



Raw Milk and Raw Milk Filter Microbiological Surveillance Programme (12NS2)

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ABBREVIATIONS

FSAI	Food Safety Authority of Ireland
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit(s)
DAFM	Department of Agriculture, Food and Marine
DSL	Dairy Science Laboratory
EU	European Union
VFSL	Veterinary Food Safety Laboratory (Cork County Council)
VTEC	Verocytotoxin producing <i>Escherichia coli</i>



SUMMARY

Over a 12-month period from June 2012 to June 2013, 600 samples of raw milk and in-line raw milk filters from 211 farms across Ireland were tested for a range of pathogens and indicator organisms including *Salmonella* species, verocytotoxin producing *Escherichia coli* (VTEC), *Listeria monocytogenes*, *Campylobacter* species, coagulase-positive staphylococci and *Escherichia coli*.

Listeria monocytogenes and *Campylobacter* spp. were the most commonly isolated pathogens from both raw milk filter and raw milk samples. Approximately 22% and 20% of raw milk filter samples were positive for *Campylobacter* spp. and *L. monocytogenes* respectively. While 7% and 3% of raw milk samples were positive for *L. monocytogenes* and *Campylobacter* spp., respectively.

Only 1% of raw milk filters and 0.5% of raw milk samples were positive for the presence of *Salmonella* spp. *E. coli* O26 (VTEC) was detected in 6% of raw milk filter samples. More than one pathogen type was detected in approximately 8% of raw milk filter samples. Only one pathogen type was detected in the raw milk samples.

In general, the isolation rates for all pathogens examined were higher for in-line raw milk filters than in the corresponding raw bulk tank milk samples. The same species of pathogen was found on both the raw milk filter and its corresponding raw milk sample on 12 of the farms sampled. The most commonly found pathogens on raw milk filters and corresponding raw milk samples were *L. monocytogenes* and *Campylobacter* spp.

There was no correlation between herd size, herd species, or season and the detection of pathogens in raw milk or on raw milk filters. There was no statistically significant correlation between the presence of the indicator organism's coagulase-positive staphylococci and *E. coli* and the presence of pathogens in raw milk samples.

Based on the findings of this survey, the FSAI continues to recommend that the sale of raw milk for direct human consumption should be prohibited in Ireland and the farm families who drink milk produced on their own farm, should pasteurise it first using a home pasteuriser or boil it before use.

ACKNOWLEDGEMENTS

The Food Safety Authority of Ireland (FSAI) would like to thank all its collaborative partners: the Department of Agriculture, Food and Marine (DAFM) and its Dairy Science Laboratory (DSL), Cork County Council's Veterinary Food Safety Laboratory (VFSL), the National Reference Laboratory for *Salmonella*, *Shigella* and *Listeria*, University Hospital Galway School of Public Health and various dairy industry processors, advisors and suppliers for their participation in this survey.

The FSAI would like to extend particular thanks to the dairy inspectors from DAFM who ensured that the required samples were collected and delivered to the Veterinary Food Safety and Dairy Science laboratories. The FSAI would also like to thank Carol Nolan, Veterinary Inspector, Cork County Council (formerly of the FSAI) who drafted the initial document outlining the surveillance programme.

GLOSSARY

A **bulk tank** is a large stainless steel storage tank used for cooling and holding raw milk from individual animals, e.g., cow, doe (female goat) or ewe (female sheep). The bulk tank serves the purpose of agitating and storing the milk on the farm and maintaining it at an appropriate temperature until the milk is collected for transportation to the milk processing plant.

A **milk filter** serves the purpose of filtering large particulate debris, e.g. faeces, soil, bedding and feed particles, that may inadvertently enter milk from the milking process. The filter is usually placed in the milk pipeline, which transports the milk from the animal to the bulk tank. The filter is not fine enough to remove bacteria from the raw milk.

Pasteurisation is a process of heating raw milk to a specific temperature for a pre-defined length of time (at least 72°C for 15 seconds or equivalent) and then immediately cooling it after it is removed from the heat. This process results in the destruction of harmful microorganisms and slows spoilage due to microbial growth in the food.

Raw milk means any milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect¹⁻².



BACKGROUND

In 2008, the FSAI's Scientific Committee published a report examining zoonotic tuberculosis and food safety in which it reviewed all available data on bovine tuberculosis and transmission to humans³. It concluded that the transmission of zoonotic tuberculosis through milk derived from infected herds has, in the past, been a major public health problem. However, it stated that this issue was largely solved by the introduction of milk pasteurisation and the national programme for the eradication of tuberculosis in cattle. The Scientific Committee recommended that *"the sale of unpasteurised milk intended for human consumption, originating from all farm animals, should be prohibited"*³.

In 2010, the FSAI's Scientific Committee published another report on the prevention of VTEC infection⁴. Highly virulent strains of *Escherichia coli* such as *E. coli* O157:H7 have only been recognised as foodborne pathogens since the 1980s. These organisms can be present as part of the normal gut flora of healthy cattle and can inadvertently contaminate milk during the milking process. Surveillance studies carried out in Ireland in recent years also indicate that these pathogens are present in low numbers in raw milk from some farms⁵⁻⁸. Upon evaluating all evidence, the Scientific Committee again concluded that consuming raw milk poses an unacceptable risk to health and recommended that *"the public sale of raw milk intended for human consumption in the raw state, originating from cattle, sheep or goats, should be prohibited"*⁴. Subsequently, the FSAI has advised Ministers that in the interest of protecting consumer health, the sale of raw milk for direct human consumption should be prohibited.

It was against this background that the FSAI coordinated a year-long study between June 2012 and June 2013 to establish the prevalence of pathogens in raw milk and raw milk filters from bovine, ovine and caprine dairy farms. In collaboration with dairy inspectors from DAFM, the VFSL, the National Reference Laboratory for *Salmonella*, *Shigella* and *Listeria*, University Hospital Galway School of Public Health and various dairy industry processors and suppliers, samples were tested for a range of pathogens and indicator organisms including *Salmonella* spp., verocytotoxin producing *Escherichia coli* (VTEC), *Listeria monocytogenes*, *Campylobacter* spp., coagulase-positive staphylococci and *Escherichia coli*.



INTRODUCTION

In the mid-twentieth century, the main illnesses associated with the consumption of raw milk were brucellosis caused by *Brucella melitensis* or *B. abortus* and tuberculosis (TB) caused by *Mycobacterium bovis*. Although sporadic cases still occasionally occur (mainly within farming families consuming their own raw milk) these diseases have largely been eradicated in developed countries through control programmes and more importantly, through pasteurisation of milk⁹⁻¹⁰.

