Field Observations on the Variation of *Streptococcus uberis* Populations in a Pasture-Based Dairy Farm

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ABSTRACT

Microbiological and molecular tools were used to monitor *Streptococcus uberis* populations on farm tracks and paddocks on a dairy farm during different seasons of a year to identify and profile potential environmental niches of *Strep. uberis* in a pasture-based dairying system. Farm tracks of high or low cow traffic were sampled every 2 wk for an entire year and *Strep. uberis* numbers were enumerated from a selective medium. During each season of the year, paddocks were sampled for the presence of *Strep. uberis* before and after grazing by dairy cows. Farm tracks of high cow traffic generally had greater concentrations of *Strep. uberis* isolated compared with tracks with less cow traffic, but there was also significant variation in the concentrations of *Strep. uberis* contamination among seasons, being highest in winter and lowest in summer. The bacterium was detected in paddocks only after cow grazing had occurred, but the bacteria could still be detected in soil for up to 2 wk following grazing in winter. Multilocus sequence typing showed great heterogeneity, with some commonality between farm track and milk isolates, which may help explain cow-to-environment or environment-to-cow transmission of the bacterium in the dairy setting.

Key words: *Streptococcus uberis*, environment, mastitis, multilocus sequence typing

INTRODUCTION

Environmental streptococci are the leading cause of clinical mastitis (CM) in pasture-based systems (McDougall, 2002). In general, environmental streptococcal IMI tend to be of short (Todhunter et al., 1995) or long duration (Zadoks et al., 2003; Pullinger et al.,

2007) and are of high prevalence in the dry and calving periods (Williamson et al., 1995). The prevalence of environmental mastitis, in particular *Streptococcus uberis*, is high in pasture-based seasonal-calving dairying countries such as New Zealand (McDougall, 2002). In these types of herds, the prevalence of contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* has decreased over the past 3 decades because of improvements in milking hygiene and increased use of dry cow therapy (McDougall, 1998). A number of reports have indicated that the relative importance, but not the absolute prevalence, of environmental mastitis increases as contagious mastitis is brought under control (Smith, 1983; Erskine et al., 1988).

Although the mechanisms by which *Strep. uberis* establish an IMI in the dry or lactating gland are unknown, bacteria must first evade the teat canal defenses to enter the mammary gland. Bramley et al. (1981) reported a high IMI rate when teat ends were contaminated with *Escherichia coli*. During lactation, pre- and postmilking disinfection of teats greatly reduces bacterial contamination, but during the dry period, when teat sanitation is not used, teat ends have increased contamination with environmental pathogens (Smith, 1983; Shearer and Harmon, 1993). An association was established between teat-end contamination during the dry period and heifer *Strep. uberis* IMI at calving (Lopez-Benavides et al., 2006). Cow factors such as age and mastitis history (Zadoks et al., 2001), udder edema, teat edema, and milk leakage (Waage et al., 2001) have been linked to an increased risk of mastitis postcalving. Microbial factors have also been suggested as important determinants of pathogenesis (Oliver et al., 1998; Almeida et al., 2006). Environmental factors may also play an important role in teat-end exposure to *Strep. uberis*. Lopez-Benavides et al. (2005) reported associations between solar radiation, soil and air temperatures, and soil moisture conditions and *Strep. uberis* contamination of farm tracks in different seasons. We

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hypothesized that greater concentrations of *Strep. uberis* in farm tracks in the colder months may provide a greater bacterial challenge to teat ends. We also speculated that the practice of spreading dairy effluent on pastures could play an important role in the transmission of *Strep. uberis* in the environment.

The recent development of a multilocus sequence typing (MLST) system for *Strep. uberis* permits objective, repeatable, and accurate typing of this bacterium (Coffey et al., 2006), and was designed to overcome the shortfalls of other molecular typing techniques. The MLST for bacteria was originally developed to provide a tool to investigate populations and population dynamics on a global scale; however, such a system is equally applicable to investigations at a herd level, where objective and accurate typing of isolates is required. Data from isolates obtained from cattle in New Zealand indicated that most sequence types (ST) identified were unique, although some ST in the environment were similar to those isolated from mammary glands (Pullinger et al., 2006). This may suggest that the presence of *Strep. uberis* in certain environmental niches may lead to teat-end colonization and subsequent IMI.

