster of two cases of campylobacteriosis in 2012 was retrospectively linked to consumption of raw milk. There was strong evidence to link two of these outbreaks to the raw milk:

- 2009, 15 cases in a school group visiting a farm (Bai *et al.*, 2010). The attack rate for drinking raw milk was 56%, while the attack rate for those who did not drink raw milk was 1.6% (X^2=34.23, P$\leq$ 0.001). *Campylobacter* spp. were detected in two milk samples (the strains were not identical to that isolated from a clinical sample, but the milk samples were taken almost two weeks after the farm visit and multiple strains may have been present in the milk).

- 2011, a cluster of nine sporadic cases that was retrospectively identified as an outbreak based on *Campylobacter* spp. strain typing. The outbreak was associated with a single supplier of raw milk (Müllner *et al.*, 2013) (see also Section 3.3.1.3).

Each year since 2008, foodborne campylobacteriosis outbreaks have represented approximately 10% of all reported foodborne outbreaks of enteric disease and raw milk has been reported as a risk factor in up to 29% of the foodborne campylobacteriosis outbreaks (Table 4).

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[8] This excludes 39 cases linked to outbreaks.

Table 4: **Drinking raw milk as a risk factor in reported foodborne outbreaks of campylobacteriosis, 2006-2012**

Year	Number of foodborne outbreaks			Number of cases associated with foodborne outbreaks			Reference
	All causative agents[1]	Campylobacteriosis	Campylobacteriosis: Raw milk reported as a risk factor	All causative agents	Campylobacteriosis	Campylobacteriosis: Raw milk reported as a risk factor	
2006	146	32	0	909	135	0	(ESR, 2007b; Pirie *et al.*, 2008)
2007	74	12	0	611	35	0	(ESR, 2008b; Williman *et al.*, 2008)
2008	89	8	1[2]	1206	36	2	(ESR, 2009b; Williman *et al.*, 2009)
2009	84	7	2	651	39	18	(ESR, 2010b; Lim *et al.*, 2010)
2010	141	14	3	936	62	9	(ESR, 2011a; Lim *et al.*, 2011)
2011	122 (102)	11	2	656	53	12	(ESR, 2012a; Lim *et al.*, 2012)
2012	110 (92)	11	3	967	51	11	(ESR, 2013a; Lopez *et al.*, 2013)

[1] All reported outbreaks where "foodborne" was selected as a mode of transmission. More than one mode of transmission can be reported for outbreaks. From 2011 the mode of transmission could be assigned as the "primary mode" (most likely or confirmed mode), and the number of outbreaks where "foodborne" was the primary mode are included in brackets in the table.

[2] Williman *et al.* (2009) reports this as milk but raw milk is reported in the associated EpiSurv outbreak report.

3.3.1.3   Case control and source attribution studies

A case control study of campylobacteriosis that considered raw milk as a risk factor was conducted in New Zealand during 1994/95 (Eberhart-Phillips *et al.*, 1997). The study found there was a significant risk of campylobacteriosis from consumption of any unpasteurised milk in the previous 10 days (multivariate analysis odds ratio 2.69 (95% confidence interval 1.38-5.23)). Consumption of raw milk was reported by 36/621 cases and 15/621 controls.

Given the few isolations of *Campylobacter* spp. from raw milk it is not possible to examine the links between types in human cases and those in raw milk isolates. However, New Zealand data have been produced to show links between the types in dairy cow faeces and those in human cases.

A comparison of the subtypes of 147 *C. jejuni* isolates from dairy cows (89) and human cases (58) in the Matamata-Piako district found that 25 human isolates were of subtypes indistinguishable from 19 dairy cow/calf isolate genotypes, showing that dairy cows harbour human pathogenic sub-types (Gilpin *et al.*, 2008b). Earlier data had shown indistinguishable *C. jejuni* subtypes isolated from 89 dairy cow faeces and 61 human cases from the Ashburton area (Devane *et al.*, 2005; Garrett *et al.*, 2007). However, such studies cannot be conclusive about transmission as overlaps also exist between the types from human cases and a number of other potential sources.

A sentinel site study in the Manawatu has provided a detailed picture of *Campylobacter* spp. sources for human infections in that region (French *et al.*, 2010; Müllner *et al.*, 2009a; Müllner *et al.*, 2010b). A study report from 2012 identified a cluster of eight cases infected with the same subtype of *C. jejuni* over a two-week period between 23rd May and 7th June 2011 (French and the Molecular Epidemiology and Public Health Laboratory, 2012). All reported drinking raw milk, with seven of the cases obtaining their milk from the same farm. A further case was identified after the report was prepared, making 9 cases in all, as reported in Section 3.3.1.2.

Analysis of risk factor data from Manawatu cases of campylobacteriosis collected during 2005-2012 found that raw milk consumption and infection with a cattle-associated strain of *Campylobacter* spp. were associated (relative risk of approximately 4) (Professor Nigel French, Massey University, pers. comm., March 2014).[9]   Furthermore, a stronger link between raw milk consumption and infection with a cattle-associated genotype was observed for cases who did not have contact with farm-animals (a possible alternative source of infection): 54.5% (12/22) of those who consumed raw milk and had no farm contact were infected with a cattle-associated genotype, compared to 12.9% (53/410) of those who did not drink raw milk and had no reported farm-animal contact (p<0.0001).

Although this analysis cannot exclude other pathways of infection with ruminant associated subtypes, this result does support raw milk being the source of infection for these cases.

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[9] Relative risk is the ratio of the probability of the event occurring in the exposed group versus a non-exposed group.

3.3.2 *Campylobacter* spp. infection in New Zealand

Campylobacteriosis has consistently been the most commonly reported infectious intestinal disease in New Zealand.[10]   Figure 2 shows annual notifications and notification rates for 2000-2012. The marked reduction in rate from 2006-2008 coincided with efforts to reduce *Campylobacter* spp. contamination on poultry produced in New Zealand (Sears, 2009).

Annual notifications are characterised by a summer peak and winter trough. The age distribution of notified cases is bimodal, with peaks in the 1-4 and 20-29 year groups (Lopez *et al.*, 2013).

Figure 2:   Campylobacteriosis notifications and notification rate by year, 2000-2012
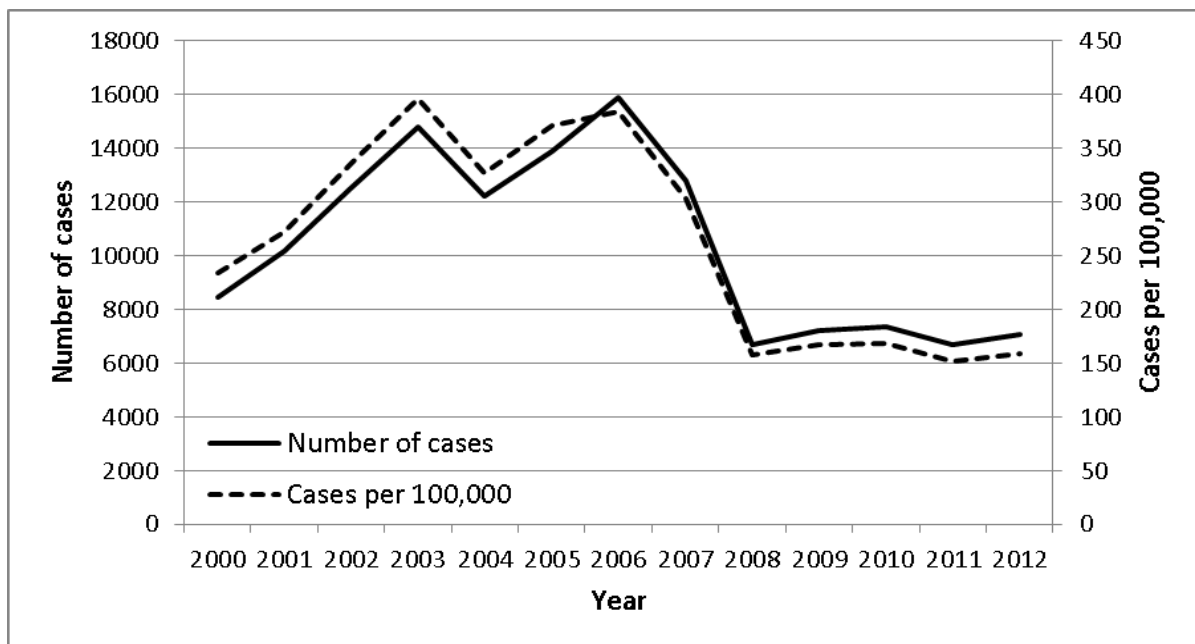
Note to Figure 2: Data are from (ESR, 2004; 2005; 2006; 2007a; 2008a; 2009a; 2010a; 2011b; 2012b; 2013b; Lopez *et al.*, 2001; Sneyd *et al.*, 2002; Sneyd and Baker, 2003).

Hospitalisation rates for notified cases of campylobacteriosis in New Zealand are approximately 10% each year, and three deaths have been reported amongst notified cases from 2003 to 2010 (0.01% of cases in each year that a death was reported). These outcomes are not always reported for each case, therefore percentages are expressed in terms of the number of cases for which outcomes are known.

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[10] Details of notified cases of campylobacteriosis are reported each year in Annual Surveillance Summaries available from: https://surv.esr.cri.nz/surveillance/surveillance.php

3.4   *Campylobacter* spp. Infection Overseas

KEY FINDINGS

There are numerous campylobacteriosis outbreaks reported from developed countries overseas that are linked to consumption of raw cows' milk, and some linked to consumption of raw goats' milk.

Results from case control studies overseas that included raw milk consumption as a potential risk factor for campylobacteriosis have been mixed, with some studies finding raw milk to be a significant risk factor and others not.

Despite significant reductions in recent years, New Zealand's rate of reported campylobacteriosis remains high when compared to rates from other countries but this may be due to different reporting practices.

Appendix 2 contains detailed data summarised in this section.

4   EVALUATION OF RISK

4.1   Existing Risk Assessments

KEY FINDINGS

Risk assessments conducted in New Zealand and overseas consistently conclude that there is a risk of campylobacteriosis associated with the consumption of raw milk, some assigning this risk as high. The risk reduces along the distribution chain as the concentration of the organism reduces with time.

Appendix 2 contains detailed data summarised in this section.

4.1.1   New Zealand risk assessment

MPI has completed a microbiological risk assessment for the consumption of raw milk in New Zealand (MPI, 2013a).   The assessment focussed on raw cows' milk and used quantitative modelling to estimate the risk per random daily serve of raw milk to consumers from *Campylobacter* spp. (and other pathogenic microorganisms).