Almost all liquid milk consumed in Ireland is now pasteurised, and as such, illness associated with its consumption is very rare¹¹. However, there is well documented Irish^{4-8, 12-28} and international²⁹⁻⁶⁹ scientific literature supporting the risks associated with drinking unpasteurised milk attributed to the pathogens *Listeria monocytogenes*, *Campylobacter* spp., VTEC, *Salmonella* and others.

While on-farm hygiene and animal health on Irish farms have improved immensely over recent years, farms remain a significant reservoir for pathogens. Even under the best hygiene standards, it is possible that raw milk can become contaminated. Improving on-farm sanitation and hygiene will improve the quality of raw milk but will not always guarantee safety. Opportunities for contamination of raw milk are diverse but include the milk-producing animals, i.e. cows, goats and sheep, wildlife, e.g. birds, rodents and insects, humans and structures and equipment present on the farm.

External environmental factors such as temperature control and storage duration of raw milk will influence the growth and survival of pathogens which may be present in raw milk. However, the main sources of contamination of raw milk will include^{8, 38, 41-43}.

- The udder of an infected, lactating animal, e.g. due to mastitis predominately caused by *Staphylococcus aureus* (udder infection with *L. monocytogenes* is most commonly reported in sheep and goats)⁴⁴
- The external surface of the udder due to contamination from animal faeces, bedding materials or mud
- Silage particularly in relation to *L. monocytogenes*
- Human handling
- On-farm milking equipment which has been inadequately maintained, cleaned or sanitised
- Contaminated water
- Contaminated air entering the milking plant, i.e. especially via clawpiece air bleeds

Pasteurisation is the simplest, most reliable and most acceptable method to ensure that milk is safe for consumers to drink⁹. According to a study carried out by the Centers for Disease Control and Prevention (CDC) between 1993 and 2006, more than 1,500 people in the United States became sick from drinking raw milk or eating cheese made from raw milk^{45, 62}. In addition, the CDC reported that unpasteurised milk is 150 times more likely to cause foodborne illness and results in 13 times more hospitalisations than illnesses involving pasteurised dairy products⁴⁵.

The European Food Safety Authority (EFSA) also reported recently that between 2007 and 2013, there were 27 outbreaks of foodborne illness associated with drinking raw milk in the European Union (EU)⁶⁶. Twenty-four of these outbreaks were caused by either *Campylobacter* (21/27), *Salmonella* (1/27) or VTEC (2/27) with the remainder caused by tick-borne encephalitis virus (3/27)⁶⁶.

As with all microbiological testing of food, there are limitations to the testing of raw milk for the presence of pathogens including ⁹:

- Contamination occurs sporadically
- Pathogens tend to be present at low levels and may not be evenly distributed in the milk
- Numbers of pathogens in a sample may be below the limit of detection of the microbiological method used to detect them but still large enough to produce illness due to their low infectious dose and the volume of milk consumed
- Pathogens present but below the limit of detection of the microbiological method used may grow to unsafe levels after testing
- Environmental conditions ⁴⁸

The detection of pathogens in raw milk filters, i.e. routinely used in modern milking systems to trap debris including particles of faeces, is well documented in the research literature ^{12, 35, 37, 47} and also highlights the inherent risks associated with the consumption of unpasteurised milk. Furthermore, while in-line milk filters trap debris, their pore size, i.e. typically 100 - 150 µm, is too large to prevent pathogenic bacteria, i.e. typically 1 - 10 µm, passing through the filter and therefore, it is very likely that a positive raw milk filter indicates that the raw bulk tank milk has been contaminated.

OBJECTIVE

The objective of this survey was to collect baseline information on the prevalence of the pathogens *Listeria monocytogenes*, *Campylobacter* spp., VTEC, *Salmonella* spp., and the hygiene indicators *Escherichia coli* and coagulase-positive staphylococci on a random selection of bovine, ovine and caprine dairy production holdings, by analysing raw milk filters and raw milk from bulk storage tanks used for cooling and holding raw milk on farms.

METHODS

Sample Collection

Over a 12-month period from June 2012 to June 2013, authorised officers from DAFM, in conjunction with local dairy advisors, identified farms for sampling purposes from four regions countrywide, i.e. North, South East, South West and Mid West, using milk processors registered milk supplier's lists ¹⁶.

Samples of raw milk filters and raw milk from bulk storage tanks used for cooling and holding raw milk on farms were then taken solely by authorised officers from DAFM. To ensure the integrity of the samples, sampling was undertaken by authorised sampling officers in line with an agreed survey protocol, i.e. 12NS2 protocol ²⁶.

Raw milk filters (**Samples A**) were taken aseptically from the milking lines directly after milking along with two raw milk samples (**Samples B** and **C**) from each farm's bulk storage tank, with a minimum volume of 100mls of raw milk taken for each sample.

Sample Analysis

The raw milk filter sample and one raw milk sample were sent to the Veterinary Food Safety Laboratory, Cork County Council (VFSL). The second raw milk sample was submitted to one of three DAFM regional dairy science laboratories (DSL) located in counties Cork, Limerick and Kildare.

In the VFSL, each raw milk filter sample was tested for the presence of *L. monocytogenes*, *Campylobacter* spp., VTEC (O157 and O26), and *Salmonella* spp. The first raw milk sample was only tested for *Campylobacter* spp. (Table 1). In the DSL, the second raw milk sample was tested for the presence of *L. monocytogenes*, *Salmonella* spp., *E. coli* and coagulase-positive staphylococci (Table 1).

Table 1: Overview of Sample Analysis carried out in each Laboratory*

Parameter	VFSL*		DSL*
	Raw Milk Filter	Raw Milk	Raw Milk
<i>L. monocytogenes</i> (Detection Only)	✓	X	✓
<i>Campylobacter</i> spp.	✓	✓	X
VTEC (O157 and O26)	✓	X	X
<i>Salmonella</i> spp.	✓	X	✓
Coagulase positive staphylococci	X	X	✓
<i>E. coli</i>	X	X	✓

* ✓ = Tested; X = Not tested

A detailed overview of all laboratory methods used in both the VFSL and DSL is given in Appendix 1.