The aim of this study was to survey and quantify the concentrations of *Strep. uberis* in several ecological niches of a New Zealand dairy farm during different seasons of the year. Using a novel selective medium developed in our laboratory (Pullinger et al., 2006), coupled with accurate bacterial typing techniques, we were able to gain a better understanding of the spatial and temporal niches of *Strep. uberis* so that more effective management practices can be developed to control environmental populations and mitigate teat-end exposure.

MATERIALS AND METHODS

Farm Details and Management

The Dexcel Lye farm is located in Hamilton, New Zealand, and is divided into 93 paddocks of ryegrass and white clover, each with an area of 1 ha. One side of each paddock is adjacent to a 5-m-wide track, composed of a 400-mm base of compacted "rotten rock" (Greywacke-type hard rock) overlaid with 50 mm of compacted pit sand. For the purpose of this study, farm tracks that were close to the dairy and that received daily cow movement were designated "high traffic" (HT). Those that were not used frequently because of their distance and location were designated "low traffic" (LT). Up to 300 cows passed along the HT farm tracks as often as 4 times per day, whereas between 50 and 300 cows passed along the LT tracks on a less frequent basis, typically on a weekly or monthly basis.

A 30-bail rotary milking platform milked 340 cows during the study period (July 2003 until November

2004). Cows calved between July and September each year and were milked twice daily through until May. During the dry period (May to July 2004), cows remained on the farm and were brought to the dairy once a week for weighing. A group of 50 animals were milked twice daily over this dry period, thus sustaining some regular movement of cows along the HT and LT tracks. At milking there was minimal udder or teat cleaning, and cows were sprayed with an iodine-based teat sanitizer postmilking. A gland was diagnosed with CM when farm staff noticed abnormal changes in milk appearance in the foremilk. The foremilk of all cows was stripped and checked for abnormalities before each milking for the first 5 d after calving, then weekly during the following 2 mo, and then when udder swelling or hardness was observed. Each case of CM was recorded and a milk sample was collected aseptically for bacteriological analysis (National Mastitis Council, 1999). The presence of IMI was diagnosed from bacteriological analysis of foremilk samples collected aseptically from a group of 150 cows on 4 occasions during lactation, which were before the first milking after calving, at 3 and 5 mo after calving, and at dry-off. A gland was diagnosed with IMI when a pathogen was observed in any of these milk samples.

Cow Presence and Bacterial Counts on Farm Tracks

A total of 4 HT and 3 LT farm tracks were monitored for the presence of *Strep. uberis* between December 2003 and November 2004. Every 2 wk, each farm track was sampled at 3 permanent sampling points. A farm track, which ranged in length from 50 to 200 m, was divided into 3 parts of approximately equal length, and the midpoint of each length was used as a reference point for sampling. A sample was always collected from the center of the track, at this midpoint.

Tracks of HT were the main arterial tracks providing access to the farm dairy and sustained cow movement as often as 4 times per day. Generally, these tracks were characterized by their muddy appearance, especially in autumn and winter, because of wet weather and repeated cow fecal depositions. In contrast, LT tracks were generally located at the periphery of the farm, and track material was compact and dry, with little fecal deposition present. Weather parameters, including solar radiation, soil and air temperature, and relative humidity, were also recorded and are reported elsewhere (Lopez-Benavides et al., 2005). Cow presence was quantified for each track by the movement of at least 50 cows along the track during 1 or more of the 3 d immediately preceding sampling, for each of the 5 to 8 samplings per season. Tracks of HT type had cow traffic

for typically 50% or more of these days (28% in winter), whereas tracks of LT type had cow traffic on fewer than 50% of these days.

Samples were collected and analyzed as follows: approximately 10 g of surface material from an area of approximately 25 cm² was scooped into a sterile plastic vial and transported to the laboratory for processing. At the laboratory, 1 g of wet weight material from each farm track sample was added to 9 mL of 0.1% peptone diluent. Samples were mixed vigorously for 2 min, and a 100- μ L volume was spread onto a selective *Strep. uberis* medium (Pullinger et al., 2006). Plates were incubated at 37°C for 72 h, and *Strep. uberis* colonies were identified and counted (Pullinger et al., 2006). The *Strep. uberis* counts were expressed as colony forming units per gram of wet weight and were log₁₀ transformed for analysis.