The predicted number of campylobacteriosis cases decreases as the supply chain lengthens because *Campylobacter* spp. die off in raw milk held at 4°C.   The predicted number of campylobacteriosis cases associated with obtaining milk from a vending machine is slightly lower than obtaining milk from the farm gate because the storage period in the vending machine is an additional step before consumption, lengthening the time period during which the decline in numbers may occur.   Similarly, for the retail store food chain there is a longer period between the farm gate and consumer purchase, allowing additional time for decline in the number of cells.

The risk assessment concluded that the risk for transmission of *Campylobacter* spp. to humans through consumption of raw milk is high, with the greatest risk being for milk obtained and consumed closest to the milking point.

4.1.2   Risk assessments from other countries

Risk assessments for *Campylobacter* spp. in raw milk have been published for Australia, Italy, the UK, Norway and Belgium (see Appendix 2, Section 8.4).   In summary:

- Australia (raw cows' milk (modelling), raw goats' milk (qualitative)): For cows' milk, the mean predicted number of cases of illness from *Campylobacter* spp. infection per 100,000 daily serves of raw milk was highest when milk was consumed from farm bulk milk tanks, decreased for milk consumed after farm gate sales; and decreased further for milk consumed after retail purchase. The decrease as the supply chain lengthens is a result of the inactivation of *Campylobacter* spp. in chilled raw milk. The goats' milk assessment rated the risk to public health and safety from *Campylobacter* spp. in raw goats' milk as "low".

- Italy (modelling of the risk of campylobacteriosis from raw milk sold through vending machines): The predicted number of campylobacteriosis cases per 10,000-20,000 consumers per year linked to consumption of refrigerated raw milk was 6.6. Modelling assumed 57% of consumers boiled the milk before consumption.

- UK (revision of evidence): Maintained the view that there were significant risks to human health from consumption of raw drinking milk.

- Norway (raw cows' milk and cream, raw milk from other animals): The probability of transmission of *C. jejuni* to humans via raw cows' milk and cream and the risk of illness were considered high. The risk of illness from milk from other animals was also considered high but supporting data were lacking.

- Belgium (raw cows' milk, risk/benefit evaluation): *Campylobacter* spp. were among the main bacteria that can be transmitted through raw milk to humans.

4.2   Evaluation of Risk for New Zealand

KEY FINDINGS

The currently available evidence suggests that the risk of *C. jejuni/coli* infection for consumers of raw milk in New Zealand is high. The risk will be greatest for milk obtained and consumed closest to the point of milking, as *Campylobacter* numbers will decline during storage.

The strongest evidence supporting this evaluation is human health surveillance data from New Zealand. This includes strong evidence from two campylobacteriosis outbreak investigations to link illness with drinking raw cows' milk, identification through intensive surveillance in the Manawatu of a cluster of sporadic cases linked to drinking raw milk, and a 1994/95 case control study.

Data on the carriage of *C. jejuni/coli* by New Zealand dairy cows indicates substantial potential for bacteria to be present in raw milk through faecal or environmental contamination.

Campylobacteriosis outbreaks caused by drinking raw milk have been reported in many developed countries overseas.

The dose-response data for *Campylobacter* spp. indicate that the risk of infection and subsequent illness is high from low numbers of cells.

4.2.1   Risk associated with raw milk

The currently available evidence suggests that the risk of *C. jejuni/coli* infection for consumers of raw milk in New Zealand is high. The risk will be greatest for milk obtained and consumed closest to the point of milking, as *Campylobacter* numbers will decline during storage.

1. The strongest evidence supporting this evaluation is human health surveillance data from New Zealand. There is strong evidence from two campylobacteriosis outbreak investigations to link illness with drinking raw cows' milk. Drinking raw milk has also been reported as a risk factor in up to a third of foodborne campylobacteriosis outbreaks each year since 2006. Consumption of raw milk has also caused sporadic cases of campylobacteriosis. The proportion of sporadic cases of campylobacteriosis reporting consumption of raw milk (amongst other risk factors) in New Zealand is small, but this information is not routinely collected and is almost certainly under reported. A case control study conducted in New Zealand during 1994/95 found there was a significant

risk of campylobacteriosis from consumption of any raw milk in the previous 10 days. Recent intensive surveillance of campylobacteriosis cases in the Manawatu region identified a cluster of cases that comprised an outbreak caused by drinking raw milk, and a strong link between drinking raw milk and infection with a cattle-associated strain of *Campylobacter* spp. for cases that did not have contact with farm animals (Section 3.3.1.3).

2. Data on the carriage of *C. jejuni* and *C. coli* by New Zealand dairy cows indicates substantial potential for bacteria to be present in raw milk through faecal or environmental contamination, even though prevalences found in raw milk surveys were <1%. Because these pathogens die off in raw milk held under refrigeration, the time between milking and testing raw milk samples for the presence of *Campylobacter* spp. will reduce that prevalence that can be detected. Much higher prevalence estimates (92% for at least one *Campylobacter* cell in bulk milk tank) have been derived from data on total bacterial counts in raw milk, faecal concentration, and within and between herd prevalences.

3. Campylobacteriosis outbreaks caused by drinking raw milk have been reported in many developed countries overseas.

4. The dose-response relationship derived from available data for *Campylobacter* spp. indicate that the probability of infection is high (50%) from relatively low numbers of cells (approximately 800).

5. *Campylobacter* spp. have been detected in a New Zealand survey of raw goat milk, and have been detected at higher prevalence in the faeces of dairy goats. These data are consistent with the pattern for raw cows' milk, suggesting that there is a risk of exposure to *Campylobacter* spp. from raw goats' milk.

There are insufficient data to evaluate the risk from raw milk from sheep and buffaloes in New Zealand.

This evaluation of risk is made on the basis of currently available data and agrees with the findings of the MPI risk assessment (MPI, 2013a). Data gaps identified in this document are summarised in Section 4.5.

4.2.2   Risks associated with other foods

Studies prior to 2007 showed that poultry was by far the leading vehicle for campylobacteriosis in New Zealand (Müllner *et al.*, 2009b; Müllner *et al.*, 2010a; Müllner *et al.*, 2010b). The incidence of campylobacteriosis has declined in New Zealand and this has been attributed to interventions in the poultry industry (Müllner *et al.*, 2013). The relative importance of ruminant associated strains of *Campylobacter* from human cases has increased (absolute numbers of ruminant associated strains have not increased but the number of poultry associated strains has declined) (French and the Molecular Epidemiology and Public Health Laboratory, 2012). Raw milk may be a source of exposure to ruminant associated strains but exposure to untreated drinking water, animals, the rural environment and recreational water are also possible transmission routes (Lake *et al.*, 2011; McBride *et al.*, 2011). Red meat consumption is not considered an important transmission route (Lake *et al.*, 2011).

4.3 The Burden of *Campylobacter* Infection in New Zealand

KEY FINDINGS

On a national scale (and on the basis of existing information), the burden of disease from drinking raw milk contaminated with *Campylobacter* spp. is considered to be low because the size of the consuming population is small and other exposures to *Campylobacter* spp. are relatively more common. The burden of disease from foodborne *Campylobacter* spp. infection in New Zealand is second on a ranked list of six enteric foodborne diseases, based on an estimate from 2011.

4.3.1 Burden of disease from raw milk contaminated with *Campylobacter*

On a national scale (and on the basis of existing information), the burden of disease from raw milk contaminated with *Campylobacter* spp. is considered to be low because:

- Currently the size of the consuming population is small. An estimated 1% of adults and 0.5% of children in the New Zealand population consume raw milk in any one day, although a case control study suggested that consumption of raw milk in New Zealand may have increased since 2007 (Jaros *et al.*, 2013).

- Other exposures to *Campylobacter* spp. are relatively more common (see Section 4.2.2).

While poultry still appears to be the principal vehicle for campylobacteriosis, the relative proportion of infections with ruminant associated *Campylobacter* spp. subtypes has increased. This result has been demonstrated by the study of cases in the Manawatu since 2005 (French *et al.*, 2010; French and the Molecular Epidemiology and Public Health Laboratory, 2012). One of the pathways for exposure to ruminant associated subtypes is consumption of raw milk, and the importance of this pathway may be increasing.

4.3.2 Burden of disease from all *Campylobacter* infection

A recent study has estimated the total number of campylobacteriosis cases for New Zealand, accounting for cases that do not come to the attention of the medical reporting systems (Cressey and Lake, 2011). The annual number of domestically acquired foodborne campylobacteriosis cases was estimated as 190,092 (90% CI: 93,748-297,938) based on data for the period 2000-2009, which represented 34% of the total estimated cases caused by 24 pathogens that may be foodborne. The proportion was 25% when data from 2009 were analysed separately.

It has been estimated by expert consultation that 56% (95% CI: 26-82) of campylobacteriosis incidence are due to foodborne transmission (Lake *et al.*, 2010). A recent analysis of the burden of foodborne disease in disability adjusted life years (DALYs) used data from 2011 and multipliers from recent studies (e.g. (Scallan *et al.*, 2011)) to estimate the level of underreporting in the health system (Cressey, 2012). The total burden of disease from campylobacteriosis and sequelae was calculated as 1,046 DALYs, with sequelae (GBS, reactive arthritis and inflammatory bowel disease) accounting for 23% of this figure. Of the total DALYs, 587 DALYs (5th-95th percentile 425-781) were attributed to foodborne infection. For comparison, the only larger DALYs estimate for foodborne-associated disease was for norovirus infection (873, 5th-95th percentile 675-1,083). When a criterion that

reflects the perceived trivial nature of very mild episodes of gastroenteritis (such as that caused by norovirus infection) was applied, foodborne campylobacteriosis became the enteric illness with the greatest burden.

An estimate of the total economic cost to New Zealand of six foodborne diseases has been published (Gadiel, 2010). This estimate converted the individual burden in DALYs to an economic value and was based on data from 2009. Of the estimated total cost including direct and indirect medical costs and the burden on individuals ($161.9m), campylobacteriosis accounted for $36.0 million (22%). Of the $36m, almost half was due to the costs associated with loss of output, which was a much higher proportion than for the other five diseases.

These estimates cover all potential food vehicles. There are no separate estimates for transmission of *Campylobacter* spp. via raw milk.

4.4 Summary of Risk

KEY FINDINGS

Campylobacter spp. can contaminate raw milk in New Zealand and the absence of pasteurisation means that there is no control measure that will eliminate *Campylobacter* spp. from this food. The currently available evidence suggests that the risk of *C. jejuni/coli* infection for consumers of raw milk in New Zealand is high.