Statistical Analysis

A chi-squared test was used to determine if there was a seasonal variation in the occurrence of the pathogens detected. Logistic regression was used to determine if the number of lactating animals on the farm influenced the occurrence of pathogens. This statistical analysis was carried out in R.^a

^a Personnel communication Prof. Francis Butler, University College, Dublin

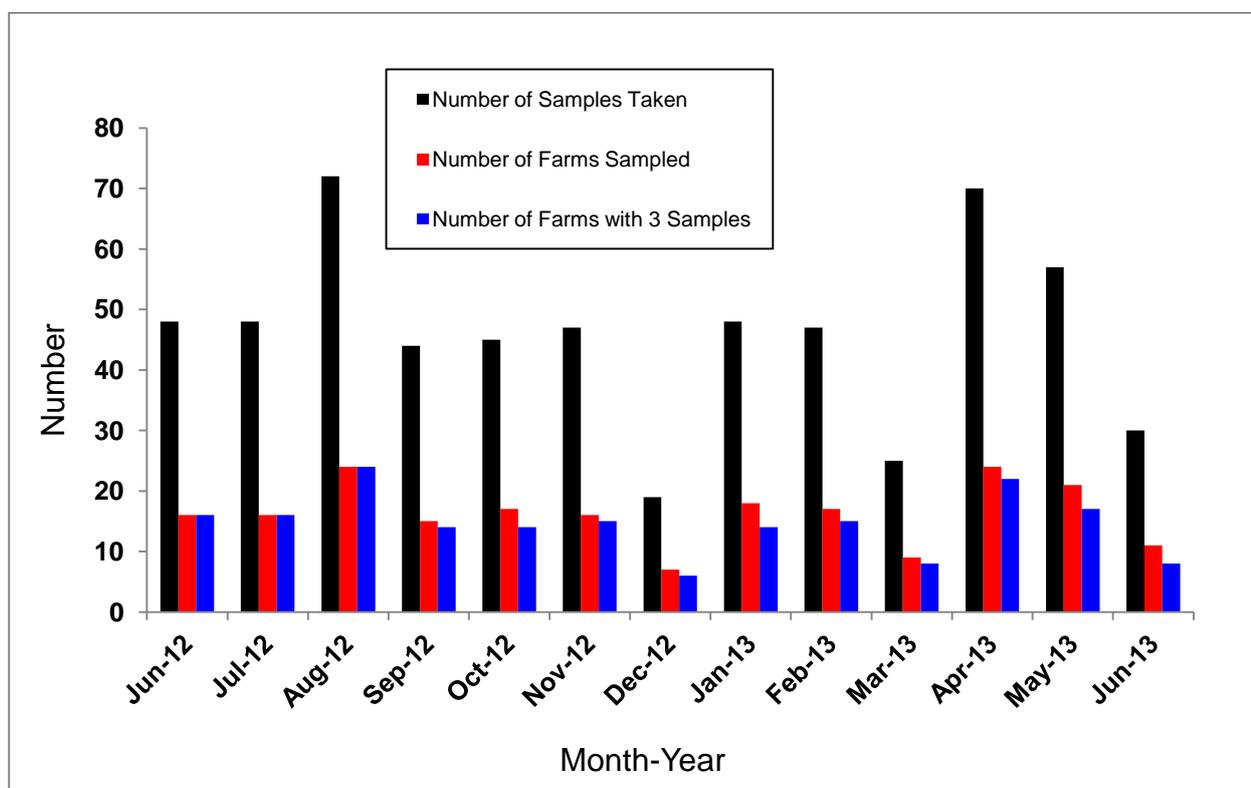
RESULTS AND DISCUSSION

General

A total of 600 samples were collected nationally from 211 dairy farms, i.e. 94% (199/211) cow, 5% (10/211) goat and 1% (2/211) sheep, between June 2012 and June 2013, and microbiologically tested. These 600 samples comprised 32% (190/600) raw milk filter samples and 68% (410/600) raw milk samples (Figure 1).

Approximately 54% (323/600) of samples analysed were collected in 2012 and 46% (277/600) in 2013, with the highest and lowest number of samples collected during the months of August, 2012, i.e.72, and December, 2012, i.e.19 (Figure 1).

Figure 1: Breakdown of Samples Collected and Analysed (n=600)



On a regional basis, all four regions were similar in terms of sample numbers taken, with approximately 25% of total sample taken from each region, i.e. 151/600, 140/600, 154/600 and 155/600 from the North, South East, South West and Mid West, respectively.

Of the 211 dairy farms sampled nationally, approximately 81% supplied large scale milk processors, i.e. > 1,001 tonnes per/year, 11% supplied small-medium scale milk processors, i.e. <100 to 1,000 tonnes per/year, and 8% supplied both categories. One dairy farm did not specify the size of the processor it supplied.

In terms of the herd size of dairy farms sampled, approximately 54% (113/211) of herds had less than 70 lactating animals while 46% (98/211) had more than 70 lactating animals. The largest herd of lactating animals was caprine, comprising 300 lactating animals in a herd of 312. The smallest herd of lactating animals was also caprine, comprising 10 lactating animals in a herd of 34.

Microbiological Analysis

Listeria monocytogenes and *Campylobacter* spp. were the most commonly isolated pathogens from both raw milk filter and raw milk samples. Approximately 22% (42/190) and 20% (38/190) of raw milk filter samples were positive for *Campylobacter* spp. and *L. monocytogenes* respectively. While 7% (15/208) and 3% (6/200) of raw milk samples were positive for *L. monocytogenes* and *Campylobacter* spp. respectively (Table 2).

Table 2: Overview of Pathogen Detection Rates across Samples Tested

Test Parameter	Percentage Detection Rate ^a	
	Raw Milk Filters	Raw Milk
<i>L. monocytogenes</i> (Detection Only)	20% (38/190)	7% (15/208)
<i>Campylobacter</i> spp.	22% (42/190)	3% (6/200)
VTEC (O157 and O26) ^b	6% (12/190)	Not Tested
<i>Salmonella</i> spp.	1% (2/185)	0.5% (1/206)
More than one pathogen detected in same sample	8% (15/190)	0

^a Percentage value based on numbers of samples tested for each pathogen

^b Isolates of *E. coli* O157:H7 and *E. coli* O26 which had at least one verocytotoxin gene, VT1 or VT2 detected

More than one pathogen type was detected in approximately 8% of raw milk filter samples (Table 2) and two of these raw milk filter samples had three pathogens detected *L. monocytogenes*, VTEC O26 and *Campylobacter* spp. Only one pathogen type was detected in the raw milk samples.

