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Pathogen profile of clinical mastitis in Irish milk-recording herds reveals a complex aetiology

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Abstract

Effective mastitis control requires knowledge of the predominant pathogen challenges on farm. In order to quantify this challenge the aetiological agents associated with clinical mastitis in 30 milk-recording dairy herds in Ireland over a complete lactation were investigated. Standard bacteriology was performed on 630 pre-treatment quarter milk samples, of which 56% were culture-positive, 42% culture-negative and 2% contaminated. Two microorganisms were isolated from almost 5% of the culture-positive samples. The bacteria isolated were *Staphylococcus aureus* (23%), *Streptococcus uberis* (17%), *Escherichia coli* (9%), *Streptococcus spp* (6%), coagulase negative staphylococci (4%) and other species (1%). A wide variety of bacterial species were associated with clinical mastitis, with *S. aureus* the most prevalent pathogen overall, followed by *S. uberis*. However, the bacterial challenges varied widely from farm to farm. In comparison with previous reports, in the present study the contagious pathogens *S. aureus* and *S. agalactiae* were less commonly associated with clinical mastitis, whereas, the environmental pathogens *S. uberis* and *E. coli* were found more commonly associated with clinical mastitis. While *S. aureus* remains the pathogen most commonly associated with intramammary infection in these herds, environmental pathogens such as *S. uberis* and *E. coli* also present a considerable challenge.

Introduction

Intramammary infection (IMI) or mastitis is estimated to be the most economically costly disease to the dairy industry both in Ireland and internationally (More and others 2010). Costs include veterinarian fees, medicinal treatment, discarded milk, production losses caused by reduced milk yield and lower milk price, higher culling rate and potentially increased mortality (Geary and others 2012). A recent survey of dairy industry stakeholders by Animal Health Ireland (AHI) identified udder health/milk quality as a priority area for improvement (More and others 2010). This was instrumental in the establishment of the CellCheck programme, the AHI-led initiative to improve udder health and reduce the somatic cell count (SCC) of the national herd. In 2004 the national average bulk tank somatic cell count (BTSCC) was estimated at almost 251,000 cells/ml (Berry and others 2006).

Mastitis is defined as any inflammatory process occurring in the mammary gland and can be sub-clinical or clinical. A variety of bacterial species are implicated in bovine mastitis with the most common being the Gram positive staphylococci and streptococci and the Gram negative enterobacteriaceae (Bannerman 2009). Isolation of a bacterial pathogen from milk of an infected cow is considered the definitive diagnosis of mastitis and identification of the causative organism can help to indicate suitable treatment and control strategies. Mastitis pathogens are classically referred to as contagious or environmental species, depending on how they behave within dairy herds. Contagious pathogens generally spread from cow-to-cow, with the infected udder being the primary source of the pathogen. Contagious pathogens include *Staphylococcus aureus* and *Streptococcus agalactiae*. Environmental pathogens, which include coliform bacteria and *Streptococcus uberis* are found in the environment where the cow lives, and spread directly from there to the udder. However, it is more recently recognised that many mastitis pathogen species contain strains of bacteria that may demonstrate contagious and/or environmental transmission patterns (Schukken and others 2012).

Current protocols for mastitis control include hygienic milking and housing conditions, routine milking machine maintenance, post-milking teat disinfection, dry cow therapy, isolation of infected animals and cow culling (Kelly and others 2009a, b). Some control strategies target cow-to-cow transmission of bacteria, while other

strategies reduce the risk of infection from bacteria with an environmental source. In order to evaluate current control strategies and devise required modifications, the local on-farm bacterial challenges must be identified and quantified.