4.5 Data Gaps

KEY FINDINGS

There are many data gaps identified in this report. Data on the amount of raw milk consumed in New Zealand, the behaviour of *C. jejuni/coli* in raw milk, and the concentration of *C. jejuni/coli* in raw milk generally and milk implicated in campylobacteriosis cases will improve the exposure assessment and therefore have the most impact on the evaluation of risk

Data gaps identified in this report are:

- Differences, if any, in pathogenicity between strains of *C. jejuni/coli*;

- The prevalence of mastitis in dairy animals caused by *Campylobacter* spp. infection;

- The prevalence of *C. jejuni/coli* in raw milk from sheep and buffaloes;

- The concentration of *C. jejuni/coli* in raw milk;

- The prevalence of *C. jejuni/coli* among milking sheep and buffaloes in New Zealand (as an indicator of the potential for milk contamination);

- Survival of *C. jejuni/coli* in raw milk at temperatures other than 4°C;

- Storage temperatures and holding times for raw milk along different stages of the supply chain from production to consumption;

- The amount of raw milk consumed in New Zealand;

- The proportion of the population consuming raw milk in New Zealand and the demographics of this population; and

- The concentration of *C. jejuni/coli* and the volume of raw milk consumed by campylobacteriosis cases where raw milk was the source of infection.

Surveillance data would be improved if all laboratory-confirmed campylobacteriosis cases were routinely asked about consumption of raw milk.

5   AVAILABILITY OF CONTROL MEASURES

KEY FINDINGS

Under current legislation, a milk producer may sell raw milk to any person if it is sold at the producer's dairy premises and in a quantity not exceeding 5 litres at any one time, and the person intends the milk for consumption by the person or the person's family.

There are no on-farm practices that can guarantee that milk will be free from pathogens but there are practices that will reduce opportunities for milk contamination.

Consumer advice on raw milk is available.

5.1   Current Control Measures

5.1.1   Controls concerning the production and sale of raw milk

The rules for the production and sale of raw milk are set by the *Animal Products Act 1999* and Section 11A of the *Food Act 1981*. MPI has stated how these rules apply to raw milk for direct human consumption in their risk assessment (MPI, 2013a). In short:

- A milk producer may sell raw milk to any person if it is sold at the producer's dairy premises and in a quantity not exceeding 5 litres at any one time, and the person intends the milk for consumption by the person or the person's family.
- All milk producers must operate under a registered Risk Management Programme (RMP). If a dairy farmer produces milk primarily for direct human consumption then the RMP must adequately manage risks, and it is the farmer's responsibility to see that it does. If a dairy farmer primarily supplies milk for another use (e.g. for pasteurisation), then the RMP will not necessarily manage the risks to consumers who buy small volumes of this milk for drinking raw.

5.1.2   MPI Risk Management Strategy

A risk management strategy developed by MPI for *Campylobacter* spp. for the period 2010-2013 has been extended with interim strategy for the period 2013-2014 (MPI, 2013b). Most of the activities focus on the meat and poultry industries. Enhanced surveillance in the Manawatu sentinel site has been the source of information on campylobacteriosis associated with raw milk and the results from this work are mentioned elsewhere in this Risk Profile (Section 3.3.1.3).

5.1.3   Controls in other countries

Sales of raw milk for direct human consumption are prohibited in Scotland and Canada (Gleadle, 2012; Government of Canada, 2013; Scottish Parliament, 2006). Appendix 3 contains information on controls in some European countries and the states of Australia and the USA where the sale of raw milk is permitted. Italy and Australia are the only countries with standards for *Campylobacter* spp. in raw milk. Several countries also require labels instructing consumers to boil the raw milk before consumption.

5.2   Additional Options for Risk Management

The absence of a pathogen elimination step for raw milk means that control measures for reducing the risk of *Campylobacter* spp. contamination must be implemented by the raw milk producer.   MPI has reviewed on-farm control options for managing pathogenic microorganisms and did not identify any animal husbandry practices which guarantee that milk will be free from pathogens (MPI, 2013a).   Measures to improve animal health and milking hygiene can reduce microbiological contamination of raw milk.   Some additional information on on-farm controls is included below.

5.2.1   <u>On-farm control options: *Campylobacter* spp.</u>

Control options to reduce the risk of contamination of raw milk by pathogens and other faecal bacteria have been examined as part of the risk assessment process conducted by MPI (MPI, 2013a).

Mastitis caused by the human pathogens *Campylobacter* spp., STEC and *L. monocytogenes* appears to be uncommon, and these bacteria are not mentioned in a review of mastitis control prepared for Dairy NZ.[11]   Nevertheless, mastitis control will reduce the risk from this occasional source of pathogen contamination, and a number of management tools are available via the Dairy NZ website.

Changes in dairy production practices are occurring in New Zealand, particularly the increasing use of feed pads, stand-off pads, and sheltered housing. These practices increase the potential for faecal contamination of the udder and teats. This makes hygiene controls at milking more important. Such controls can include pre-milking teat dips, cleaning and drying of teats before milking, stripping of foremilk and clipping of udder hair. These measures are time consuming, which would be a barrier for implementation. Effective equipment cleaning is another aspect of milking hygiene which can reduce the risk of contamination of raw milk, through control of the formation of biofilms.

Contaminated supplementary feed may increase the risk of carriage and shedding of pathogens by livestock (Crump et al., 2002). It is important that feed is properly treated to eliminate pathogens.

The potential for microbiological testing to be a component of risk management for raw milk will be limited by the time required to conduct such testing. A rapid test such as that offered by the Bactoscan instrument (less than 10 minutes) could be used for microbiological monitoring of bacterial numbers that would be an indicator of faecal contamination events.[12] This could enable diversion of milk with high bacterial counts (potentially from a faecal contamination event) to pasteurisation. The cost of such an instrument and consumables could be a barrier to its use by individual farms.

A 2008 social study on raw milk products found that the term "raw milk" was not well understood, and for labelling purposes, the term "unpasteurised milk" was favoured over "raw milk" and "non-heat treated milk" (NZFSA, 2009). Consumer education to more clearly define categories of milk may help risk communication.

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[11] http://www.dairynz.co.nz/file/fileid/27234 accessed 20 March 2014.
[12] http://www.foss.dk/industry-solution/products/bactoscan-fc accessed 21 March 2013

5.2.2   Consumer advice

The authors of a review of US consumer safety in relation to raw milk and raw milk cheeses debated some of the options for risk management (Yilmaz *et al.*, 2009). They argued that imposing an outright ban on all sales of raw milk would require too much time and resources to enforce, and may not be completely effective at preventing illegal sales. This is supported by the FoodNet-based study of raw milk consumption in the United States, where the probability of raw milk consumption was not related to the legal status of sales in individual states (Buzby *et al.*, 2013). Yilmaz *et al.* (2009) recommended providing education to dairy producers and consumers, and implementing the use of warning labels on raw milk packaging.

MPI has published advice to consumers on the safety of raw milk.[13]   The advice includes instructing consumers to "keep raw milk under refrigeration (4°C or less) and discard if it has spent more than two hours at room temperature".

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[13] http://www.foodsmart.govt.nz/food-safety/high-risk-foods/raw-milk/ accessed 13 May 2013

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7   APPENDIX 1: HAZARD AND FOOD

7.1   *C. jejuni* and *C. coli*

General information on the growth, survival and inactivation of *C. jejuni* and *C. coli* is presented in the microbiological data sheet available from:
http://www.foodsafety.govt.nz/elibrary/industry/Campylobacter-Organism_Causes.pdf

Characteristics of *C. jejuni* and *C. coli* that are important for this Risk Profile are:

- Does not grow below 32°C, and will not grow under refrigeration, even if other conditions are optimal.

- Requires low oxygen conditions to grow.

- Survives better under refrigeration than at room temperature.

- Inactivated during freezing and slowly inactivated under subsequent frozen storage.

- May enter a "viable non-culturable" state under stressful conditions (so will not be detected through normal culturing methods in the laboratory).

- Can be found in the intestinal tract of a wide variety of wild and domesticated warm-blooded animals which show no sign of disease (Wallace, 2003).

7.1.1   *Campylobacter* spp. typing methods

Serotyping using agglutination reactions according to the Penner system (Penner and Hennessy, 1980), once used as the principal international reference typing scheme, is now rarely applied.

C. jejuni and *C. coli* have two flagellin genes, *fla*A and *fla*B. The ends of these genes are highly conserved, while there is considerable sequence variation in the region in-between. Typing based on this characteristic is known as amplified fragment length polymorphism (AFLP) or restriction fragment length polymorphism (RFLP).

The most commonly applied methods of typing of *Campylobacter* spp. in New Zealand have been PFGE and MLST.

As the enzymes used and the conditions under which the gel electrophoresis is undertaken can have a marked influence on the end result of PFGE typing, standardised protocols are essential. The PulseNet USA network was established in 1996 by the Centers for Disease Control and Prevention and now involves several international networks using PFGE typing.[14] New Zealand is part of the PulseNet Asia Pacific branch through the participation of ESR.[15]

MLST involves amplification and sequencing of seven "housekeeping" genes, i.e. genes which are conserved in all strains of *Campylobacter* spp. but which exhibit sufficient variation to enable differentiation between strains. This technique has been extensively

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[14] http://www.pulsenetinternational.org/ (accessed 29 January 2014).
[15] http://www.pulsenetinternational.org/networks/asiapacific/ (accessed 29 January 2014).

applied in the sentinel site study in the Manawatu. Data from MLST studies can be compared with types found in other laboratories using an International database, hosted by Oxford University.[16]

7.2 *Campylobacter* spp. in Raw Milk and among Dairy Animals Overseas

Recent surveys investigating the prevalence of *Campylobacter* spp. among dairy animals or in raw milk from countries that are less comparable to New Zealand are not included in this Risk Profile except when data are scarce (e.g. sheep and buffaloes' milk). This includes surveys conducted in Asian countries (e.g. China, Japan), African countries and Middle Eastern countries (e.g. Turkey).

7.2.1   Detection of *Campylobacter* spp. in raw milk overseas

7.2.1.1   Cows' milk

Food Standards Australia New Zealand (FSANZ) has summarised 25 studies published between 1983 and 2007 that measured the prevalence and/or concentration of *Campylobacter* spp. in raw cows' milk in various countries (see Table 3.1, page 49 of (FSANZ, 2009a)). The prevalence values ranged 0-18.2% for milk collected from farms, with a median value of 1.5%. Prevalences for samples taken at retail were 1.7, 5.9 and 40%. Note that these studies used different approaches to sampling and testing, so the results may not be strictly comparable. Another review representing data from 21 countries reported a mean prevalence in raw milk of 3.2% with a range of 0 to 9.2% (Humphrey *et al.*, 2007).

Studies published since 2007 are presented in Table 5. Where *Campylobacter* spp. were isolated, the prevalence ranged 0.4-5% of raw milk samples, except for one Italian survey (all were *C. jejuni*).

A survey of milk filters taken from the milking systems of 33 farms in Italy found similar prevalences of *C. jejuni* when the filters were tested by cultural methods (8/378, 2.1%) or PCR (18/378, 4.8%) (Giacometti *et al.*, 2012c). In another Italian survey, *C. jejuni* was detected on milk filters collected from 4/13 (31%) farms (Serraino *et al.*, 2013).