In general, the isolation rates for all pathogens examined were higher on in-line raw milk filters than in the corresponding raw bulk tank milk samples (Table 2) which broadly agrees with the findings of similar international studies ³³. However, in some cases, the detection rates in raw milk samples were higher than those internationally reported ³³.

On 12 farms sampled (all bovine), the same pathogen type was detected on both the raw milk filter and in the corresponding raw milk sample (Table 3). The most commonly detected pathogens on both raw milk filters and in the corresponding raw milk sample were *L. monocytogenes* and *Campylobacter* spp. (Table 3).

Table 3: Number of Farms where the same Pathogen Type was detected on both Raw Milk Filter and in Raw Milk Samples

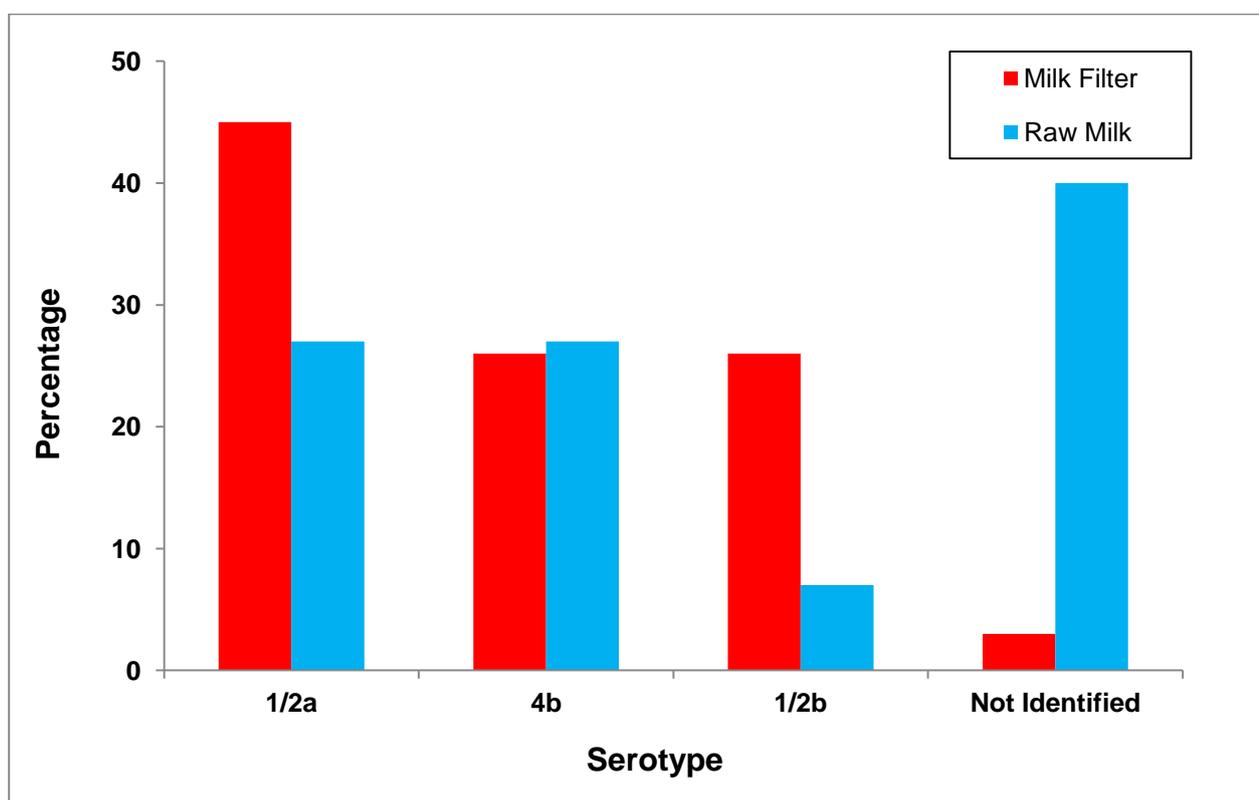
Isolated on Both Raw Milk Filter and Raw Milk Samples	Number of Establishments (Farms)
<i>L. monocytogenes</i>	6
<i>Campylobacter</i> spp.	5
<i>Salmonella</i> spp.	1

However, as VTEC (O157 and O26) was not tested for in raw milk samples (Tables 1 and 2), a full comparison is not possible

Listeria monocytogenes was isolated from 20% (38/190) of raw milk filter samples and 7% (15/208) of raw milk samples (Table 2). These results indicate a higher detection rate for *L. monocytogenes*, particularly in raw milk, than a number of international studies³³. All raw milk filters (n=38) and the majority of raw milk (14/15) samples positive for *L. monocytogenes* came from bovine herds. The remaining positive raw milk sample came from a caprine herd. However, it should be noted that as most of the sampling came from bovine herds, no conclusions can be drawn regarding the significance of this finding.

No association was detected between the number of lactating dairy animals in the herd and the detection of *L. monocytogenes* in the samples using logistic regression analysis. On chi-squared analysis, the season of the year had no effect on the detection of *L. monocytogenes* in the samples. Approximately 89% (47/53) of samples positive for *L. monocytogenes* were typed by conventional serological agglutination to determine their serotypes. Three serotypes of *L. monocytogenes*, i.e. 1/2a, 4b and 1/2b, were identified in both raw milk filter and raw milk samples as outlined in Figure 2⁷⁵.

Figure 2: Distribution of *L. monocytogenes* Serotypes in Isolates Analysed^a



^a Milk filter samples (n=38); Raw milk samples (n=9)

The majority of human outbreaks are caused by 4b strains with 1/2a, 1/2b and 4b accounting for 95% of the isolates from human illness^{26, 63, 74-75}. The most commonly identified serotype was 1/2a isolated from 45% and 27% of positive raw milk filters and raw milk samples, respectively. Serotype 4b was isolated from 26% of positive raw milk filters and 27% of raw milk samples (Figure 2).

In the current survey, Pulse Field Gel Electrophoresis (PFGE) was carried out on 47 of the *L. monocytogenes* positive samples. Two enzymes, i.e. Ascl and Apal, were used to subtype *L. monocytogenes* which generated two PFGE profiles for each *L. monocytogenes* strain. All the PFGE profiles were then submitted to the European Reference Laboratory for *L. monocytogenes* database and pulso-numbers were generated for a considerable number of the profiles. Table 4 provides an overview of the distribution of pulso-numbers for both Ascl and Apal⁷⁵.