Paddock Bacterial Counts After Grazing and Spreading of Effluent

Three paddocks, considered typical for the farm in terms of area, pasture type, and soil type, were used to evaluate the presence of *Strep. uberis* before and after grazing. During spring (November 2003), autumn (May 2004), and winter (August 2004), paddocks were sampled at 4 points in time: 1 d before grazing, and 1 d, 1 wk, and 2 wk after grazing. Cows typically grazed a paddock over a 3-d period. At each sampling, 9 samples each of grass, surface soil, and subsoil material were collected and analyzed for the presence of *Strep. uberis*. Sampling points were approximately 100 m from each other and covered the entire paddock.

A similar approach was used to evaluate the presence of *Strep. uberis* on paddock material following application of farm dairy effluent to paddocks, during the years 2004 to 2005 or 2005 to 2006. During spring (November 2004), autumn (March 2006), and winter (July and August 2006), 3 paddocks considered typical for the farm in terms of area, pasture type, and soil type were selected and dairy effluent was applied 3 d after grazing. Nine samples each of grass, surface soil, and subsoil material were collected before grazing, 1 d following grazing, and then 24 h, 48 h, and 1 to 3 wk after effluent was applied. Effluent was applied 2 or 3 d after cows grazed the paddock. Sampling points were 100 m from each other and covered the entire paddock. Samples were collected as follows: grass leaves were cut with sterile scissors from a 200-cm² area, leaving a 5-cm grass residual, and collected into a sterile plastic bag. To obtain the soil surface material, a 100-cm² area was cleared of grass, and soil was scooped (approximately 10 g) into a sterile plastic vial. Subsoil samples were collected in a similar manner, except that a 5-cm layer

of soil was first discarded before the sample was collected. All samples were analyzed for the presence of *Strep. uberis* according to the same procedures as described for farm track samples.

Genotyping of *Strep. uberis* Isolated from Farm Tracks and CM Cases

A total of 64 *Strep. uberis* isolates were randomly selected from media plates of farm track samples obtained over the summer, autumn, and winter seasons (December 2003 to August 2004). One colony was chosen from the plate and genotyped at 7 housekeeping genes by MLST, as described by Pullinger et al. (2006). Similarly, 58 isolates obtained from IMI (including CM cases) of 48 cows were randomly selected and genotyped by using the same methodology. Mastitis isolates were selected from cases that occurred between July 2003 and September 2004. The PCR protocols for MLST typing of *Strep. uberis* are described in the “MLST of *Streptococcus uberis*” home page (<http://pubmlst.org/suberis/info/protocol.shtml>). The allelic profile of an isolate was designated as an ST.

Data Analysis

The concentration of *Strep. uberis* (cfu/g) in farm track material was analyzed by using REML (Patterson and Thompson, 1971) to fit a model with season of the year and type of farm track (HT or LT) and their interactions as fixed effects, and individual farm track and the interaction between season of the year and farm track as random effects. Because *Strep. uberis* was not commonly detected in paddocks and bacterial concentrations varied according to time of the year and time of sampling relative to cow grazing, data are presented as raw means of *Strep. uberis* detected in paddock material for each season.

RESULTS

Contamination of Farm Tracks by *Strep. uberis*

Farm track contamination by *Strep. uberis* varied widely, depending on the season of the year ($P < 0.001$) and type of farm track ($P = 0.005$), with an interaction between season of the year and type of farm track ($P < 0.001$). Mean seasonal differences were $P < 0.001$ (HT) and $P = 0.051$ (LT). For the HT tracks, concentrations of *Strep. uberis* were generally similar in autumn, winter, and spring but were lowest in summer (Table 1), whereas for the LT tracks, concentrations of *Strep. uberis* were generally similar in summer, autumn, and spring but were greatest in winter.

Table 1. Average $\log_{10}$ *Streptococcus uberis* (cfu/g of wet weight $\pm$ SD) counts observed in farm tracks of high (HT) or low (LT) cow traffic in different seasons