Only one study was located with data on the concentration of *Campylobacter* spp. in raw cows' milk. Of nine samples of milk from two farms containing *C. jejuni*, the mean concentration was 16 MPN/100 ml (Humphrey and Beckett, 1987). A concentration of 100 MPN/100 ml (1 MPN/ml) was measured in one sample.

7.2.1.2   Goats' milk

FSANZ summarised the results of six surveys for *Campylobacter* spp. in raw goats' milk in Australia (see Tables 2 and 3, page 132 of (FSANZ, 2009b)). *Campylobacter* spp. were only detected in one of these surveys, at a prevalence of 5.3% (6/113). Six other surveys of goats' milk have been summarised in Table 6. Only one of these, conducted in Austria, reported *C. jejuni* detection, but the results were not reported separately from sheep milk samples also collected during this survey.

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[16] http://pubmlst.org/campylobacter/ (accessed 29 January 2014).

7.2.1.3   Sheep milk

The two surveys of sheep milk reported in the literature did not detect the pathogen. *Campylobacter* spp. were not detected in 26 samples (some frozen) of raw sheep milk from nine producers, as sampled at the farm or retail outlets in England and Wales during the 1990s (Little and De Louvois, 1999). *Campylobacter* spp. were also not detected in 63 samples of raw sheep milk collected from the bulk milk tanks of farms across Switzerland in 2002 (Muehlherr *et al.*, 2003). *C. jejuni* may have been detected in raw sheep milk from Austria but the results were not reported separately from goats' milk also collected during this survey (see Table 6).

7.2.1.4   Buffalo milk

Campylobacter spp. were not detected on 14 milk filters collected over seven weeks from a buffalo dairy farm in Italy (Serraino *et al.*, 2013).

Table 5:   Prevalence of *Campylobacter* spp. in raw cows' milk overseas (studies published from 2007)

Study location	Study period	Sample source	Number of samples	Prevalence: Number positive (% positive)	Reference
Australia (WA)	2007	NR	183	ND	(FSANZ, 2009a)
Finland	2011	Bulk tank milk (177 farms)	177	ND	(Ruusunen *et al.*, 2013)
Finland	2006/07	Bulk tank milk (3 farms)	15	ND	(Hakkinen and Hänninen, 2009)
Germany	2004	Bulk tank milk (4 farms)	209	1 (0.5) [1]	(Messelhäusser *et al.*, 2008)
Italy	2009-11	131 vending machines	618	9 (1.5) [1]	(Bianchi *et al.*, 2013)
Italy	2010	60 vending machines	99	1 (1.0) [1]	(Giacometti *et al.*, 2012b)
Italy	2010-12	Bulk tank milk (282 farms)	282	34 (12) [1]	(Bianchini *et al.*, 2014)
Norway	2006	Bulk tank milk samples ("from different farms")	262	1 (0.4) [1]	(Løvseth *et al.*, 2007)
Poland	NR	Bulk tank milk	150	7 (5) [1]	(Wysok *et al.*, 2011)

ND, Not detected

[1] All isolates identified as *C. jejuni*.

Table 6:   Prevalence of *Campylobacter* spp. in raw goats' milk overseas

Study location	Study period	Sample source	Number of samples	Prevalence: Number positive (% positive)	Reference
Austria	NR	Bulk tank sheep and goat milk (53 farms) [1]	160	2 (1.3) [1]	(Schoder *et al.*, 2010)
Australia	2001-2006	Frozen bulk milk (3 farms)	63	ND	(Eglezos *et al.*, 2008)
Czech Republic	NR	Bulk tank milk(1 farm)	48	ND	(Cupáková *et al.*, 2012)
UK (England, Wales)	NR	Farm and retail (56 producers). Some samples were frozen.	100	ND	(Little and De Louvois, 1999)
Spain	2003	Bulk tank milk (11 farms)	11	ND	(Cortés *et al.*, 2006)
Switzerland	2004	Bulk tank milk	344	ND	(Muehlherr *et al.*, 2003)

ND, Not detected

[1] All isolates identified as *C. jejuni*. The authors do not report the results for the sheep, goats' or mixed sheep and goats' milk samples separately.

7.2.2 *Campylobacter* spp. among dairy animals overseas

7.2.2.1 *Dairy cows*

FSANZ summarised 16 studies published between 1982 and 2007 that measured the prevalence of *Campylobacter* spp. among cows in various countries using rectal or faecal samples (see Table 3.2, page 50 of (FSANZ, 2009a)). The in-herd prevalence values ranged 0-83%, with a median value of 20.7%. Studies published since 2007 are presented in Table 7. The prevalence of *C. jejuni* among individual adult dairy cows ranged 19-50%, and the prevalence of *C. coli* was lower (range 2-15%).

Some studies have enumerated *Campylobacter* spp. in faecal matter from dairy cows:

- Of rectal grabs from 120 adult cows in 24 dairy herds in Denmark, 11 samples yielded *Campylobacter* spp. and the mean concentration of these samples was 2.1 $\log_{10}$ CFU/g (Nielsen, 2002).

- In a study of 474 cattle in Scotland (mix of dairy and beef), enumeration of *Campylobacter* spp. in faecal samples showed that most animals were shedding low concentrations. Only 4% of cattle faeces contained >100 CFU/g (Rotariu *et al.*, 2009).

- A study of 61 cows on three farms in Lithuania found the average concentration of *Campylobacter* spp. to be 3.7 $\log_{10}$ CFU/g (Ramonaitė *et al.*, 2013). Statistically significant higher concentrations were recorded in faeces from calves and heifers from these farms.

7.2.2.2 *Dairy goats*

Campylobacter spp. were not isolated from faecal samples from 222 healthy dairy goats reared on 12 farms in Spain (Cortés *et al.*, 2006).

7.2.2.3 *Dairy sheep*

Data were only available for *Campylobacter* spp. in non-dairy sheep. In Scotland, the in-herd prevalence was 97/389 (25%), and prevalence as assessed at the farm level was 48/88 (55%) (Rotariu *et al.*, 2009). The same study showed that most animals were shedding low concentrations as only 11% of sheep faeces contained numbers of bacteria that could be counted (>100 CFU/g). The average concentration of *Campylobacter* spp. in these samples was 2×10^5 CFU/g). A study of 120 sheep herds in Spain found 34 (28%) herds were positive for *C. jejuni* and 10 (8%) for *C. coli*, and in four of the positive herds, the prevalence of *Campylobacter* spp. among individual animals (44-50 per herd) ranged 2-18% (Oporto *et al.*, 2007). A study in England estimated a "pat prevalence" for *C. jejuni* of 17% by sampling 960 freshly voided faeces across four sheep farms over two years, and found the prevalence peaked in summer (Grove-White *et al.*, 2010).

7.2.2.4 *Buffaloes*

Data were only available for buffaloes in Laos at slaughter. *C. jejuni* was isolated from 2/184 (1%) of caecal samples (Boonmar *et al.*, 2007).

Table 7: **Prevalence of *Campylobacter* spp. in dairy cows overseas (faecal samples or rectal grabs), studies published from 2007**

Study location	Study period	Sample source	Number of samples	Prevalence: No. positive (% positive)		Reference
				C. jejuni	*C. coli*	
Animal-level studies (in-herd prevalence)						
Chile	NR	Faecal swab samples	80	15 (19)	2 (3)	(Fernández and Hitschfeld, 2009)
Finland	2006/07	Fresh faecal samples from the floor, 3 farms	340	169 (50)	11 (3)	(Hakkinen and Hänninen, 2009)
Italy	2010-12	Rectal grabs, 3 herds	82	25 (30)	ND	(Bianchini *et al.*, 2014)
Lithuania	2012	Rectal samples, 3 farms	59 calves 80 heifers 61 cows	27 (46) 51 (64) 26 (43)	10 (17) 19(24) 9(15)	(Ramonaitė *et al.*, 2013)
Spain	NR	Faecal grabs, 2 herds	96	43 (45)	ND	(Oporto *et al.*, 2007)
UK	2006-08	Fresh faecal samples, 14 farms	3,300	19%[1]	NR	(Grove-White *et al.*, 2010)
USA	2009	Fresh faecal samples, 11 farms	227	79 (35)	5 (2)	(Sanad *et al.*, 2013)
Farm-level studies						
Spain	NR	Faecal grabs, 30 animals per herd, pooled	82 herds	12 (15)	3 (4)	(Oporto *et al.*, 2007)

NR, not reported; ND, not detected.

[1] Estimated "pat-prevalence" based on Huber–White robust standard error estimates. Actual prevalence not reported.

7.2.3   Recalls

Recalls are not necessarily linked to human illness, but recall information provides an indication of how often *Campylobacter* spp. are detected in raw drinking milk sold for direct human consumption. Recall information is only relevant for countries where the sale of raw milk for direct human consumption is legal.

7.2.3.1   European Union

The Rapid Alert System for Food and Feed portal was used to retrieve recall records regarding pathogenic microorganisms in milk and milk products.[17] There are 32 countries participating in this system (including all EU member states and Lichtenstein, Iceland, Norway and Switzerland). The search retrieved 260 records dating from 1985 to September 2013. There were no recalls issued for raw milk on the basis of contamination with *Campylobacter* spp.

7.2.3.2   Australia

Raw cows' milk is not permitted for sale in Australia, but raw goats' milk is allowed to be sold in some Australian states. All food recalls recorded by FSANZ from 2000 to May 2013 were scanned for relevant records.[18] No recalls for raw goats' milk were issued during this period.

7.2.3.3   United States

The regulations for the sale of raw milk vary between States and recalls are issued by appropriate State Departments. There is no centralised database available for retrieving data.

7.2.4   Consumption of raw milk

7.2.4.1   North America

The US Foodborne Diseases Active Surveillance Network (FoodNet) monitors foodborne illness in 10 state health departments, covering 15% of USA's population. FoodNet's activities include surveys of the people living in these areas. In a 2006/07 survey, a total of 17,372 people were asked whether they had consumed any unpasteurised milk in the past seven days, and 528 (3%) had (CDC, 2007). Estimates for the proportion of farming families and farm workers who consume raw milk range from 35 to 60% (Oliver *et al.*, 2009).

A more recent analysis combined results from the 2006/07 FoodNet survey (above) and from two other FoodNet surveys carried out in 1998/99 and 2002/03 (Buzby *et al.*, 2013). Across all years of the survey, 3.4% (1,004/29,753) of respondents reported consuming unpasteurised milk at some point in the previous seven days. Of those who reported consuming raw milk, only 6.5% lived on a farm and only 14.8% lived in a rural area. Just

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[17] https://webgate.ec.europa.eu/rasff-window/portal/. Search function parameters entered: Product type: Food; Notification type: Alert; Product category: Milk and milk products; Hazard category: Pathogenic micro-organisms. (Accessed 10 July 2013)

[18] The FSANZ website (http://www.foodstandards.gov.au/) only contains recent recalls. The full dataset was kindly provided by FSANZ.

under half of raw milk consumers (44.9%) lived in a State where all sales of unpasteurised milk were prohibited (some States permitted cow shares).