International studies have identified large variation in the pathogens deemed to be the predominant cause of mastitis depending on geographical location, production system and local mastitis control programmes (Zadoks and Fitzpatrick 2009). There is a dearth of information on the aetiology of mastitis on Irish farms. A study carried out over 30 years ago (1978-1980) on autumn calving cows, profiled the pathogens then associated with both sub-clinical and clinical mastitis at drying-off on farms in Ireland (Egan and O'Dowd 1982). *Staphylococcus aureus* was identified as the predominant mastitis-causing pathogen and was responsible for over 60% of mastitis cases. The Department of Agriculture, Food and the Marine (DAFM) Regional Veterinary Laboratories (RVLs) compile annual mastitis surveillance reports which show the relative frequency of mastitis pathogens isolated from voluntarily submitted samples. Sample submission to the RVLs peaks in autumn just before most cows are dried-off. In each of the years 2005 to 2010 *S. aureus* was the microorganism most commonly isolated from milk samples and accounted for up to 40% of samples submitted (DAFM 2006-2009, 2010). In a recent study of the prevalence of sub-clinical mastitis-associated pathogens on dairy farms in Ireland, *S. aureus* was found to be the most common pathogen followed closely by *S. uberis*. These pathogens accounted for 21% and 19% of isolates respectively (Barrett and others 2005). This *S. uberis* frequency was much higher than the values reported by Egan and O'Dowd (1982) or the recent DAFM RVL surveillance reports. *S. uberis* is also the most common major pathogen associated with both clinical and sub-clinical mastitis in England and Wales, clinical mastitis in New Zealand and was the pathogen most commonly isolated from milk samples submitted to diagnostic labs in the United Kingdom and New Zealand (Bradley and others 2007; DAFM 2010; McDougall 2003; McDougall and others 2007; Petrovski and others 2011; Randall 2010) which may be associated with different production systems.

The objective of this study was to profile the bacterial pathogens currently responsible for clinical mastitis in milk recording dairy herds in Ireland over a full lactation.

Materials and Methods

Sample collection

All commercial farmers from 2 discussion groups in the South of Ireland were contacted and invited to participate in the study (n = 42) and 28 (67%) self-selected. Discussion group work focuses on management and breeding strategies to maximise profit. Results from the Teagasc Profit Monitor show that these discussion group members are within the top 10% of Irish dairy farmers in profit terms. An additional three research dairy herds from Teagasc were also included. Participating farmers were expected to sample any cow in lactation that presented with clinical mastitis during the study period. Sampling commenced on 14/2/2010 and ceased on 14/2/2011. These dates were chosen to cover a complete lactation for spring, split spring/autumn and autumn-calving herds. Farmers were instructed at discussion group meetings on how to aseptically collect samples and were given a milk sampling pack consisting of gloves, sterile tubes, and a protocol for aseptic milk sampling. Clinical mastitis was diagnosed by the farmer based on the usual criteria (clots, flecks or blood in the milk or heat or swelling in the udder). Quarter milk samples were taken aseptically before antibiotic treatment commenced. Before sampling, hands were washed in disinfectant or fitted with disposable gloves. Then the teat ends were disinfected with methylated spirits and allowed to dry. Teat canal contaminants were removed by discarding the first three strips of milk from the infected quarter. Two strips of milk (~10ml) were then collected in a sterile container held at approximately a 45° angle to the teat to avoid contamination. Sample bottles were capped and labelled with the cow tag number, the date and the quarter. Animals with repeat cases of mastitis were re-sampled unless they had been sampled within the previous 10 days (lag time before a new case could be declared). Samples were frozen until taken to the monthly discussion group meetings. Farmers received a weekly communication (by e-mail and Short Message Service (SMS) text message) to thank them for samples submitted at the previous meeting, and to remind them to continue collecting and submitting samples.

Sample editing

While a total of 820 milk samples were received, 89 of these had been taken outside the study dates (14/2/2010 to 14/2/2011) while 16 were repeat samples (from the same quarter of the same cow less than 10 days apart). These samples were removed from

the study. In the course of the discussion group meetings it was found that one farmer submitted sub-clinical mastitis samples (based on California Mastitis Test) and these samples (n = 85) were also excluded from the study. In total 630 samples from 30 dairy herds (3 research herds and 27 commercial herds) located primarily in the South of Ireland, were available for analysis.