Table 4: Distribution of Pulso Numbers for Ascl and Apal^a

No. of Isolates with same Ascl Profile	Ascl Profile	No. of Isolates with same Apal Profile	Apal Profile
3	GX6A16.0010.EU	4	GX6A12.0016.EU
3	GX6A16.0017.EU	3	GX6A12.0021.EU
2	GX6A16.0032.EU	2	GX6A12.0087.EU
2	GX6A16.0040.EU	2	GX6A12.0165.EU
4	GX6A16.0057.EU	2	GX6A12.0171.EU
2	GX6A16.0145.EU	2	GX6A12.0176.EU
3	GX6A16.0170.EU	5	GX6A12.0208.EU
7	No Match - EURL Data base	2	GX6A12.0222.EU
		3	GX6A12.0246.EU

^a Profiles have the same pulso number when two profiles are indistinguishable. Profiles can be very closely related but not an exact match. These profiles are grouped together on dendograms and are considered closely related when they are 90% or greater similar.

The European Reference Laboratory for *L. monocytogenes* is currently developing a database for *L. monocytogenes*. The benefit of this is that there will be one database for food, feed and animal samples in Europe and the submitted PFGE gels will be stored with a unique pulso-number assigned. This will allow information to be exchanged across Europe without having to share PFGE gel images⁷⁵.

Salmonella species

One percent (2/185) of raw milk filters and 0.5% (1/206) of raw milk samples were positive for the presence of *Salmonella* spp. (Table 2).

Salmonella Kentucky [Antigenic Structure 8,20:i:z6] was isolated from a raw milk filter sample taken from a bovine herd. Laboratory testing, i.e. antibiogram, of the *S. Kentucky* isolate indicated no antibiotic resistance.

Another raw milk filter and its corresponding raw milk sample, from a different bovine herd, were both positive for *Salmonella*. However, while both samples were *Salmonella* spp. positive, only the raw milk filter sample was serotyped. It was identified as *S. Dublin*. Previously published international research has shown milk filters as useful indicators of the presence of salmonellae^{35, 65, 71}.

Campylobacter species

Twenty-two percent (42/190) of raw milk filter samples and 3% (6/200) of raw milk samples tested positive for the presence of *Campylobacter* spp. (Table 2).

Of the samples positive for *Campylobacter* spp., approximately 16% (30/190) of raw milk filter samples and 1% (2/200) of raw milk samples were *C. jejuni*. Approximately 3% (5/190) of raw milk filter samples and 0.5% (1/190) of raw milk samples were *C. coli*. One raw milk filter sample was positive for *C. lari*. All positive *Campylobacter* spp. samples were taken from bovine herds. All the *Campylobacter* spp. detected in the raw milk filter and raw milk samples have been associated with human illness.

Five of the raw milk samples (5/200) positive for *Campylobacter* spp. had a *Campylobacter* spp. identified in the corresponding raw milk filter sample. Three raw milk samples (3/200) positive for *C. jejuni* and *C. coli* had the same *Campylobacter* spp. identified in the corresponding raw milk filter sample.

On chi-squared analysis, the season of the year had no effect on the detection of *Campylobacter* spp. on the raw milk filter samples. No association was detected between the number of lactating dairy animals in the herd and the detection of *Campylobacter* spp. on raw milk filter samples using logistic regression analysis.

VTEC (O157 and O26)

Raw milk filter samples were tested for the presence of VTEC (O157 and O26) (Table 1). Approximately 7% (13/190) of these samples were positively identified as *E. coli* O157:H7 or *E. coli* O26. However, only 6% (12/13) of these raw milk filter samples positively identified as *E. coli* O157:H7 or *E. coli* O26 had at least one verocytotoxin gene, VT1 or VT2 detected (Table 2).

One *E. coli* O157:H7 isolate was detected which was deficient for both the verocytotoxin genes, VT1 and VT2 and the virulence genes *eaeA*, i.e. gene associated with the attaching and effacing lesion of enterocytes and *hlyA*, i.e. the plasmid located enterohaemolysin-encoding gene¹². However, in the same raw milk filter sample, a verocytotoxigenic *E. coli* O26 isolate was also detected which had both the verocytotoxin genes, VT1 and VT2 in addition to the virulence genes *eaeA* and *hlyA*.

Eighteen *E. coli* O26 isolates were detected, of which 67% (12/18) had at least one of the verocytotoxin genes, VT1 or VT2 (Table 5):

Table 5: Overview of some Virulence Genes Identified in *E. coli* O26 Isolates ^a

Number of <i>E. coli</i> O26 Isolates	VT1	VT2	<i>eaeA</i>	<i>hlyA</i>
5	✓	✓	✓	✓
2	X	✓	✓	✓
1	X	X	✓	✓
5	X	X	X	X
5	✓	X	✓	✓

