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Associations between *Streptococcus uberis* populations on farm races and climatic changes during a twelve-month period

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ABSTRACT

Streptococcus uberis is an important mastitis-causing pathogen in cows managed under pastoral conditions. The influence of environmental conditions on *S. uberis* ecology in dairy farms is unknown. To evaluate associations between climatic parameters and population levels of the bacterium on the farm, farm races were monitored for *S. uberis* levels on a fortnightly basis from November 2003 to November 2004 at the Dexcel Lye research farm. Higher *S. uberis* contamination was observed in races where cow traffic was frequent and lower in areas where usage was minimal. Highest *S. uberis* contamination occurred in the month of August (630 cfu/g) and lowest in January (2 cfu/g). Significant associations were observed between *S. uberis* levels and relative humidity (r = 0.6), soil temperature at a depth of 10 cm (r = -0.8), solar radiation (r = -0.7) and maximum air temperature (r = -0.7). Around 70% of *S. uberis* clinical mastitis cases occurred in the non-lactating and calving periods, when *S. uberis* levels in races were above 100 cfu/g and solar radiation levels were low. It is suggested that weather conditions have a significant impact on the survival of *S. uberis* in the dairy farm environment.

Keywords: mastitis; *Streptococcus uberis*; environment; weather.

INTRODUCTION

Streptococcus uberis is an environmental Gram positive bacterium that causes around 70% of clinical mastitis (CM) cases in dairy farms in New Zealand (Williamson *et al.*, 1995; Pankey *et al.*, 1996; McDougall, 1998). This bacterium has been isolated from many sites around the bovine dairy environment and the cow itself (Cullen, 1966; Cullen *et al.*, 1969; Sharma & Packer, 1970; Bramley *et al.,* 1979; Bramley, 1982; Kruze & Bramley, 1982). However, little information exists on the population levels of the bacterium in the farm and its associations with climatic conditions.

The ecology of *S. uberis* in the dairy environment has not been elucidated to a great extent, but work reported in Britain by Cullen (1966) highlighted the presence of *S. uberis* in different parts of the body and possible associations with weather and cow events. Studies in the United States of America by Sharma and Packer (1970) reported a recovery of *S. uberis* in increased numbers from milk and skin of cows during the winter months.

Cullen (1966) suggested that weather and stage of lactation are important determinants of *S. uberis* populations. The isolation of *S. uberis* from the lips (Cullen, 1966), rumen and soil (Cullen & Little, 1969), rectum and vulva (Bramley *et al.*, 1979), faeces (Bramley, 1982) and bedding material (Bramley *et al.*, 1979) suggest that this bacterium can survive for certain periods of time in different cow body sites and the environment. Furthermore, Cullen (1966) proposed that the lips were the primary reservoir of the bacterium and that the isolation in rectal swabs reflected the occasional passage of the bacterium through the digestive system. Later studies showed that *S. uberis* was only found in wet and muddy soil where cows congregated (Cullen & Little, 1969).

Previous findings show that *S. uberis* is associated with cow presence in the dairy environment. We have been investigating *S. uberis* in several locations in the pastoral dairying environment, and have consistently isolated the bacterium from farm race material. This information provided us with sufficient population data to determine associations with climatic parameters, which then enabled us to explore interactions between *S. uberis* in the environment, climatic events and incidence of clinical mastitis.

MATERIALS AND METHODS

Raceways selection and sampling

Six farm races of the Dexcel Lye research farm were selected for the study. Selection was on the basis of cow traffic, with three chosen from areas of high traffic and three of low traffic. Three permanent sampling points were selected and identified per race. On a fortnightly basis, a sterile plastic vial was used to scoop (approx. 10 g) surface material from the centre of each race sampling point. Samples of race material were collected individually and analysed quantitatively for the presence of *S. uberis*. Samples were processed within one hour after collection. Race sampling started in mid November 2003 and finished in mid November 2004.

Raceways were 5 m wide and their construction was as follows. After a layer of top soil was removed, a layer of 'rotten' rock was added and compacted down to 400 mm. This was followed by a layer of pit sand that was compacted to 50 mm.

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Bacteriology of race material

From each race sample, 1 g of wet weight race material was added to 9 ml of 0.1% peptone diluent. Samples were mixed vigorously for 2 minutes and 100 µl was spread plated onto a selective *S. uberis* medium (Pullinger *et al.* 2005 in preparation). Plates were incubated at 37^{o}C for 72 h and *S. uberis* colonies were identified on the basis of colony morphology, esculin reaction, inulin fermentation and β-glucuronidase activity (Pullinger *et al.* 2005 in preparation). Colony forming units were expressed as cfu/g wet weight and $\log_{10}$ transformed for analysis.