Bacteriological analysis

In order to identify the pathogen causing intramammary infection, standard laboratory methods recommended by the National Mastitis Council were used (National Mastitis Council 1999). Briefly, 10 µl of milk was plated directly on trypticase soy agar (TSA) containing 5% defibrinated sheep or calf blood. The plates were examined after overnight incubation at 37 °C in aerobic conditions. Plates without visible colonies were incubated for a further 24 hours and if there were still no visible colonies they were deemed negative for mastitis-associated pathogens. Plates that contained three or more colony types were deemed contaminated and discarded. For a small number of plates two colony types were found and both were identified. Colonies were putatively identified based on colony morphology, haemolysis, Gram stain, catalase test and growth on MacConkey lactose indicator agar, Baird-Parker agar and Mannitol Salt Agar. Putative *Staphylococcus aureus* colonies were distinguished from coagulase negative staphylococci (CNS) by the above tests in addition to the coagulase test. *Streptococcus uberis* was identified by its ability to hydrolyse aesculin while remaining streptococci were identified using the API 20 Strep strips (BioMerieux).

BTSCC calculation

For 29 of the herds involved in the study (processor data for 1 herd was unavailable) we obtained a single BTSCC value and milk volume per month. The BTSCC value was the arithmetic mean of the available BTSCCs, as tested by the relevant milk processor, weighted by milk volume for that month. An annual BTSCC was estimated for each herd in the study as the arithmetic mean of the monthly BTSCCs weighted by milk volume. The BTSCC across all herds involved in the study was calculated as the geometric mean of the annual BTSCCs for all herds irrespective of milk volume produced as herd was considered the experimental unit. Monthly BTSCC and milk volume data for all milk-recording herds was provided by the Irish Cattle Breeding Federation (ICBF). A national monthly BTSCC for all milk recording herds was calculated as the geometric mean of the monthly BTSCC across

herd weighted by milk volume. An annual national BTSCC of milk-recording herds was estimated from this data as the arithmetic mean of the monthly BTSCC weighted by milk volume.

Mastitis incidence calculation

For each herd the total number of cow days at risk during lactation was calculated. All herds involved in the study were milk recording with data stored in the ICBF database. Individual cow lactation records were obtained from ICBF and the risk period for each cow was calculated as the number of days between calving and either drying off or leaving the herd. For those animals which had a case of clinical mastitis the lag time, (10 day period before a new case could be declared) was subtracted from the days at risk. The period pre-calving and post-drying off was not included in the days at risk calculation as farmers did not sample outside of lactation. For each herd total days at risk was the sum of the individual cow days at risk. The lactation period clinical mastitis incidence, expressed as the number of cases per 10,000 cow days at risk, was calculated for each herd as

$$\text{Incidence} = \frac{\text{total number of cases}}{(\text{Days at risk}/10{,}000)}$$

The study was carried out over a one year period and hence incidence, expressed as cases/100 cows calving/year was also calculated as

$$\text{Incidence} = \frac{\text{total number of cases}}{\text{Herd size}/100}$$

Results

Herd profile

Thirty dairy herds, predominantly located in the South of Ireland participated in the study. The mean number of calvings per herd per year was 213, with a median of 209 and a range of 60 to 575 (Table 1). The herds were a combination of spring calving (n = 26), autumn calving (n = 1) and split calving herds (n = 3) reflecting the fact that dairy farmers in Ireland operate largely a spring calving system in order to maximise milk production from grazed grass. The estimated annual BTSCC of the herds involved in the study was 224,515 cells/ml (95% C.I. = 205,090 – 245,781 cells/ml) and 76% of the herds had an annual BTSCC >200,000 cells/ml.