Farm track	Summer 2003 to 2004 (Dec. to Feb.)	Autumn 2004 (Mar. to May)	Winter 2004 (Jun. to Aug.)	Spring 2004 (Sep. to Nov.)
HT				
HT-A	2.0 $\pm$ 1.4	4.2 $\pm$ 0.7	4.1 $\pm$ 0.3	3.4 $\pm$ 0.8
HT-B	1.7 $\pm$ 0.9	2.8 $\pm$ 1.7	3.3 $\pm$ 0.5	2.2 $\pm$ 0.9
HT-C	0.5 $\pm$ 0.6	2.2 $\pm$ 1.5	2.6 $\pm$ 0.8	1.7 $\pm$ 0.8
HT-D	0.7 $\pm$ 1.1	1.8 $\pm$ 1.5	1.6 $\pm$ 0.4	1.9 $\pm$ 0.5
Mean HT	1.2 $\pm$ 1.2	2.8 $\pm$ 1.6	2.9 $\pm$ 1.1	2.3 $\pm$ 1.0
LT				
LT-A	0.8 $\pm$ 1.1	0.6 $\pm$ 0.6	1.7 $\pm$ 1.7	0.1 $\pm$ 0.3
LT-B	0.5 $\pm$ 0.7	0.0 $\pm$ 0.0	2.9 $\pm$ 1.3	0.4 $\pm$ 0.7
LT-C	0.5 $\pm$ 0.6	1.0 $\pm$ 0.6	1.1 $\pm$ 1.3	0.6 $\pm$ 0.7
Mean LT	0.6 $\pm$ 0.8	0.5 $\pm$ 0.6	1.9 $\pm$ 1.5	0.4 $\pm$ 0.6

Overall, HT tracks had greater *Strep. uberis* concentrations than LT tracks across the seasons. On the HT tracks, *Strep. uberis* was found at concentrations greater than $\log_{10}$ 2.0 cfu/g in autumn, winter, and spring, whereas for the LT tracks, contamination close to $\log_{10}$ 2.0 cfu/g was observed only in winter (Table 1). Frequent cow traffic occurred along the HT tracks, because they were located in proximity to the farm dairy. The HT-A track received the most frequent passage of cows (Figure 1), with at least 50 cows passing along the track for 100% of the 3 d immediately preceding each sampling occasion of the season, and this track also had the greatest contamination by *Strep. uberis*, reaching concentrations greater than $\log_{10}$ 4.0 cfu/g in autumn and winter (Table 1). Track HT-B was further away from the dairy but still received frequent cow movement (range 50 to 90% of the days preceding sampling), and *Strep. uberis* was isolated on more than 80% of the sampling occasions (Figure 1). Track HT-C was also used frequently, and the isolation of *Strep. uberis* ranged from 40 to 100%. In summer, *Strep. uberis* on tracks HT-C and HT-D was detected on 40% of the sampling occasions, with concentrations below $\log_{10}$ 1.0 cfu/g, although cow presence was observed on more than 50% of the days preceding each sampling occasion.

On LT tracks, isolation of *Strep. uberis* was less frequent and average concentrations were usually below $\log_{10}$ 1.0 cfu/g, with the exception of winter, when concentrations were greater than $\log_{10}$ 1.7 cfu/g in LT-A and LT-B (Table 1; $P = 0.072$). For LT-B in autumn, *Strep. uberis* could not be detected on any of the 7 sampling occasions, although the highest LT value was observed in winter for LT-B ($\log_{10}$ 2.90 cfu/g), which was comparable to values observed in HT tracks in the same season.

Populations of *Strep. uberis* in Paddocks

Effect of Grazing. Cow presence and season were important factors for the detection of *Strep. uberis* in

paddocks. We observed that, in general, *Strep. uberis* could not be detected in paddocks when cows were not present in the previous 24 h (Table 2); however, the bacterium was detected the day after cows grazed a paddock and remained at detectable concentrations for up to 2 wk, depending on the season and sampling location. In summer, *Strep. uberis* was detected on the soil surface only after cows grazed the paddock, with concentrations below $\log_{10}$ 1.0 cfu/g. In autumn, the bacterium was detected in grass and soil surface material, but in concentrations below $\log_{10}$ 1.0 cfu/g. The greatest concentration of *Strep. uberis* was found in winter, and especially on soil surface material, where values greater than $\log_{10}$ 2.0 cfu/g were observed up to 2 wk postgrazing. Winter was the only season in which the bacterium could be detected in subsoil material at concentrations greater than $\log_{10}$ 1.0 cfu/g.