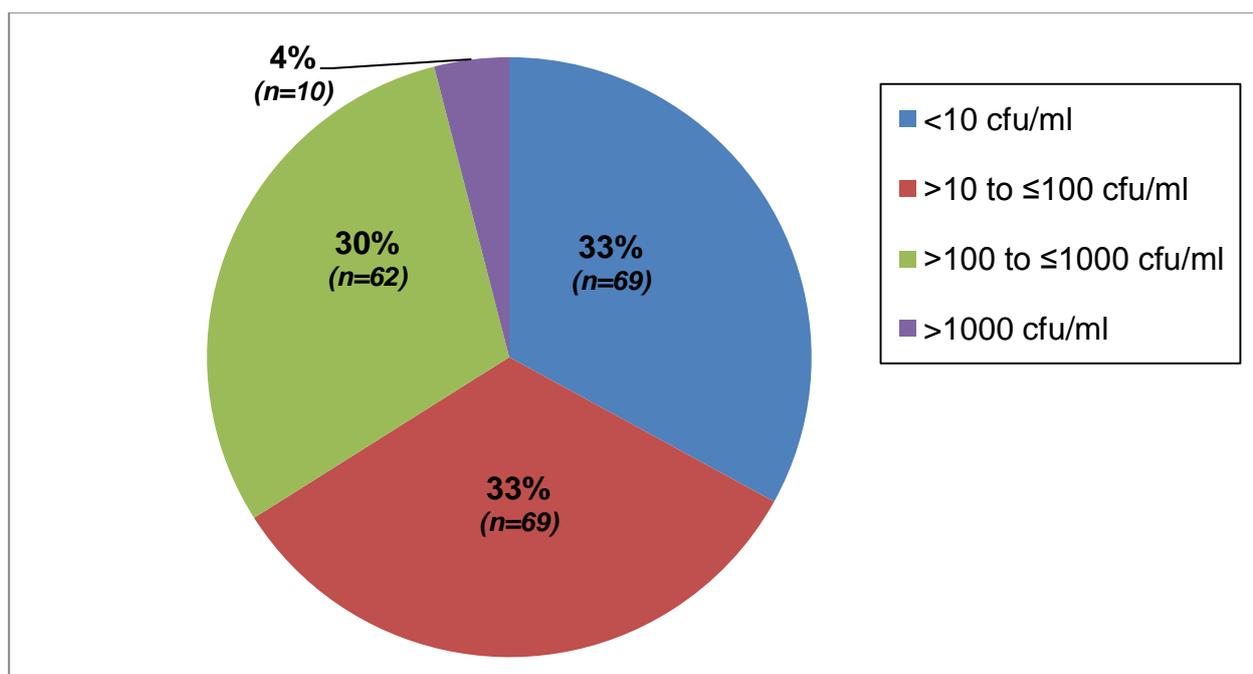
^a ✓ Gene present; X Gene not present

Coagulase-Positive Staphylococci

Coagulase-positive staphylococci such as *Staphylococcus aureus* are often found in raw milk due to contamination caused by poor hygiene conditions, or milk which has come from cows with mastitis ⁴⁸. As such, coagulase-positive staphylococci can be used as an indicator of dairy hygiene during milking when testing the microbiological safety and quality of raw milk ⁴⁰.

Over 66% of raw milk samples tested had counts of coagulase-positive staphylococci < 100cfu/ml indicating a good standard of hygiene, i.e. specified by the International Commission on Microbiological Specifications for Foods (ICMSF) (Figure 3). ⁴⁰

Figure 3: Enumeration of Coagulase-Positive Staphylococci in Raw Milk Samples (n=210)



While no raw milk sample contained sufficient numbers, i.e. > 100,000 cfu/ml, of coagulase-positive staphylococci required for enterotoxin formation, isolates from 120 raw milk samples that had a coagulase-positive count when enumerated were submitted for further laboratory analysis to determine the prevalence of genes encoding staphylococci enterotoxins ¹⁷.

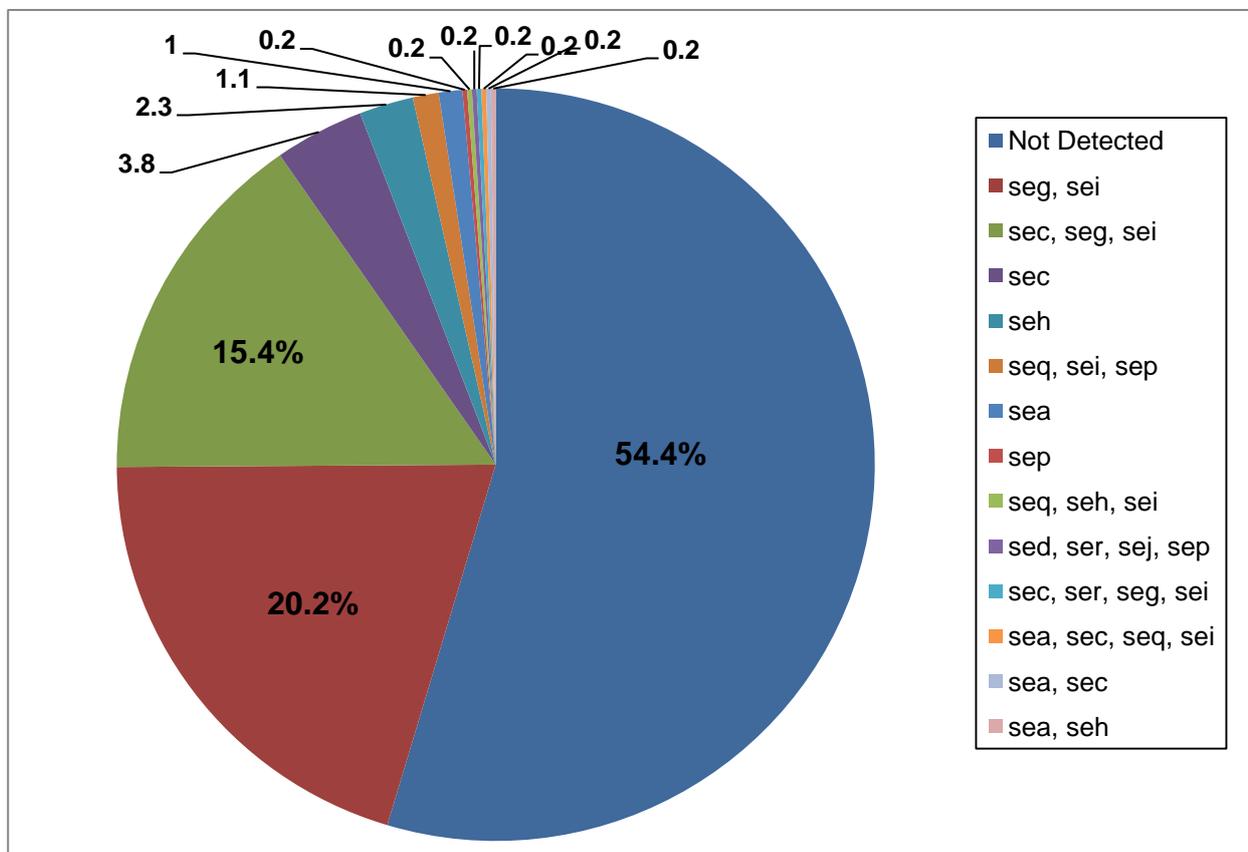
Laboratories submitted coagulase-positive staphylococci strains found during analysis up to a maximum of 5 coagulase-positive staphylococci strains per raw milk sample. A total of 526 coagulase-positive staphylococci strains were then analysed by polymerase chain reaction (PCR) to determine the prevalence of genes encoding staphylococci enterotoxins ¹⁷.

All the coagulase-positive staphylococci strains tested contained the 23S rDNA which is specific to *Staphylococcus aureus* ¹⁷.