Bacteriological profiling

In total, 630 milk samples were received from 30 validated herds. The number of samples per herd ranged from 4 to 73 (Table 1). The seasonal distribution of milk sample submission is shown in Figure 1. The bacteriological profiling results are presented in Table 1 and summary results of the relative frequency of isolation of the major mastitis pathogens as a total of all submitted samples and as a total of culture positive samples are shown in Figure 2 and Table 2. In total, 265 samples (42.1%) resulted in no growth on blood agar and so did not yield any bacterial isolate for pathogen identification. An additional 15 samples were deemed contaminated and excluded from the analysis, leaving 350 milk samples from which mastitis pathogens were identified. For 30 samples a mixed culture of 2 organisms was obtained (Table 3) resulting in the identification of 380 pathogens from 350 milk samples. In agreement with previous studies *Staphylococcus aureus* was the most prevalent species recovered and was found in 144 milk samples (22.9%) from 27 different herds. *Streptococcus uberis* was the next most abundant pathogen and was found in a total of 109 milk samples (17.3%) from 26 herds. A considerable number of coliforms (n = 58), other streptococci species (n = 35) and coagulase negative Staphylococci (CNS) (n = 27) were also recovered. The other streptococci species identified were predominantly *S. dysgalactiae* but also included Enterococcus spp, *S. agalactiae* and catalase negative species that could not be identified using the BioMerieux API 20 Strep strips. For 7 milk samples the isolated pathogen would not grow on media without blood and could not be reliably identified. Preliminary identification indicated this pathogen was *Trueperella pyogenes*. In summary, of the

milk samples evaluated, no pathogen was identified in 44.4% due to lack of growth or contamination. *S. aureus* was recovered from 22.9%, while *S. uberis* was recovered from 17.3%, coliforms were recovered from 9.2% while other streptococci and CNS accounted for 5.5% and 4.3% respectively (Table 2).

Despite the fact that *S. aureus* was the most common pathogen isolated it was not the most common pathogen in the majority of herds. Of the 30 herds studied, *S. aureus* was the most common pathogen in 10, *S. uberis* was the most common in 13, while 2 had equal numbers of *S. uberis* and either *S. aureus* or *S. dysgalactiae*. In a further 4 herds, coliforms were most commonly associated with clinical mastitis cases and a further herd had equal numbers of coliforms and *S. dysgalactiae*. This indicated that the bacterial challenge varied widely from herd to herd (Table 1).

Incidence of clinical mastitis

The mean clinical mastitis incidence across all herds was 5.01 cases per 10,000 cow days at risk or 13.2 cases per 100 cows calving per year. The median clinical mastitis incidence was 3.59 cases per 10,000 cow days at risk or 8.6 cases per 100 cows calving per year. The clinical mastitis incidence during lactation varied from 0.52 cases per 10,000 cow days at risk for herd 19 to 29.31 cases per 10,000 cow days at risk for herd 18 (Table 1). The incidence varied widely from herd to herd with a number of herds with extremely high or low incidence. Clinical mastitis incidence decreased as herd size increased, however the relationship was not significant on exclusion of herd 18 (high incidence but very high number of no growth samples).

Discussion

Clinical mastitis is the most costly form of mastitis due to discarded milk, treatment and other costs (Halasa and others 2007). In order to develop an effective mastitis control strategy the incidence of mastitis should be known and the on-farm bacterial challenges identified and quantified. In a recent study Barrett et al (2005) examined the prevalence of pathogens associated with sub-clinical mastitis in Ireland, however the aetiological agents associated with clinical mastitis have not been quantified in over 30 years and no prospective study has looked at the pathogenic challenges over an entire lactation. In the present study we identified the bacterial challenges associated with clinical mastitis over a full lactation. The study herds were milk-recording herds predominantly located in the South of Ireland as the dairy industry is most heavily concentrated in this region. The herds were larger than the average milk-recording herd (~80 cows calving) however, they did not differ significantly from the national average in the most common udder health measure, SCC. This was shown by the mean BTSCC of the study herds (224,515 cells/ml), which was not significantly different from the estimated national mean BTSCC of all milk-recording herds (230,881 cells/ml).