Clinical mastitis records

Clinical mastitis (CM) cases that occurred in the Dexcel Lye Farm research herd were analysed in the Mastitis Research Laboratory, and cases caused by *S. uberis* were recorded and used in the analysis. During the study period, approximately 340 cows were milked off a 90 ha area. Planned start of calving was programmed for July 7, 2004. All cows, except a group of 50 that were milked through the winter period, received dry cow therapy in late April or early May 2004.

Data analysis

Each fortnight, log *S. uberis* cfu from all races were pooled and averaged to generate a whole farm value. Correlations were made between log *S. uberis* cfu and each climate variable that had been averaged over *x* consecutive days prior to sampling. The duration of *x* varied from one day to a maximum of ten days prior to sampling. The climate variables analysed included: relative humidity (%), maximum air temperature (^{o}C), earth temperature (^{o}C at a depth of 10 cm), solar radiation (MJ/m^2), soil moisture (%) and rainfall (mm), that were obtained from the Ruakura Weather Station, located about 5 km from the race sampling points. Statistical analysis was conducted using STATISTICA 6.1 (StatSoft, Inc., Tulsa, OK, USA) and means are expressed with their respective standard error (SEM).

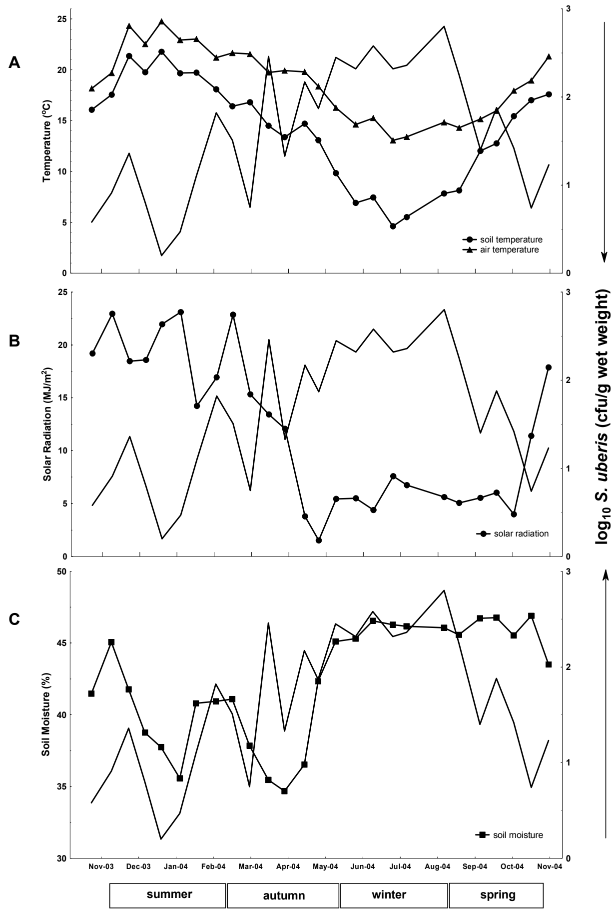
RESULTS

Climate and *S. uberis* associations

The highest correlations ($P < 0.05$) between farm race, log *S. uberis* and climate records were observed when individual climate variables were averaged from one until six days prior to sampling. The resulting correlations were 0.6 (relative humidity), -0.7 (maximum air temperature), -0.8 (soil temperature), -0.7 (solar radiation) and 0.4 (soil moisture). Rainfall was not significantly correlated with *S. uberis* levels.

Average relative air humidity was above 70% for all sampling periods. Air temperatures above 21^{o}C were observed in the summer months, while they were below 16^{o}C in the winter months. The soil temperature during the winter months only reached a maximum of 8^{o}C (Figure 1A). Solar radiation levels were below 8 MJ/m^2 during the months of May to October, when the majority of fortnightly *S. uberis* levels were above 100 cfu/g (Figure 1B). Finally, soil moisture levels were above 45% in the winter months, which coincided with *S. uberis* levels above 100 cfu/g (Figure 1C).

FIGURE 1: Fortnightly mean log *S. uberis* cfu/g (solid line) isolated from race material and climatic variables (averaged six days prior to sampling) recorded from a local weather station.