Effect of Dairy Effluent. Dairy effluent spread on paddocks contained *Strep. uberis* at concentrations that varied from $\log_{10}$ 2.2 cfu/mL in spring to $\log_{10}$ 4.4 cfu/mL in autumn, and $\log_{10}$ 3.0 cfu/mL in winter (Table 3). Typically, effluent was spread onto paddocks 3 d after grazing, and cows would not return again to graze the paddock for at least 21 d. In general, after 24 h of effluent application, *Strep. uberis* could be detected in grass, soil surface, and subsoil material. The exception was in spring, when it could be detected only in subsoil material. Further, in spring, *Strep. uberis* was detected only in the soil surface 48 h after effluent application, with concentrations greater than $\log_{10}$ 0.3 cfu/g. In autumn and winter, the greatest *Strep. uberis* concentrations were found in the soil surface after 24 h, and were greater than $\log_{10}$ 3.6 cfu/g. Progressively, this contamination decreased, but a detectable presence of *Strep. uberis* persisted for up to 2 wk after effluent application. *Streptococcus uberis* could not be detected at any time 3 wk after the initial effluent application.

Figure 1. Frequency of *Streptococcus uberis* isolation and cow presence in farm tracks of high (HT) or low (LT) cow traffic across seasons.

MLST of *Strep. uberis* Isolates

The *Strep. uberis* ST found in farm tracks or the milk of mastitic cows were highly heterogeneous, with 39 ST found among 64 farm track isolates and 40 ST found among 58 isolates obtained from 48 infected cows. Approximately 70% of the farm track ST were observed only once during the 1-yr monitoring period (Figure 2). Others such as ST-85 and ST-91 were isolated 5 times

from farm tracks of HT or LT (Table 4). Of the 39 ST isolated from farm tracks, 7 were also identified in cows with an IMI or CM (Tables 4 and 5). Sequence type-85 was isolated from 3 different tracks across 3 seasons and was also isolated from an IMI for cow 8217 (Table 5). Sequence type 91 was isolated 5 times on 4 different tracks during the year, and was also isolated from 3 IMI and 2 CM cases in 4 different cows (Table 5). In total, 40 different ST of *Strep. uberis* were responsible

Table 2. Average *Streptococcus uberis* levels ($\log_{10}$ cfu/g wet weight $\pm$ SD) found in different paddock components before and after grazing by dairy cows

Season	Sampling time (relative to grazing)	Grass (n ¹ = 9)	Soil surface (n = 9)	Subsoil (n = 9)
Summer	1 d before	ND ²	ND	ND
	1 d after	ND	0.35 $\pm$ 1.04	ND
	1 wk after	ND	ND	ND
	2 wk after	ND	ND	ND
Autumn	1 d before	ND	ND	ND
	1 d after	0.60 $\pm$ 1.19	0.53 $\pm$ 1.06	ND
	1 wk after	0.22 $\pm$ 0.67	ND	ND
	2 wk after	ND	ND	ND
Winter	1 d before	ND	ND	ND
	1 d after	1.45 $\pm$ 1.44	2.18 $\pm$ 1.30	1.01 $\pm$ 1.22
	1 wk after	ND	2.82 $\pm$ 1.29	ND
	2 wk after	ND	2.18 $\pm$ 1.73	1.61 $\pm$ 1.31

¹n = number of replicates per sampling.

²ND = not detected.

Table 3. Average *Streptococcus uberis* levels ($\log_{10}$ cfu/g of wet weight $\pm$ SD) found in different paddock components before and after effluent spreading on recently grazed paddocks

Season	Sampling time	Grass (n ¹ = 9)	Soil surface (n = 9)	Subsoil (n = 9)
Spring	1 d before grazing	ND ²	ND	ND
	1 d after grazing	2.5 $\pm$ 1.4	2.2 $\pm$ 1.8	1.3 $\pm$ 1.6
	24 h after effluent	ND	ND	0.3 $\pm$ 0.8
	48 h after effluent	ND	0.3 $\pm$ 0.8	ND
	1 wk after effluent	ND	0.8 $\pm$ 1.1	ND
	2 wk after effluent	ND	1.0 $\pm$ 1.2	ND
	3 wk after effluent	ND	ND	ND
Autumn	1 d before grazing	ND	ND	ND
	1 d after grazing	0.6 $\pm$ 1.2	ND	ND
	24 h after effluent	1.4 $\pm$ 1.5	4.0 $\pm$ 0.5	1.9 $\pm$ 1.4
	48 h after effluent	0.5 $\pm$ 1.0	3.7 $\pm$ 0.6	2.0 $\pm$ 1.3
	1 wk after effluent	ND	ND	ND
	2 wk after effluent	ND	ND	ND
	3 wk after effluent	ND	ND	ND
Winter	1 d before grazing	ND	ND	ND
	1 d after grazing	0.7 $\pm$ 1.4	2.0 $\pm$ 1.6	ND
	24 h after effluent	0.2 $\pm$ 0.7	3.7 $\pm$ 0.4	1.6 $\pm$ 1.5
	48 h after effluent	ND	2.2 $\pm$ 1.3	0.6 $\pm$ 1.1
	1 wk after effluent	ND	1.3 $\pm$ 1.6	ND
	2 wk after effluent	ND	0.6 $\pm$ 1.2	ND
	3 wk after effluent	ND	ND	ND