That *S. aureus* was the most common pathogen overall is in agreement with previous reports (Barrett and others 2005; DAFM 2006-2009, 2010; Egan and O'Dowd 1982) but it was the most common pathogen in only 33% of herds. The high number of *S. aureus* isolates overall was due largely to the relatively high number of *S. aureus* positive samples from 3 herds (9, 11 and 30). Nevertheless the frequency of *S. aureus* isolation was substantially less than reported by Egan and O'Dowd in the early 1980s but was in line with that found by Barrett *et al* (2005). The environmental pathogen *S. uberis* was the second most commonly isolated pathogen overall and the most commonly isolated pathogen in 13 herds. This frequency of *S. uberis* isolation (17%) is in marked contrast to the early 1980s when aesculin-positive streptococci, such as *S. uberis*, accounted for only 2-3% of mastitis pathogens (Egan and O'Dowd 1982). It is also considerably higher than reported in the DAFM surveillance reports which found *S. uberis* in only 5-10% of submitted milk samples in each of the years 2005-2010 (DAFM 2006-2009, 2010). However, the *S. uberis* isolation frequency reported here agrees with that reported by Barrett *et al*, although their study included a higher number of split-calving herds which were more likely to have an *S. uberis*

infection (Barrett and others 2005). In the early 1980s a sizable *S. agalactiae* challenge existed, however, in this study only a single *S. agalactiae* isolate was found indicating that the prevalence of this pathogen has declined. This result supports the finding of Barrett *et al*, (2005) who also found *S. agalactiae* in only a single herd and the DAFM surveillance reports which also show a low frequency of *S. agalactiae* isolation.

In the present study no bacteria were recovered from 42% of samples and so the aetiological agent of infection could not be identified. It has previously been reported that at the quarter level, approximately 10-40% of milk samples from cases of clinical mastitis yield no growth (Lam and others 2009). The culture negative rate reported here is higher than in some previous studies (Garmo and others 2010; Hogan and others 1989; Sargeant and others 1998) although it is similar to the findings of a study examining the incidence rate of clinical mastitis on Canadian dairy farms (Olde Riekerink and others 2008). However, it can be difficult to compare culture negative rates between studies due to variability in sample processing, such as whether samples were frozen, duration of freezing, inclusion or not of cryopreservatives and whether samples were pre-incubated before plating. Milk samples for this study were frozen immediately and collected monthly. This was unlikely to have any major effect on the relative frequency of pathogen isolation as it has previously been demonstrated that freezing milk for up to 6 weeks has no effect on the viability of the major mastitis pathogens (Murdough and others 1996). Furthermore, freezing for up to 16 weeks had no effect on *S. aureus* and streptococci isolation rates although it did increase the number of CNS positive samples and decrease the number of *E. coli* positive samples (Schukken and others 1989). As our samples were submitted monthly it is considered that freezing had no effect on the relative frequency of the isolated mastitis pathogens.

In this study, the mean clinical mastitis incidence during lactation was 5.01 cases per 10,000 cow days at risk. This is lower than previous estimates of 42.4 cases per 100 cows and 54 cases per 100 cow years at risk (Kinsella and Austin 1990; More and others 2012). There are a number of possible reasons for this difference such as the fact the different methods were used to calculate clinical mastitis incidence in each study and that the high risk periods pre-calving and post-drying off were not included in the present study. Additionally, farmers in the present study may have failed to sample every case of clinical mastitis in their herds. Alternatively, as these farmers are within the top 10% of Irish dairy farmers they may have effective mastitis

prevention plans resulting in a low incidence of clinical mastitis. Two herds had very high incidences of clinical mastitis. For one of these herds, an extremely high number of culture negative samples were submitted indicating that clinical mastitis may have been misdiagnosed in this herd or intramammary inflammation may have been non-bacterial in nature. The second herd with a very high incidence of clinical mastitis had an expected number of culture negative samples. This herd was, however, the only autumn calving herd in the study.