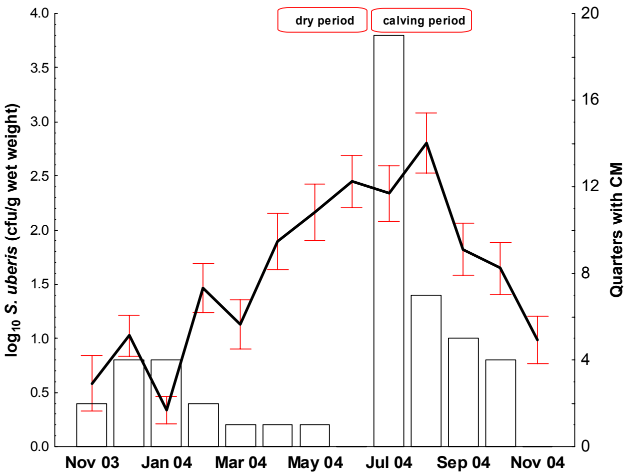


Race *S. uberis* populations and clinical *S. uberis* mastitis

Fortnightly samplings were pooled across high and low cow traffic races to obtain a whole farm value. Nevertheless, races associated with high cow traffic consistently yielded high numbers of *S. uberis* relative to the low traffic races, where only sporadic *S. uberis* isolations were observed. The average number of *S. uberis* isolated from race material was above 100 cfu/g 2.0 in the months of May to August, which coincides with the non-lactating and calving periods. Lower populations of *S. uberis* were observed in the months of November to January, which coincides with the mid lactation period (Figure 2).

Around 70% of CM caused by *S. uberis* occurred in the non-lactating and calving periods, corresponding to the months of May to September 2004. The majority of CM cases were observed in the month of July 2004 (19 cases), when most of the animals calved. The CM case observed in May was from the group of cows that were milked through winter. In summary, *S. uberis* contamination levels (cfu/g) in raceways were 10 ± 1 (summer), 63 ± 2 (autumn), 316 ± 2 (winter) and 32 ± 1 (spring).

FIGURE 2: Monthly mean log *S. uberis* cfu (± SEM) isolated from race material (solid line) and number of clinical mastitis cases diagnosed with *S. uberis* (white bar).



DISCUSSION

This study followed the population levels of *S. uberis* in the Dexcel Lye research farm races for a period of 12 months, and compared with climatic data collected locally. Using a selective medium, *S. uberis* was successfully isolated from race material.

Little information exists on the relationship between *S. uberis* and environmental factors in pastoral dairying, and particularly its impact on the incidence of new *S. uberis* intramammary infections. Since *S. uberis* was continuously isolated from farm races with high cow traffic, it is assumed that faecal matter and urine are the main sources of bacterial contamination. Contamination of bedding material with *S. uberis* and its association with faecal matter and urine has been reported (Bramley *et al.*, 1979; Bramley, 1982; Hogan *et al.*, 1989; Ward *et al.*, 2002).

Variation in the levels of *S. uberis* contamination in race material was associated with changes in soil temperature, soil moisture, solar radiation, relative humidity and air temperature. Higher race *S. uberis* numbers were observed when air and soil temperatures were low, soil moisture was high and solar radiation levels were low. In general, longevity of bacteria is enhanced when stored at low temperatures. For example, survival studies of *Enterococcus faecalis* under varying soil types and soil moisture conditions concluded that bacteria survived longer at lower temperatures and under moist conditions (Kibbey *et al.*, 1978). However, the authors also noted that the longevity of *E. faecalis* in moist and cool soil conditions might be due to the lack of antagonism (predation and competition) in the soil microflora.

Solar radiation levels of 13 MJ/m^2 reduced populations of *Escherichia coli* and *Enterococcus faecium* in estuarine water (McCambridge & McMeekin, 1981). In this study, when solar radiation levels were below 10 MJ/m^2, *S. uberis* values above 100 cfu/g were observed. Nevertheless, these times also coincided with low soil temperatures and high soil moisture.

Streptococcus uberis is the most common pathogen isolated from CM cases in the dry period and at calving (Williamson *et al.*, 1995; Pankey *et al.*, 1996; McDougall, 1998). During this study, the majority of CM caused by *S. uberis* was highest in the month of July 2004, when the majority of calving occurred. Also, the calving period was associated with high soil moisture, low soil and air temperatures, and low solar radiation levels. Although this study does not establish a cause and effect relationship between mastitis susceptibility and climate, it provides preliminary evidence of an association between certain climatic parameters, contamination of farm races with *S. uberis* and CM caused by this pathogen. Thus, higher environmental bacterial numbers during certain periods of the year allow a greater exposure of the skin of cows to *S. uberis*, thereby increasing its chances of infecting the mammary gland.

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