¹n = number of replicates per sampling.²ND = not detected.

for 38 cases of IMI and 20 cases of CM. Seven of these ST were common to IMI and CM cases (Table 4), and for 5 of these ST, the cow that developed an IMI also developed CM.

DISCUSSION

The prevalence of *Strep. uberis* was monitored in various dairy farm locations for a period of 1 yr. Bacterial

Figure 2. Frequency of isolation of sequence types found in farm track material over a period of one year.

Table 4. Number of *Streptococcus uberis* sequence type (ST) isolates obtained from cases of IMI and clinical mastitis (CM), including those common to isolates from farm track material

ST	Cases (n)		Farm track isolates (n)	Total isolates (n)
	IMI	CM		
80		1		1
81	1			1
82	1			1
83	1			1
84	1			1
85	1		5	6
89		1		1
90	1	1		2
91	2	3	5	10
94	3	3		6
104	2			2
105	1		3	4
106	1			1
107	1		2	3
126		1		1
127		1		1
129	1		1	2
130	1			1
131	1			1
132	1			1
133	1			1
134	1			1
135	1	1		2
136	1			1
137	1	2		3
138	1			1
139		1		1
140	1			1
141	1			1
142	1	1		2
143	1			1
144	1			1
145	1	1	1	3
146	1			1
147		1		1
159	1		1	2
160	1			1
205		1		1
207		1		1
215	3			3
Total isolates	38	20	18	76

concentrations were generally greater in the colder months, which may be positively associated with bacterial survival at cooler temperatures and with management factors occurring during these times, such as grazing intensity, which generates longer pasture rotation lengths during the autumn and winter months (Macdonald and Penno, 1998). Climatic variables may also play an important role in helping the bacterium to survive in the dairy environment. Lopez-Benavides et al. (2005) found a positive correlation between bacterial concentrations in farm tracks and relative humidity. Significant negative associations of farm track contamination with soil and air temperature, and solar radiation were also reported. Lesser temperatures and solar radiation are typical of colder months, when cows are

not lactating or nearing parturition. In New Zealand, the majority of dairy cows calve during late winter and early spring (Verkerk, 2003), which also coincides with the greatest incidence of *Strep. uberis* mastitis (Williamson et al., 1995; Pankey et al., 1996; McDougall, 2003).

The ability of *Strep. uberis* to survive in the environment may be enhanced by the presence of a hyaluronic acid capsule (Oliver et al., 1998), because it would serve as a means of protection from desiccation and as maintenance of a humid environment in close proximity to the bacterial cell. An analysis of isolates of *Strep. uberis* from several countries showed that the presence of genes responsible for the production of a hyaluronic acid capsule correlated with isolation of *Strep. uberis* from the mammary gland of cattle (Field et al., 2003; Coffey et al., 2006; Pullinger et al., 2006). Analysis of New Zealand isolates showed that 82% (59/72) of farm track isolates carried the *hasA* (hyaluronate synthase) gene (Pullinger et al., 2006), which is important for expression of the hyaluronic acid capsule. The role of the capsule may lie in processes that favor its ability to challenge or contaminate the teat end.

Cow presence and season of the year were important factors associated with *Strep. uberis* found in the environment. Those farm tracks that were closest to the dairy had the greatest bacterial concentrations, probably because of continuous inoculation of *Strep. uberis* through cows shedding the bacterium in the feces (Zadoks et al., 2005) or leakage of milk from infected udders. The MLST genotyping of farm tracks and milk *Strep. uberis* isolates showed a highly heterogeneous population of bacteria in the environment and in milk. From the environment, a few strains isolated on repeated occasions were also the cause of mastitis in cows. Pullinger et al. (2007) suggested that the outcome of an IMI, either CM or cure, is more dependent on host factors than on interstrain differences. Because multiple strains in the environment are capable of potentially causing IMI, it is likely that a *Strep. uberis* infection is the result of increased teat-end exposure (Lopez-Benavides et al., 2006) and a breach in the physical barriers of the teat (Seinhorst et al., 1991; Capuco et al., 1992). It should be noted that in this study, not all isolates from a media plate were typed for MLST. A recent study suggested that genotyping of at least 20 isolates from a media plate was necessary to account for 95% of ribotype variability in a soil sample (Dopfer et al., 2005). This is an area of research that needs more clarification to estimate the association between the presence of environmental isolates and occurrence of IMI in dairy cows.