The last study to examine pathogens associated with clinical mastitis in Ireland dates to the early 1980s (Egan and O'Dowd 1982). There have been substantial changes in dairy farming practices, mastitis awareness and control and the mean BTSCC since then (Berry and others 2006; Kinsella and Austin 1990; Oltenacu and Broom 2010). The pathogen profile in this study also differs from that in the 2005 to 2010 DAFM surveillance reports, in that we found a much lower frequency of *S. aureus* and coliform isolation and a higher frequency of *S. uberis* isolation. This may reflect the fact that the DAFM surveillance report is based on voluntarily submitted milk samples from both clinical and subclinical cases, the number of which peak in autumn when the majority of cows are in late lactation. This contrasts with our study, in which sample submission peaked in spring, and so the surveillance reports may not represent the pathogen profile throughout lactation. The pathogen profile reported here agrees quite closely with data from Barrett *et al* (2005) despite the fact that they sampled sub-clinical mastitis and included a higher proportion of split-calving herds, indicating that similar pathogens are causing clinical and sub-clinical mastitis. Traditionally mastitis prevention and control measures targeted those pathogens considered to have contagious transmission patterns, such as *S. agalactiae*, and *S. aureus*. Such programmes can lead to a reduction in the prevalence of these pathogens but can result in a shift in the relative pathogen frequency to environmental pathogens (Zadoks and Fitzpatrick 2009). Ireland has recently launched a national udder health programme, CellCheck. CellCheck has established best practice, and is building industry capacity to manage both contagious and environmental mastitis challenges. The findings from this study indicate the importance of such a holistic approach. In conclusion, while *S. aureus* remains the bacterium most commonly associated with mastitis in these Irish milk-recording herds, other pathogens, particularly the environmental pathogens *S. uberis* and *E. coli* also present a considerable challenge.

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Figure 1. Distribution by month of milk sample submission.

Figure 2. Frequency of isolation of the major mastitis pathogens from cases of clinical mastitis.

Table 1. Summary description of farms, and bacteriological profiling of clinical mastitis

Farm No.	No. of cows calving	Calving	Estimated annual Bulk tank SCC	No of samples	*Putative S. aureus* (%)	*S. uberis* (%)	Coliform (%)	CNS (%)	*S. agalactiae* (%)	Other Streptococci (%)	Other / Contaminated (%)	Negative (%)	Clinical Mastitis Incidence†	Clinical mastitis Incidence‡
1	255	Spring	293832	31	5[1] (16)	5[1] (16)	2 (6)	1 (3)	0 (0)	1 (3)	1 (3)	17 (55)	4.55	12.2
2	334	Spring	178278	44	10[1] (23)	11[1] (25)	3 (7)	3 (7)	0 (0)	0 (0)	2 (5)	16 (36)	4.29	13.2
3	231	Spring	175348	18	2 (11)	1 (6)	1 (6)	0 (0)	0 (0)	0 (0)	2 (11)	12 (67)	2.62	7.8
4	303	Spring	216808	22	3[2] (14)	6[2] (27)	3 (14)	0 (0)	0 (0)	0 (0)	1 (5)	11 (50)	2.90	7.3
5	86	Spring	153878	5	1 (20)	3 (60)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	2.27	5.8
6	77	Spring	302147	5	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (80)	1.95	6.5
7	251	Spring	263131	30	1 (3)	10 (33)	4 (13)	2 (7)	0 (0)	2 (7)	0 (0)	11 (37)	4.44	12.0
8	132	Spring	226287	16	3 (19)	1 (6)	2 (13)	0 (0)	0 (0)	1 (6)	0 (0)	9 (56)	4.60	12.1
9	217	Spring	288919	38	23[2] (61)	1[1] (3)	3 (8)	0 (0)	0 (0)	2[1] (5)	1 (3)	10 (26)	7.09	17.5
10	132	Spring	263823	15	6[2] (40)	7[2] (47)	0 (0)	0 (0)	0 (0)	1 (7)	0 (0)	3 (20)	4.02	11.4