In Canada, a sample of 2,332 residents of the Waterloo Region (Ontario) participated in a telephone survey of food consumption and food safety during 2005/06 (Nesbitt *et al.*, 2009). Seventeen (0.7%) respondents reported consuming raw milk in the seven days prior to being questioned. Drinking raw milk was significantly more prevalent among rural residents (9.0%) than among urban residents (0.4%, P<0.001).

7.2.4.2 Italy

A quantitative risk assessment focussed on one province of the Emilia Romagna Region in Italy estimated that 1-2% of the population were consumers of raw milk from vending machines (10,577-21,154 people of a population of 995,000) (Giacometti *et al.*, 2012a). From a consumer survey, Giacometti *et al.* (2012a) found that 57% of consumers boiled the raw milk before consumption, so the estimated proportion of the population consuming unboiled raw milk is 0.5-0.9% (4,548-9,096 people).

8 APPENDIX 2: EVALUATION OF ADVERSE HEALTH EFFECTS

8.1 GBS and Reactive Arthritis

GBS is a potentially fatal autoimmune neurological complication of *Campylobacter* spp. infection. Approximately 15-20% of patients with GBS are left with some form of disability (Mangen *et al.*, 2004) and approximately 3-5% die (Kemmeren *et al.*, 2006). From New Zealand data the mean annual incidence of hospitalisations resulting from GBS was 2.3/100,000 for the period 1988-2010, and the case fatality rate was 3% for the period 1989-2008 (Baker *et al.*, 2012). Some studies have concluded that certain serotypes of *C. jejuni*, particularly Penner Serotypes O19 and O41, are more frequently associated with GBS than other serotypes (Allos *et al.*, 1998; Wallace, 2003). Other studies have found no association between specific serotypes and GBS (Endtz *et al.*, 2000; Rees *et al.*, 1995).

An analysis of New Zealand GBS hospitalisations and campylobacteriosis data calculated an age-standardised rate of GBS in the month after hospitalisation for campylobacterosis as 810/100,000 person-years (Baker *et al.*, 2012). The study also suggested that *Campylobacter* spp. infection may be responsible for approximately 25% of GBS cases.

These figures are similar to those reported in international literature. The frequency of GBS resulting from campylobacteriosis has been reported to be 1 in 1,000 or less, e.g. a rate of <2/10,000 for the UK (Nyati and Nyati, 2013; Tam *et al.*, 2006; van Doorn *et al.*, 2008). A systematic review of 30 case-control studies investigating the relationship between *Campylobacter* spp. infection and GBS concluded that 31% of GBS cases may be attributable to previous *Campylobacter* spp. infection (Poropatich *et al.*, 2010). This finding was further supported by a more recent case-control study in India that found evidence of recent *C. jejuni* infection in 30% (15/50) of GBS patients compared to 8% (3/40) of controls ($p<0.005$) (Sharma *et al.*, 2011).

The frequency of reactive arthritis has been estimated as 0.9-1% of all campylobacteriosis cases (Ajene *et al.*, 2013; Altekruse *et al.*, 1999) or 3-16% of more serious (GP attending) campylobacteriosis cases (Hannu *et al.*, 2002; Johnsen *et al.*, 1983; Locht and Krogfelt, 2002; Rees *et al.*, 2004).

8.2 Dose Response

To describe the dose-response relationship for *Campylobacter* spp. modelling has been split into two parts: Estimating the probability of infection caused by ingestion of variable numbers of cells, and estimating the probability of disease, given infection.

Three dose response challenges have been conducted with volunteers, and data for both infection and sickness are shown in Table 8. In these studies:

- A volunteer became infected at day 2, and ill at day 4, after ingesting 500 *C. jejuni* cells in 180 ml pasteurised milk (Robinson, 1981). The isolate was from an outbreak caused by raw milk consumption.

- Experiments were performed with 111 adult volunteers who were administered varying numbers of two *C. jejuni* isolates in nonfat milk (Black *et al.*, 1988). It was noted that the propensity to develop symptoms did increase with dose, but that that dose response

relationship was not clear. The lowest dose administered, 8×10^2 CFU (one isolate only) caused 50% of the 10 participants to shed *C. jejuni* in their faeces but only 10% developed symptoms. These data illustrate that people can become infected by the organism but not become ill from it. The data also showed a difference in pathogenicity between the two isolates.

- A trial of 23 subjects (all of whom had not had recent *Campylobacter* spp. infection and were immunologically naïve) receiving 1×10^6 or 1×10^5 CFU of *C. jejuni* found that 100% and 93% of subjects became ill (i.e. attack rate), respectively (Tribble *et al.*, 2009). All shed the organism in their faeces. Repeat doses administered to some of the participants provided some evidence for acquired immunity (see Section 8.2.1).

Table 8: **Dose response data for human volunteers consuming different concentrations of *C. jejuni***

Dose (CFU)	Medium[1]	No. (%) infected[2]		No. (%) ill[3]		Reference
5×10^2	PM	NR		1	(100)	(Robinson, 1981)
8×10^2 strain A3249	NFM	5/10	(50)	1/10	(10)	(Black *et al.*, 1988)
8×10^3 strain A3249	NFM	6/10	(60)	1/10	(10)	(Black *et al.*, 1988)
5×10^4 strain CG8421	BB	8/8	(100)	8/8	(100)	(Tribble *et al.*, 2009)
8×10^4 strain CG8421	BB	7/7	(100)	6/7	(86)	(Tribble *et al.*, 2009)
9×10^4 strain A3249	NFM	11/13	(85)	6/13	(46)	(Black *et al.*, 1988)
1×10^5 strain 81-176	BB	5/5	(100)	3/5	(60)	(Tribble *et al.*, 2010)
8×10^5 strain A3249	NFM	8/11	(73)	1/11	(9)	(Black *et al.*, 1988)
1×10^6 strain CG8421	BB	8/8	(100)	8/8	(100)	(Tribble *et al.*, 2009)
1×10^6 strain A3249	NFM	15/19	(79)	2/19	(11)	(Black *et al.*, 1988)
1×10^6 strain 81-176	NFM	7/7	(100)	3/7	(43)	(Black *et al.*, 1988)
1×10^7 strain 81-176	BB	5/5	(100)	2/5	(40)	(Tribble *et al.*, 2010)
1×10^8 strain A3249	NFM	5/5	(100)	0/5	(0)	(Black *et al.*, 1988)
1×10^8 strain A3249	BB	4/4	(100)	2/4	(50)	(Black *et al.*, 1988)
2×10^8 strain 81-176	NFM	10/10	(100)	6/10	(60)	(Black *et al.*, 1988)
1×10^9 strain 81-176	BB	36/36	(100)	33/36	(92)	(Tribble *et al.*, 2010)
2×10^9 strain 81-176	NFM	22/22	(100)	9/22	(41)	(Black *et al.*, 1988)

[1] NR = not recorded, PM = pasteurised milk, NFM = non-fat milk, BB = bicarbonate buffer.
[2] Two stools positive for *C. jejuni* at ≥24 h post-inoculation.
[3] Confirmed as infected and developed diarrhoea.

The data from Black *et al.* (1988) have been investigated for the purpose of modelling the dose-response relationship (Medema *et al.*, 1996; Teunis *et al.*, 1999; Teunis and Havelaar, 2000), with an overview reported by an expert group assembled by the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) (FAO/WHO, 2002). Infection, where the microorganism is reproducing in the body, was modelled separately from illness, which is less frequent. The FAO/WHO hazard characterisation (FAO/WHO, 2002) explored the idea that there is a conditional probability of disease in humans resulting from infection. This model predicts that in the vast majority of cases where people become

infected there is >20% and <50% chance of the person subsequently becoming sick, with the maximum probability at 33%.

To illustrate the probability of human disease given a variety of doses, Figure 3 shows results from application of the FAO/WHO model using a fixed 33% probability of becoming ill after infection has occurred.

Figure 3:   FAO/WHO dose response model; probability of illness fixed at 33%
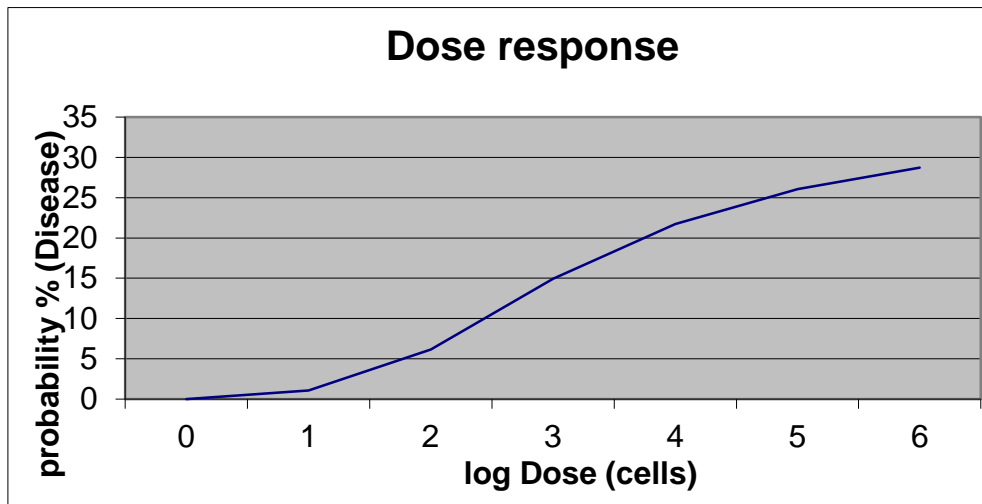
8.2.1   Acquired Immunity

There are several pieces of evidence suggesting that regular exposure to *Campylobacter* spp. provides a measure of immunity to repeated illness. A study with a specific *Campylobacter* subtype found that subjects given a second high dose exposure 28-49 days after a first dose (which caused illness) became infected but did not develop symptoms of illness (Tribble *et al.*, 2010). Attenuated illness occurred in subjects challenged again one year later indicating that immunity was not permanent and decreased over time. The attack rates were 92% in immunologically naïve subjects, 0% in those given a repeat dose after a short time, and 57% of those given a second dose a year later. However, another study using a different subtype of *C. jejuni* did not find the same effect (Kirkpatrick *et al.*, 2013). Of 15 subjects receiving an initial challenge, 14 experienced campylobacteriosis. Of the eight subjects who were given a second challenge 3 months later, all experienced campylobacteriosis with similar severity.