Analysis of these coagulase-positive staphylococci strains, i.e. *S. aureus*, for the presence of 11 genes, i.e. *sea*, *seb*, *sec*, *sed*, *see*, *seir*, *seg*, *seh*, *sei*, *sej* and *seip*, which encode for staphylococcal enterotoxins indicated that 54% did not possess the genes. Of the remaining 46% that did possess the genes for producing enterotoxins, *seg* and *sei* were the most prevalent ^{17,70}.

Approximately 38% of *S. aureus* strains tested had *seg* and *sei* genes either together or in association with other enterotoxin genes. A full overview of the prevalence of staphylococcal enterotoxin genes in the *S. aureus* strains is given in Figure 4.

Figure 4: Percentage Prevalence of Genes Encoding for Staphylococcal Enterotoxins in Coagulase Positive Strains



It is important to note that the presence of large numbers of staphylococci is not sufficient to correlate a specific food such as raw milk for example, as the vehicle of a food poisoning incident because not all staphylococci are enterotoxigenic.

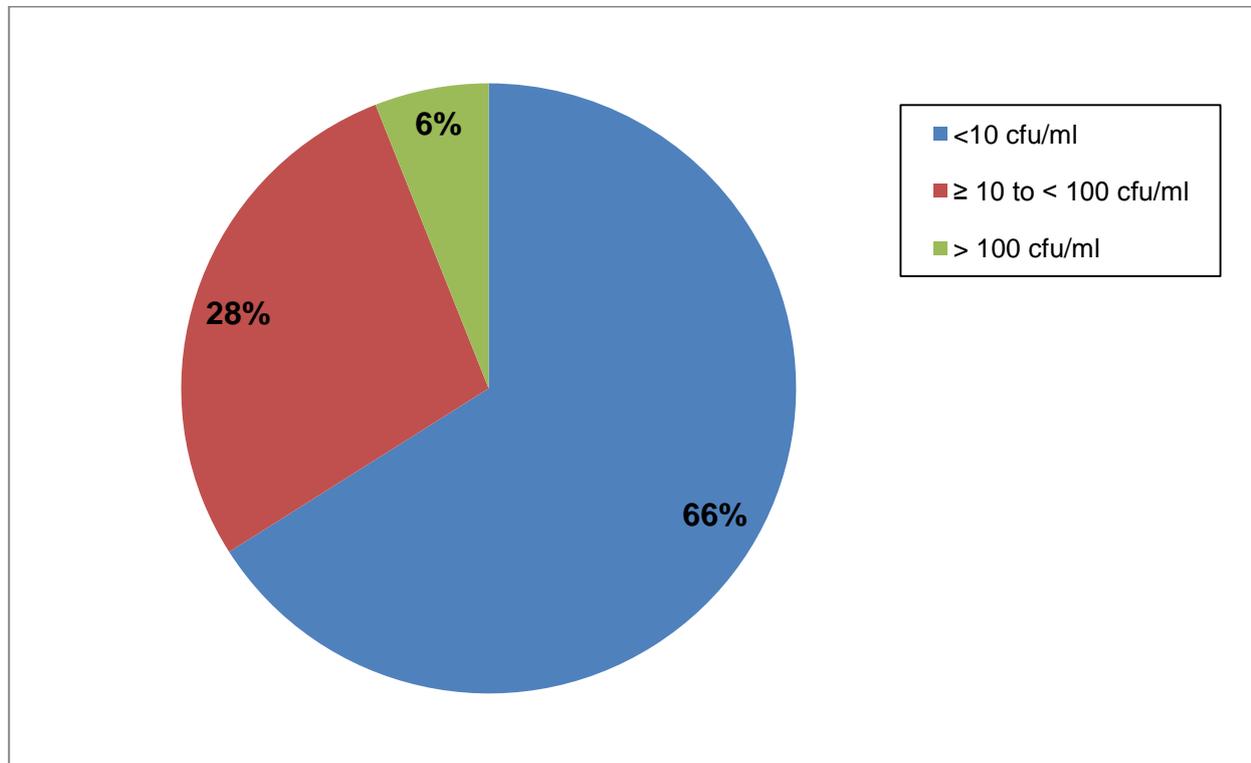
In addition, demonstration of enterotoxigenicity of food isolates is only circumstantial evidence of enterotoxigenic staphylococcal contamination and the potential for causing food poisoning cannot be ascertained without demonstrating the actual presence of the enterotoxins in a suspect food⁷². Conversely, neither the absence of coagulase-positive staphylococci nor the presence of small numbers of bacteria is complete assurance that the food is safe (because the staphylococcal enterotoxins are extremely resistant and may survive processes which kill the bacterial cells such as pasteurisation)^{68, 72}.

Although coagulase-positive staphylococci are used as indicators of dairy hygiene, overall, there was no statistically significant correlation between coagulase-positive staphylococci numbers and the presence of pathogens in raw milk samples.

Escherichia coli

Escherichia coli are one of the most common indicator organisms used to assess the potential risk of enteric pathogens being present in food. *E. coli* is considered an indicator of faecal contamination and levels can be related to farm hygiene conditions, the condition and effectiveness of cleaning of milking equipment as well as the temperature that the milk is held at in bulk storage tanks¹⁸. Ninety-four percent of raw milk samples tested in the current survey had *E. coli* count < 100 cfu/ml, while 66% had counts <10 cfu/ml as outlined in Figure 5.

Figure 5: Enumeration of *Escherichia coli* in Raw Milk Samples^a



^a Only raw milk samples, i.e. sample C (n=210), were tested for the presence of *E. coli*

However, the presence of *E. coli* doesn't always predict the presence of pathogens in a food. In a recent study which examined raw bulk tank milk, 16% of samples were positive for *E. coli*. However, pathogens such as *E. coli* O157:H7 and *Salmonella* were not detected in the same raw bulk tank milk⁶⁹. In another study by Berry *et al.* the majority of raw milk samples which tested positive for *L. monocytogenes* (while not a faecal pathogen) had very low *E. coli* counts and would be considered to have satisfactory dairy hygiene practices¹⁸.

Overall, there was no statistically significant correlation between *E. coli* numbers and the presence of pathogens in raw milk samples.

DISCUSSION AND CONCLUSIONS

The risks of acquiring foodborne illnesses such as salmonellosis, campylobacteriosis, listeriosis, VTEC infection and others from the consumption of raw milk have been well documented internationally²⁹⁻⁶⁹. In Ireland, there are many well documented cases of pathogens found in raw milk and foodborne illness associated with consumption of raw milk^{4-8, 12-28}. In the United Kingdom during November 2014, three separate incidents involving six cases of *E. coli* O157, including five cases in children, were potentially linked to the consumption of raw milk⁶⁴.