11	91	Spring	284518	26	18 (69)	1 (4)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	6 (23)	10.96	28.6
12	115	Autumn	199937	73	14^{1} (19)	8^{1} (11)	15 (21)	2 (3)	0 (0)	3 (4)	5 (7)	27 (37)	22.71	63.5
13	132	Spring	263069	7	3 (43)	1 (14)	0 (0)	1 (14)	0 (0)	1 (14)	0 (0)	1 ((14)	0.85	5.3
14	235	Spring	233280	21	2^{1} (10)	3 (14)	2 (10)	2 (10)	0 (0)	4^{1} (19)	0 (0)	9 (43)	4.13	8.9
15	365	Spring	224502	9	1^{1} (11)	3^{1} (33)	0 (0)	1^{1} (11)	0 (0)	2^{1} (22)	0 (0)	4 (44)	1.39	2.5
16	118	Spring	226128	19	3 (16)	6^{1} (32)	0 (0)	2^{2} (11)	0 (0)	3^{1} (16)	0 (0)	7 (37)	5.80	16.1
17	575	Split	227582	58	10^{2} (17)	16^{2}(28)	7^{2}(12)	4^{2} (7)	1 (2)	4 (7)	5 (9)	15 (26)	3.45	10.1
18	60	Spring	267573	41	6^{1} (15)	2^{1} (5)	0 (0)	4^{1} (10)	0 (0)	2^{1} (5)	0 (0)	29 (71)	29.31	68.3
19	224	Spring	286573	4	1^{1} (25)	2^{1} (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	0.52	1.8
20	284	Spring	103630	5	1 (20)	0 (0)	2^{1} (40)	0 (0)	0 (0)	1^{1} (20)	0 (0)	2 (40)	0.65	1.8
21	186	Split	282565	13	0 (0)	3 (23)	1 (8)	1 (8)	0 (0)	0 (0)	2 (15)	6 (46)	2.50	7.0
22	450	Spring	234361	8	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	1 (13)	0 (0)	6 (75)	0.71	1.8
23	461	Spring	207747	7	3 (43)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (57)	0.53	1.5
24	200	Split	NA	28	4 (14)	2 (7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	22 (79)	3.74	14.0
25	121	Spring	197709	10	2 (20)	1 (10)	4 (40)	1 (10)	0 (0)	0 (0)	0 (0)	2 (20)	3.25	8.3

26	105	Spring	222288	17	1^{1} (6)	4^{2} (24)	2^{1} (12)	0 (0)	0 (0)	1 (6)	1 (6)	10 (59)	6.24	16.2
27	98	Spring	151188	9	3^{1} (33)	4^{2} (44)	1^{1} (11)	1 (11)	0 (0)	1 (11)	1 (11)	0 (0)	3.73	3.8
28	223	Spring	253499	17	5^{1} (29)	6^{1} (35)	0 (0)	2^{2} (12)	0 (0)	2^{2} (12)	0 (0)	5 (29)	3.08	7.6
29	109	Spring	201220	15	0 (0)	1 (7)	2 (13)	0 (0)	0 (0)	1 (7)	1 (7)	10 (67)	4.75	13.8
30	231	Spring	244443	19	12 (63)	1^{1} (5)	3^{1} (16)	0 (0)	0 (0)	0 (0)	0 (0)	4 (21)	3.38	8.2

NA = not available

†Incidence expressed as number of cases per 10,000 cow days at risk

‡Incidence expressed as number of cases per 100 cows per year

Number in superscript denotes number of times the pathogen was isolated in combination with a second species. As 2 species were isolated from some samples the total number of diagnoses is greater than the number of samples tested (>100%).

Table 2. Summary of bacterial isolates recovered from cases of clinical mastitis in Ireland.

Pathogen	No. of isolates	% of total samples*	% of culture positive samples	No of herds (%)
S. aureus	144	23	37.9	27 (90)
S. uberis	109	17	28.7	26 (87)
Coliforms	58	9	15.3	18 (60)
Other streptococci	35	6	9.2	19 (63)
CNS	27	4	7.1	14 (47)
Other	7	1	1.8	5 (17)
No growth/contaminated	280	44	-	30 (100)

* The number of diagnoses exceeds the total number of samples (>100%) as 2 pathogens were isolated from 30 samples.

Table 3. Clinical mastitis samples with more than one isolated pathogen.

Pathogens	**Number**
S. aureus/*S. uberis*	14
S. uberis/coliform	4
S. dysgalactiae/CNS	4
S. aureus/*S. dysgalactiae*	3
S. uberis/CNS	2
coliform/CNS	1
S. aureus/CNS	1
S. dysgalactiae/coliform	1

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