Acquired immunity may explain the very high rates of diarrhoeal illness found in young children in developing countries where the illness is rare in adults (Havelaar *et al.*, 2009). Frequent and multiple exposures at a very young age to a wide variety of *Campylobacter* subtypes is thought to generate host immunity, although the illness also causes significant mortality and morbidity in those children (Kotloff *et al.*, 2013).

In developed countries widespread immunity is considered to be absent, but may occur in people with regular exposure (Havelaar *et al.*, 2009). There are two examples in the literature reporting a proportion of people involved in an outbreak where raw milk was the vehicle who did not become ill, apparently because they had been regularly exposed to *Campylobacter*.

- In an outbreak of campylobacteriosis at a US farm retreat where raw milk was identified as the vehicle, 19 of the 29 college students who consumed raw milk during the visit developed acute *C. jejuni* enteritis (Blaser *et al.*, 1987). Ten other people (four students and six farm workers) who consumed the raw milk but did not become ill were identified as regular raw milk consumers. The habitual consumers of raw milk had elevated levels of anti-*C. jejuni* antibodies (compared to the non-habitual raw milk consuming students who did consume the milk but did not become ill). These data, particularly for the farm workers, suggest preceding chronic exposure to *Campylobacter* in raw milk.

- In the UK, 77/300 agricultural college students developed campylobacteriosis after consuming raw milk (Jones *et al.*, 1981). The cases occurred over a period of about three weeks, and it was inferred that the raw milk was contaminated intermittently. Overall, students regularly drinking greater quantities of the raw milk were more likely to become ill compared to low consumers. However, a proportion of those consuming larger quantities did not become ill. Bactericidal antibodies to *Campylobacter* were found in a high proportion (63%) of these asymptomatic high consumers, as well as the symptomatic cases.

8.3 *Campylobacter* spp. Infection Overseas

8.3.1 Incidence

Table 9 shows the reported incidence of campylobacteriosis for several countries for the year 2011 (the most recent year for which data were available for all countries). New Zealand's 2011 campylobacteriosis rate of 151.9 per 100,000 is at the high end of the range of rates listed in Table 9 (range 0.9-178), closest to rates reported in the Czech Republic, Australia, the United Kingdom and Switzerland. Comparisons of campylobacteriosis rates between countries must be made cautiously, as reporting practices may differ (e.g. in Canada, *Campylobacter* infections are not routinely reported to the provincial or central reference laboratories so are underrepresented in national figures (NESP, 2013), and the New South Wales state of Australia only reports outbreaks of campylobacteriosis (Australian Government, 2013)).

The European Food Safety Authority lists the reported cases of human campylobacteriosis for 26 EU Member States and 3 Non-Member States for the year 2011 (European Food Safety Authority and European Centre for Disease Prevention and Control, 2013). The incidence varied from 0.3 (Latvia) to 178 (Czech Republic) cases per 100,000. The overall incidence in the EU was 50 cases per 100,000.

Data on the incidence of campylobacteriosis for the period 1997-2012 shows that the rates in Australia, and the USA and have remained stable over the most recent decade, in contrast to the rate in New Zealand (Figure 4). However, the EU has reported a significant increase in the number of cases from 2008-2011 (European Food Safety Authority and European Centre for Disease Prevention and Control, 2013). Rate changes are less obvious for very large populations, such as those of the USA and EU. Rates are also influenced by population estimates for the year in which they are calculated.

In 2011, 43 deaths resulting from campylobacteriosis were reported in the EU, 34 of these occurred in the UK. The case fatality rate was 0.04% of the cases where this information was provided.

Table 9: **Reported incidence data for notified cases of campylobacteriosis overseas**

Country	Year	Incidence (cases/100,000)	No. of notified cases	Reference[1]
Australia (excluding New South Wales)	2010	114.1	16,986	a
	2011	117.2	17,725	a
	2012	101.6	15,653	a
North America				
USA[2]	2011	14.3	6,785[2]	b
Canada	2011	5.6	1,938	c
EU countries[3]				
EU notifications	2011	50.3	220,209	d
Austria	2011	16.0	1,345	d
Belgium	2011	70.5	7,716	d
Czech Republic	2011	178.0	18,743	d
Denmark	2011	73.0	4,060	d
Finland	2011	79.3	4,262	d
France	2011	8.5	5,538	d
Germany	2011	86.6	70,812	d
Ireland	2011	54.3	2,433	d
Netherlands	2011	50.9	4,408	d
Poland	2011	0.9	354	d
Spain	2011	47.4	5,469	d
Sweden	2011	87.2	8,214	d
United Kingdom	2011	115.4	70,298	d
Non-EU countries[3]				
Iceland	2011	38.6	123	d
Norway	2011	61.1	3,005	d
Switzerland	2011	100.8	7,964	d

[1] References:
   a. (Australian Government, 2013)
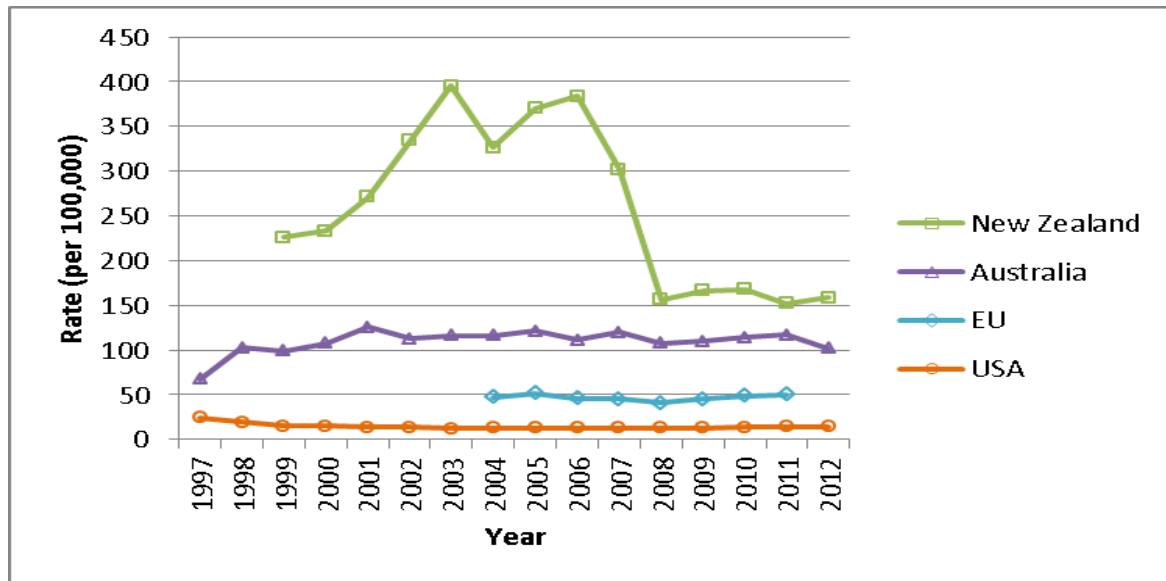   b. (CDC, 2012)
   c. (NESP, 2013)
   d. (European Food Safety Authority and European Centre for Disease Prevention and Control, 2013)

[2] Data is for the 10 sentinel states monitored by FoodNet, not the whole of the USA.

[3] Data are for confirmed cases only. An additional 4,535 cases were notified but unconfirmed.

Figure 4: Reported incidence of campylobacteriosis in New Zealand, Australia, the EU and the USA, 1997-2012

<u>Note to Figure 4</u>: Data were only available for the EU for the period 2004-2012
References:
New Zealand: See note to Figure 2.
Australia: (Australian Government, 2013)
USA: (CDC, 2013)
EU: (European Food Safety Authority, 2005; European Food Safety Authority and European Centre for Disease Prevention and Control, 2006; 2007; 2009; 2010; 2011; 2012; 2013)

8.3.1.1   Community level estimates

The number of notified campylobacteriosis cases only represents a proportion of total cases, since not all cases will come into contact with public health agencies. Estimates for the annual number of community campylobacteriosis cases and annual rates of infection have been published:

- USA: 1,058,387 (90% Credible Interval (CrI):   423,255-2,019,498) cases of domestically-acquired campylobacteriosis cases, of which 80% were estimated as being foodborne (845,024 cases, 90% CrI: 337,031-1,611,083) (Scallan *et al.*, 2011). This was based on surveillance data from 2000 to 2008. Using the 2006 USA population of 299 million, the foodborne cases correspond to a rate of 283 per 100,000.

- Canada: 447 cases of domestically-acquired foodborne campylobacteriosis cases per 100,000 people per year (Thomas *et al.*, 2013). This estimate was based on surveillance data from 2000 to 2010 plus relevant international literature, and was produced through a modelling approach that accounted for underreporting and underdiagnosis.

8.3.2   <u>Outbreaks associated with raw milk consumption.</u>

Table 10 summarises outbreaks of campylobacteriosis linked to consumption of raw cows' milk occurring from 2000 onwards where details are published in the scientific literature. See FSANZ (2009a) for outbreaks occurring before 2000. In many outbreaks, isolates from the implicated milk were not available but the association between milk consumption and disease was made using epidemiological data.

FSANZ (2009b) lists five campylobacteriosis outbreaks occurring before 2000 linked to consumption of raw goats' milk. No more recent reports were identified in the scientific literature, and neither were reports of campylobacteriosis linked to raw sheep or buffaloes' milk consumption.

It is important to note that peer-reviewed outbreak reports in the scientific literature represent a proportion of the reported campylobacteriosis outbreaks linked to raw milk. Numerous press releases from government authorities and press reports are also available on the internet and these show that campylobacteriosis outbreaks linked to raw milk consumption continually occur.[19] The US Centers for Disease Control (CDC) operate a searchable database of foodborne outbreaks and 75 confirmed outbreaks of foodborne campylobacteriosis attributed to the consumption of raw milk were reported from 1998 to 2011.[20]

Five recent reviews of surveillance data also provide evidence for raw milk as a potential vehicle for *Campylobacter* spp:

- USA, outbreaks linked to dairy products, 1993-2006: Of 121 outbreaks where the pasteurisation status of the dairy product was known, 46 (38%) were caused by raw milk (Langer *et al.*, 2012). The authors did not specify the number of raw milk outbreaks caused by *Campylobacter* spp. infection, but this pathogen was the cause of 40/73 of the outbreaks associated with all unpasteurised dairy products (milk plus cheese).[21]

- USA, campylobacteriosis outbreaks, 1997-2008: Of 158 outbreaks for which a food vehicle was implicated or confirmed, raw milk was the vehicle in 51 (32%) outbreaks (Taylor *et al.*, 2013).

- USA, review of sporadic enteric cases, 2001-2010: Identified three campylobacteriosis outbreaks linked to consumption of raw milk (raw milk consumption at a farm where a ministry group was staying in 2001, and two outbreaks in 2008, one associated with raw milk consumption at a family reunion and the other raw milk purchased from a local dairy farm) (Robinson *et al*., 2014).