In an opinion published in January 2015, EFSA stated that raw milk can be a source of harmful bacteria, mainly *Campylobacter*, *Salmonella* and VTEC, that can cause serious illness⁶⁶. EFSA further stated that implementing current good hygiene practices at farms is essential to reduce raw milk contamination, while maintaining the cold chain is also important to prevent or retard bacterial growth in raw milk. However, EFSA concluded that these practices alone do not eliminate the risks from raw milk and that boiling raw milk before consumption, i.e. pasteurise, is the best way to destroy pathogens which can make people sick⁶⁶. Infants, children, pregnant women, older people and those with a weakened immune system have a higher risk of becoming ill from drinking raw milk⁶⁶.

Another study published by the CDC also in January 2015, indicated that the average annual number of outbreaks in the United States due to consumption of raw milk has more than quadrupled since the last similar study – from an average of three outbreaks per year during 1993-2006 to 13 per year during 2007-2012⁶². Overall, there were 81 outbreaks in 26 States from 2007 to 2012 with 77% caused by *Campylobacter* spp. The outbreaks, which accounted for approximately 5% of all foodborne outbreaks in the United States with a known food source, made nearly 1,000 people ill and hospitalised 73. More than 80% of the outbreaks occurred in states where selling raw milk is currently legal⁶².

It is clear from the findings of this survey that raw milk sampled from bulk storage tanks on dairy farms in Ireland can contain pathogens which can make people sick. However, the detection rate of pathogens on in-line raw milk filter samples was generally higher than in raw milk samples, particularly in the case of *L. monocytogenes*. Other studies have also shown similarly higher isolation rates for *L. monocytogenes* and *Salmonella* spp. on in-line raw milk filter samples in comparison to corresponding bulk tank raw milk samples⁴⁹⁻⁵¹.

The presence of pathogens on in-line raw milk filters does not always correlate to the presence of pathogens in the bulk-tank raw milk. However, the presence of pathogens on the in-line raw milk filters does indicate the potential for contamination of bulk milk and is indicative of contamination of the milking parlour and/or the herd^{12, 35, 37, 47, 60}.

Additionally, as bulk tank raw milk contains milk from many different animals within a herd, only a proportion of these animals in the herd may be shedding pathogens and contaminating the raw milk⁷³. Hence, contaminated milk can be diluted manyfold with uncontaminated milk. In such circumstances, the level of pathogens in the bulk milk may be too low for a reasonable and practical sampling scheme to detect. Nevertheless, the milk may remain a risk.

RECOMMENDATIONS

1. Consumption of raw milk can increase the risk of consumers being exposed to pathogens and developing a foodborne illness. The FSAI recommends that the sale of raw (unpasteurised) milk from all farm animals for direct human consumption should be prohibited in Ireland and advises that the most effective way to protect public health is to ensure that all milk is effectively heat-treated, e.g. pasteurised or boiled, especially when served to infants, children, pregnant women, older people and those with a weakened immune system or suffering from a chronic disease.
2. Until such time as a ban on the sale of raw milk is put in place, the FSAI recommends that, in addition to the legal requirement to have the words “*raw milk*” on the label of any raw milk intended for direct human consumption ², it is recommended that the label also contains the following - “*This milk has not been heat-treated and may therefore contain organisms that are harmful to health. It is recommended to boil before consumption*”.
3. Drinking pasteurised or other heat treated milks, e.g. ultra-high temperature treatment (UHT), remains the most effective measure currently available to consumers to decrease their risk, while not substantially changing the nutritional value of milk ⁵¹. The FSAI recommends that farm families who drink raw milk produced on their own farms should pasteurise it before drinking using a home pasteuriser or boil it before use ²⁷⁻²⁸.
4. Dairy farmers should implement and maintain good hygiene practices in their operations and operate to good agricultural practices as set out in Annex I of Regulation (EC) 852/2004 ⁷⁶ and Annex III, Section IX of Regulation (EC) 853/2004 ² to reduce raw milk contamination, while maintaining the cold chain to prevent or retard bacterial growth in raw milk.



APPENDIX1: MICROBIOLOGICAL METHODS ⁶⁰

Table 3: Detection Methods used by the Veterinary Food Safety Laboratory (VFSL)

Pathogen	Method
<i>Campylobacter</i> spp. ^{a-b}	In-house method based on ISO 10272-1:2006 (E) ^c
<i>Escherichia coli</i> O26 ^d	In-house modified method based on ISO 16654:2001
<i>Escherichia coli</i> O157:H7 ^d	In-house method based on ISO 16654:2001
<i>Salmonella</i> spp. ^e	In-house method based on ISO 6579:2002; <i>Incorporating amendment 1:2007</i>
<i>Listeria monocytogenes</i> ^a	In-house method based on ISO 11290-1:1996; <i>Incorporating amendment 1:2004(E)</i>

^a *Campylobacter* spp. and *L. monocytogenes* isolates were forwarded to the NRL Backweston for serotyping and speciating respectively.

^b *Campylobacter* isolates were stored at -80°C and send in batch for speciation and AMR profiling at the end of the sampling programme to CVRL, Backweston.

^c Raw milk sample (Sample B) were only tested for *Campylobacter* spp. as per method above.

^d *E. coli* O26 and *E. coli* O157 isolates were examined for virulence traits and stored at -80°C for further DNA fingerprinting and AMR profiling by UCD at the end of the sampling programme.

^e *Salmonella* isolates from the VFSL were forwarded to the National Salmonella Reference Laboratory, NUIG.

Table 4: Detection and Enumeration Methods used by the Dairy Science Laboratories (DSL) ^{a-b}

Pathogen/ Microorganism	Method
Detection of <i>Listeria monocytogenes</i>	ISO 11290-1:1996 (<i>Incorporating amendment 1:2004</i>)
Detection of <i>Salmonella</i> spp.	ISO 6579:2002 (<i>Incorporating amendment 1:2007</i>)
Enumeration of <i>Escherichia coli</i>	Using the TBX (Pour Plate Technique) ISO 16649-2:2001
Enumeration of coagulase-positive staphylococci	Using the Pour Plate Technique ISO 6888-2:1999 (<i>Incorporating amendment A1:2003</i>)

^a Located in Cork, Limerick and Kildare

^b Raw milk sample (Sample C)



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