Dairy effluent is commonly spread onto paddocks as a means of disposing of cow manure as well as providing

Table 5. Associations between IMI or clinical mastitis (CM) milk isolates and farm track isolates

ST	Total isolates (n)	Source	Jul. to Aug. 2003 (winter)	Sep. to Nov. 2003 (spring)	Dec. 2003 to Feb. 2004 (summer)	Mar. to May 2004 (autumn)	Jun. to Aug. 2004 (winter)
85	6	Cow ID Track	8217 (IMI)		HT-A	HT-A HT-D	HT-A HT-B
91	10	Cow ID Track	9673 (IMI) 1024 (IMI/CM)	1033 (CM) 8777 (IMI)		LT-C	HT-A HT-C LT-A LT-B
105	4	Cow ID Track		6906 (IMI)	HT-B LT-B LT-C		
107	3	Cow ID Track		9682 (IMI)	HT-B HT-D		
129	2	Cow ID Track	461 (IMI)				HT-C
145	3	Cow ID Track	8705 (IMI)	1026 (CM)	HT-A		
159	2	Cow ID Track		1045 (IMI)		HT-B	
Total	30						

nutrients that are necessary for the production of plant tissue. Manure is particularly valued for its nitrogen content, but its OM content is important for water retention and for reducing leaching of organically bound nutrients (Van Horn et al., 1994). In New Zealand, the majority of dairy cow waste is slurry-based and not solid (Saggard et al., 2004). It is generally thought that the spread of this waste onto land is the least environmentally damaging disposal method, but problems such as odor and nitrate leaching into watercourses are of concern (Saggard et al., 2004; Hutchison et al., 2005). Further, the spread of zoonotic agents such as *E. coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella* spp., and *Cryptosporidium parvum* are of particular concern (Hutchison et al., 2005). In this study, the persistence of *Strep. uberis* in paddocks spread with liquid effluent was of short duration in autumn, but was detectable for up to 2 wk in spring and in winter; however, by the third week after the initial application, *Strep. uberis* was not detected in either grass, soil surface, or subsoil material. The minimum rotation length for the paddocks in this study was 3 wk. If cow contamination by *Strep. uberis* had been attributed to the ingestion of grass contaminated with the bacterium or by direct contact of the teats with *Strep. uberis*-contaminated soil, it could be concluded that the spread of effluent onto land was probably not a major risk factor for mastitis; however, cow presence in the paddocks was an important factor for bacterial survival in paddocks.

We observed that prior to grazing, all paddocks had undetectable concentrations of *Strep. uberis* but that *Strep. uberis* could be detected on paddock material for up to 2 wk after grazing. The greatest bacterial concentrations were found in winter, which coincides with a greater stocking rate, longer rotation lengths (Macdonald and Penno, 1998), and environmental conditions (Lopez-Benavides et al., 2005) that may benefit the growth and survival of the bacterium. In winter, contamination was very high because *Strep. uberis* was also detected in the subsoil, which was not commonly observed in other seasons. The specific mechanism by which cattle contaminate pasture with *Strep. uberis* is unknown but could involve defecating material containing *Strep. uberis*, because as many as 10% of cows are known to excrete live cells of *Strep. uberis* (Lacy-Hulbert et al., 2005). Transfer of contaminated farm track material into the paddock may also occur via the hooves and legs, and this study has shown this material to be consistently contaminated with *Strep. uberis*.

The current study shows the degree of variability of *Strep. uberis* isolated from diverse ecological niches in the dairy environment, and the association that exists between cow presence and seasons. We found that multiple *Strep. uberis* ST could be isolated from the environment, as well as from infected cows. This study provides insight into the potential environmental niches that should be studied in more detail for the design of targeted management practices that aim to reduce teat-end exposure to the bacterium.

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