- Australia, foodborne campylobacteriosis outbreaks, 2001-06: Of 16 outbreaks for which a food vehicle was identified, raw milk was the vehicle in two (Unicomb *et al*., 2009).

- Germany, campylobacteriosis cases, 2005-2011: Of 16 outbreaks involving five or more cases, four were associated with consumption of raw milk (Hauri *et al.*, 2013). Two of these raw milk outbreaks were linked to the same farm and involved 82 campylobacteriosis cases.

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[19] See, for example, http://outbreakdatabase.com/ and use the search parameters: Keywords = raw, unpasteurized Vehicle = milk; Organism = Campylobacter (accessed 21 January 2014).
[20] http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx accessed 7 October 2013
[21] One of these outbreaks was a mixed infection of *Campylobacter* spp. with shiga toxin-producing *E. coli*.

Table 10: **Overseas outbreaks of campylobacteriosis where raw cows' milk was an implicated vehicle (2000 onwards, reported in the scientific literature)**

Country	Year	Total cases[1]	Hospitalis-ations	Ages of non-hospitalised cases	Exposure to raw milk	Evidence linking milk to cases	Reference
USA (Alaska)	2013	18	NR	21 months to 81 years	Cow share programme at a farm producing 90% of its milk for pasteurisation (selling raw milk is illegal in Alaska).	No *Campylobacter* spp. found in milk samples, but raw milk was the only common risk factor amongst cases. Indistinguishable PFGE pattern found in isolates from cases, field manure, and calf barn.	(Castrodale *et al.*, 2013)
USA (Pennsylvania)	2013	8[2]	NR	NR	Dairy certified to sell raw milk on site, at retail and off-farm pickups.[3]	Matching PFGE pattern in patient stool, bulk tank and retail milk.	(Weltman *et al.*, 2013)
USA (multiple states)	2012	148	10	2-74 years	Raw milk with 15 day shelf life was sold on site, at 12 retail markets, 39 drop points, and home delivery by a large dairy farm certified to sell raw milk.[3]	Two unopened consumer cartons provided a *Campylobacter* spp. isolate with identical PFGE pattern to clinical isolates.	(Longenberger *et al.*, 2013)
USA (Michigan)	2010	12	NR	NR	NR	Cases drank raw milk	(FDA, 2010)
The Netherlands	2007	16	NR	NR	Lunch at dairy farm where raw milk was served.	Of the 19 persons who had consumed raw milk,16 (84%) had become ill. Of the persons who did not drink the raw milk, none became ill. A significant association was found between tasting the raw milk and being ill (risk difference=0.84, p=0.0011). Isolates from patients generated identical flaA PCR-RFLP pattern to four isolates from farm bulk milk tank.	(Heuvelink *et al.*, 2009)

Country	Year	Total cases[1]	Hospitalis-ations	Ages of non-hospitalised cases	Exposure to raw milk	Evidence linking milk to cases	Reference
Italy	2006	NR	5	NR	Raw milk distributed outside a school.	PFGE pattern of isolates from raw milk and cow faecal sample matched that of cases.	(Amato *et al.*, 2007)
The Netherlands	2005	19	NR	School children	School trip to a dairy farm.	Milk and filter samples taken 2 weeks after outbreak were negative for *Campylobacter*, but a cow rectal swab isolate matched isolates from cases (*flaA* typing).	(Heuvelink *et al.*, 2009)
USA (Utah)	2004	13	0	11 to 50 years	High school athletic team dinner of 20 people at which 15 people drank raw milk.	13/15 who drank raw milk became sick compared to 0/5 who did not.	(Peterson, 2003)
Australia	2003	13	0	NR	School students visiting farm, drank raw milk	Cohort study	(The OzFoodNet Working Group, 2004)
Finland	2002	6[4]	1	7, 11 and 13 year old children, plus parents and grandparents	Farming family consuming home milk supply.	All *C. jejuni* isolates tested (two human, two milk and 10 bovine faecal) had identical PFGE pattern.	(Schildt *et al.*, 2006)
The Netherlands	2002	30	NR	Schoolchildren	Raw milk drunk by schoolchildren on farm visit.	28/58 who drank raw milk became sick compared to 2/35 who did not.	(Van den Brandhof *et al.*, 2003)
USA (Wisconsin)	2001	70[5]	NR	2-63	Cow leasing programme, milk picked up at gate or delivered. Organic dairy farm certified Grade A for providing milk for pasteurisation.	PFGE pattern from bulk farm milk tank matched outbreak strain.	(Harrington *et al.*, 2002)

Country	Year	Total cases[1]	Hospitalis-ations	Ages of non-hospitalised cases	Exposure to raw milk	Evidence linking milk to cases	Reference
Germany	2000	31	NA	NA	Consuming raw milk during farm visit.	Identical strains between patients and raw milk.	(Thurm *et al.*, 2000)
Germany	2000	18	NR	Kindergarten age	Kindergarten children drinking certified raw milk from local dairy.	Isolates from faecal samples from children and raw milk matched by RFLP-PCR.	(Atanassova and Ring, 2001)
Germany	2005	6	NR	NR	Farm visit	Common food	(Hauri *et al.*, 2013)
Germany	2010	6	NR	NR	Children's service on farm	Only food consumed by all cases	(Hauri *et al.*, 2013)
Germany	2010	5[6]/14 exposed	NR	NR	Farm visit by several families (May 2010)	Only food consumed by all cases	(Hauri *et al.*, 2013)
Germany	2010	77/117 exposed	NR	NR	School farm visit (same farm as above, June 2010)	Only food consumed by all cases	(Hauri *et al.*, 2013)

NR, not reported. NA, not available (article not in English).

[1] Confirmed and suspected.

[2] 4 cases were ≤18 years old.

[3] The same dairy farm was the source of milk in both of these outbreaks.

[4] Repeated episodes amongst these cases.

[5] 5 secondary cases, of which 4 were mothers of sick children.

[6] Table reports 8 cases, text reports 5 cases.

8.3.3 Case control studies and reviews investigating raw milk as a risk factor

Table 11 summarises 12 case control studies which included raw milk consumption as one of the risk factors considered. Some case control studies that apparently included asking participants about raw milk consumption are excluded from the table as the reports do not provide any detail on this aspect, e.g. Tenkate and Stafford (2001). The ability of case-control studies to determine the risk of raw milk is sometimes compromised by the low numbers of cases and controls amongst the study population consuming the product, thus generating broad confidence intervals. A study in Norway in 1990 had only 2/52 cases and 0/103 controls who reported consuming raw milk so the odds ratio was not able to be calculated (Kapperud *et al.*, 1992).

The case control studies have produced mixed results. Four studies and an additional reference did not find consumption of raw milk to be a significant risk factor for campylobacteriosis (Adak *et al.*, 1995; Davis *et al.*, 2013; Effler *et al.*, 2001; Potter *et al.*, 2003; Schorr *et al.*, 1994).[22]

Six studies found consumption of raw milk to be a significant risk factor for campylobacteriosis by univariate analysis but in three of these studies, consumption of raw milk was not independently associated with campylobacteriosis when analysed by multivariate analysis (Carrique-Mas *et al.*, 2005; Kapperud *et al.*, 2003; Neal and Slack, 1997). The study in Denmark found raw milk to be a significant risk factor by multivariate analysis, but it was no longer significant after protective factors (factors with odds ratio<1 by univariate analysis) were removed from the multivariate model (Neimann *et al.*, 2003). In the two remaining studies raw milk was a significant risk factor in both univariate and multivariate analyses (Friedman *et al.*, 2004; Michaud *et al.*, 2004).

The large case control study in England during 2005/06 (1,592 cases and 3,983 controls) found that the risk of campylobacteriosis was statistically reduced for those who regularly consumed raw milk (Tam et al., 2009). The authors commented "We could not confirm participants' immunologic status; however, these results suggest that long-term exposure to these sources of *Campylobacter* spp. might confer partial immunity. In immunologically susceptible populations, however, unpasteurized milk is a well-known cause of outbreaks of infection with *Campylobacter* and potentially fatal Shiga toxin-producing *Escherichia coli*."

The potential for long term exposure to *Campylobacter* spp. to produce some degree of immunity is supported by a case-control study in Wisconsin, USA, which found the prevalence of antibodies to *C. jejuni* to be higher among farm-resident children than in non-farm resident children (Belongia *et al.*, 2003). In this study, drinking raw milk was one of several significant factors associated with the presence of *C. jejuni* antibodies when analysed by univariate analysis, but after multivariate analysis *C. jejuni* seropositivity was only associated with increasing age and living on a farm.

Case-control methodology was applied in a UK study of 3,849 reported campylobacteriosis cases (2000/01) to identify risk factors associated with potentially unreported outbreaks (Gillespie *et al.*, 2003). According to this study drinking raw milk was associated with illness in the community (Odds Ratio 2.15 95% CI: 1.33-3.49).

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[22] The numerical results from the univariate analysis were not reported by Effler *et al*., 2001 so this study was not included in Table 13.

A review of domestically-acquired, laboratory confirmed, sporadic enteric infections in Minnesota for the period 2001-10 found that of 14,339 cases analysed, 530 (4%) reported consumption of raw milk (information on raw milk consumption is routinely collected in Minnesota) (Robinson *et al.*, 2014). Of these 530 cases, 407 were (77%) were campylobacteriosis cases. Only half of the 530 cases reported contact with cattle. Persons with *Campylobacter* infection had the highest percentage of reported raw milk consumption (6.0%). The findings suggest that raw milk might be an important cause of campylobacteriosis in Minnesota.

Table 11: **Campylobacteriosis case control studies since 1990 that included raw milk as a risk factor**

Time period	Country	Risk Factor	Prevalence of risk factor		Odds ratio (95% confidence interval) by		Reference
			Cases (%)	Controls (%)	Univariate analysis	Multivariate analysis	
1990/91	England, Wales	Consumption of untreated milk	11/598 (1.8)	5/738 (0.7)	3.2 (0.9-12.2)	5.0 (0.8-32.6)	(Adak *et al.*, 1995)
1991	Switzerland	Consumption of raw milk	30/167 (18.0)	51/282 (18.1)	1.0 (0.6-1.7)	1.3 (0.7-2.4)	(Schorr *et al.*, 1994)
1994/95	Nottingham, England	Drinking unpasteurised milk	13/313 (4.2)	6/512 (1.2)	3.5 (1.3-9.4)	2.4 (0.8-7.6)	(Neal and Slack, 1997)
1995	Sweden	Drinking unpasteurised milk	17/101 (16.8)	11/198 (5.6)	3.6 (1.5-8.9)	NR	(Studahl and Andersson, 2000)
1996/97	Denmark	Drinking unpasteurised milk	20/217 (9.2)	13/236 (5.5)	1.9 (0.9-4.0)	11.8 (2.0-70.3) Excluding protective factors: 2.3 (0.9-5.9)	(Neimann *et al.*, 2003)
1998/99	USA	Drank raw or unpasteurised milk	?/1316 (2)	7/1316 (0.6)	3.8 (1.4-10.2)	4.3 (1.3-14.2)	(Friedman *et al.*, 2004)
1999/2000	Norway	Drinking unpasteurized milk	29/206 (14.1)	28/420 (6.7)	2.2 (1.3-3.9)	NS	(Kapperud *et al.*, 2003)
2000/01	Canada (Quebec)	Consuming raw milk or raw milk products	3/153 (2.0)	25/310 (8.1)	3.1 (1.8-5.5)	3.7 (2.0-6.9)	(Michaud *et al.*, 2004)
2000/01	USA (Michigan)	Consumption of raw milk in the 2 weeks before contact or illness	?/83 (10)	?/122 (11)	1.2 (0.4-3.2)	NR	(Potter *et al.*, 2003)
2005/06	England	Regularly drinks raw milk Occasionally drinks raw milk	NR	NR	NR	0.2 (0.1-0.7) 0.7 (0.3-1.5)	(Tam *et al.*, 2009)

Time period	Country	Risk Factor	Prevalence of risk factor		Odds ratio (95% confidence interval) by		Reference
			Cases (%)	Controls (%)	Univariate analysis	Multivariate analysis	
2001/02	Sweden (children)	Consumption of unpasteurised milk	14/112 (12.5)	7/267 (2.6)	5.1 (1.8-19.9)	6.9 (0.4-118.1) Excluding protective factors: 3.7 (0.9-16.1)	(Carrique-Mas *et al.*, 2005)
2009/10	USA (Washington)	Ate or drank unpasteurised dairy product	16/69 (9.5)	13/211 (6.2)	Chi-square P value = 0.23 (NS)	NI	(Davis *et al.*, 2013)

NR = not reported, NS = not significant and values not reported, NI = not included in multivariate analysis, ? = numerator not reported and cannot be calculated from percentage.

8.4   Risk Assessment and Other Activities Overseas

8.4.1   Australia

FSANZ published two microbiological risk assessments in 2009, one addressing raw cows' milk and one raw goats' milk (FSANZ, 2009a; b). Both considered the risk of illness from raw milk contaminated with *Campylobacter* spp. (as well as other pathogens). Both found that there was a risk of *Campylobacter* spp. infection if raw milk was consumed.

The raw cows' milk risk assessment included quantitative microbiological modelling to predict the number of illnesses per 100,000 daily servings of raw milk for children and adults. The mean predicted cases of illness from *Campylobacter* spp. infection per 100,000 daily serves of raw milk were:

- 19 children and 20 adults when milk is consumed from farm bulk milk tanks;

- 5 children and 5 adults when milk is consumed after farm gate sales; and

- <1 children and <1 adults when milk is consumed after retail purchase.

The maximum time period for the total supply chain was not fixed (unlike the New Zealand model).

The decrease in predicted cases as the supply chain lengthens is a result of the inactivation of *Campylobacter* spp. in chilled raw milk. Some assumptions had to be made where data gaps existed. Some important data gaps were the prevalence and concentration of pathogens in Australian dairy cows and raw milk produced in Australia, and raw milk consumption and the demographics of the consuming population in Australia. Sensitivity analysis of the model also revealed that the degree of teat soiling and the within herd prevalence of *Campylobacter* spp. were major factors influencing *Campylobacter* spp. concentration in the milk.

The raw goats' milk risk assessment, using qualitative risk rating, rated the risk to public health and safety from *Campylobacter* spp. in raw goats' milk as 'low'. The risk assessment noted that susceptible populations were likely to consume goats' milk, but the demographics of the consuming population were unknown as were the frequency and amount of consumption. Data on the prevalence and concentration of pathogens in the domestic raw goat milk supply were also scarce.

8.4.2   Italy

A quantitative risk assessment was performed to describe the risk of campylobacteriosis linked to consumption of raw milk sold in vending machines in Northern Italy (Giacometti *et al.*, 2012a). The assessment focussed on *C. jejuni* and encompassed the whole food chain from the farm to the consumer. The model also considered two storage scenarios where the milk was kept at optimal temperature (4°C) throughout the food chain or kept at variable (worst-case) temperatures as identified through another study (see (Giacometti *et al.*, 2012d)). The model predicted the median number of *Campylobacter* infections/campylobacteriosis cases per 10,000-20,000 consumers, per year, linked to consumption of raw milk as:

- 6.64/2.12 infections/cases under the best storage scenario; and

- 3.48/1.14 infections/cases under the variable storage scenario.

The lower numbers of infections/cases predicted under worst storage scenario resulted from previous experimental observations that the decimal reduction time for *Campylobacter* spp. under variable temperatures was shorter (133h) than when refrigeration temperatures were maintained (624h).

It is important to note that the model assumed that 57% of consumers boiled the raw milk before consumption.

8.4.3   United Kingdom

The Advisory Committee on the Microbiological Safety of Foods (ACMSF), who provide scientific advice to the UK Food Standards Agency (UKFSA), has considered the risks associated with raw drinking milk on several occasions in the past, and most recently in 2011. On all occasions the ACMSF concluded that there were significant risks to human health from consumption of raw drinking milk and stressed the importance of pasteurisation to ensure food safety (ACMSF, 2011a; b). The UKFSA recently completed a wider review that included new scientific and surveillance information since the 2011 review, and in January 2014 launched public consultations in England, Wales and Northern Ireland on the controls governing the sale and marketing of raw drinking milk and raw cream in these countries (Food Standards Agency, 2014a; b; c). One objective of these consultations is to harmonise raw milk labelling rules.

8.4.4   Norway

The Norwegian Scientific Committee for Food Safety has published two risk assessments, one considering raw cows' milk and one considering raw milk from other species (sheep, goat, horse and reindeer) (VKM, 2006; 2007). The Committee considered that the probability of transmission of *C. jejuni* to humans via raw milk and cream is high, and the risk of illness is also high.

8.4.5   Belgium

In 2011 the Scientific Committee for the Belgian Federal Agency for the Safety of the Food Chain (FASFC) published a risk-benefit evaluation of raw cow milk consumption (FASFC, 2011). The committee concluded that *C. jejuni* and *C. coli* were among the main bacteria that can be transmitted through raw milk to humans (these conclusions were based on wider European data because there was a lack of data specific to Belgium).

9   APPENDIX 3: CONTROL MEASURES IN OTHER COUNTRIES

This section provides a summary of controls in some European countries and the states of Australia and the USA where the sale of raw milk is permitted.

9.1.1   Australia

At the federal level, Clause 15 of the Australia New Zealand Food Standards Code Standard 4.2.4 (which only applies in Australia) requires milk that is to be sold as liquid milk or used in the manufacture of dairy products (excluding cheese) to be pasteurised (or equivalently processed) "unless an applicable law of a State or Territory otherwise expressly provides." (FSANZ, 2012).

A review of legislation for individual Australian states indicated that in some states (New South Wales, Queensland, South Australia, and Western Australia) the sale of raw goats' milk is permitted. This permission is subject to producers having a documented food safety programme or plan. The product must be labelled as unpasteurised.

9.1.2   United Kingdom

The Food Hygiene (Scotland) Regulations 2006 state that no person shall place on the market raw milk intended for direct human consumption.[23] In England, Wales, and Northern Ireland it appears that sales of raw cows' milk are permitted with restrictions specified by the UKFSA, whereas sales of other types of raw milk (sheep, goat, buffalo milk) are not subject to these restrictions but may be controlled by a local food authority (Department of Health Social Services and Public Safety, 2006; Gleadle, 2012; National Assembly for Wales, 2006; Secretary of State, 2013). The restrictions on the sale of raw cows' milk essentially allow only sales directly from the farmer to consumers (i.e. from farm gates, farm catering operations, from a vehicle used as a shop premises, and by a farmer at farmers markets).

In England and Northern Ireland all raw milk products except buffalo milk must be labelled as not heat-treated and therefore may contain organisms harmful to health. This labelling applies to all raw milk sold in Wales (Gleadle, 2012).

9.1.3   Republic of Ireland

According to the website of the Food Safety Authority of Ireland (FSAI) sales of raw milk in Ireland appear to be permitted provided the products are labelled as "raw milk", and the origin must be stated if it is not bovine (FSAI, 2008; 2010). Premises selling raw milk must be registered and approved, and general EC hygiene regulations and specific microbiological standards (plate count, somatic cell count) must be met. It appears that some of these regulations do not apply to producers who directly supply small quantities of primary products either to the final consumer or to local retail establishments directly supplying the final consumer. While allowing sales of raw milk, the FSAI advise against consumption of this product (FSAI, 2009).

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[23] http://www.legislation.gov.uk/ssi/2006/3/contents/made

9.1.4   Italy

The sale of raw milk is permitted in Italy, but its use in catering premises, including school cafeterias, is prohibited. In 2007 the Italian Government permitted the sale of raw milk via vending machines and by 2012, around 1,400 machines were in operation (Bucchini, 2012; Giacometti *et al.*, 2012a). The vending machines must be registered, only filled with milk from a single farm on a daily basis, and the milk kept at 0-4°C. If the vending machine fills bottles, the bottle must carry the label "unpasteurised raw milk". All raw milk sold must be labelled "to be used only after boiling" (for on-farm sales, the warning is to be given verbally, and it must appear on the front of vending machines). An expiry date of three days after delivery to the consumer is required.

9.1.5   France

Raw milk must be labelled with the words "raw milk, keep at +4°C maximum" and "boil before consumption for sensitive people (young children, pregnant women and people with weakened immune systems)", and carry a deadline for consumption that is three days after production (Angot, 2012; Dehaumont, 2012). Suppliers must be registered.

9.1.6   Germany

There are two classifications of raw milk in Germany. Raw milk ("rohmilch") must only be sold from the farm by the producer directly to the consumer, and the farmer must display a sign on their tank stating the product is raw milk and that it must be boiled before consumption. "Vorzugsmilch" (certified milk) is unpasteurised milk that has been produced and handled according to higher standards than those required for normal milk production including a monthly testing regime. Vorzugsmilch must be packaged for sale through retail outlets and must be labelled as "raw milk – store at a maximum of 8°C, consume up to [date]", where the date is 96 hours after milk collection (German Federal Ministry of Justice, 2007; LAVES, 2013; Tschischkale, 2011).

9.1.7   United States of America

All milk sold interstate must be pasteurised, but individual States are responsible for setting their own legislation for the sale of raw milk (FDA, 2012). It is at least technically possible to legally sell or distribute raw milk for human consumption in 30 states (National Conference of State Legislatures, 2013). Overall regulation for the USA dairy industry is the responsibility of the USFDA.