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Prevalence, seasonality, and growth of enterococci in raw and pasteurized milk in Victoria, Australia

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ABSTRACT

This study investigated the prevalence, seasonality, and species variety of enterococci present in raw milk factory silos and pasteurized milk in 3 dairying regions in Victoria, Australia, over a 1-yr period. Additionally, the growth ability of thermoduric enterococci isolated in this study (*Enterococcus faecalis*, *E. faecium*, *E. hirae*, and *E. durans*) was determined in milk at temperatures likely to occur during storage, transport, and distribution, and before domestic consumption (4 and 7°C). Enterococci were detected in 96% of 211 raw milk samples, with an average count of 2.48 $\log_{10}$ cfu/mL. Counts were significantly lower in winter than summer (average 1.84 $\log_{10}$ cfu/mL) and were different between factories but not regions. *Enterococcus faecalis* was the most prevalent species isolated from raw milk in every factory, comprising between 61.5 and 83.5% of enterococcal species across each season. Enterococci were detected in lower numbers in pasteurized milk than in raw milk and were below the limit of detection on spread plates (<10 cfu/mL) after factory pasteurization. Residual viable cells were only detected following enrichment using 100-mL samples of milk, with 20.8% of the samples testing positive; this equated to a decrease in the average raw milk enterococci count of >4 $\log_{10}$ cfu/mL following pasteurization. Although *E. faecalis* predominated in raw milk and *E. durans* was found in only 2.9% of raw milk samples, *E. durans* was the most prevalent species detected in pasteurized milk. The detection of enterococci in the pasteurized milk did not correlate with higher enterococci counts in the raw milk. This suggested that the main enterococci populations in raw milk were heat-sensitive and that thermoduric enterococci survived pasteurization in a small numbers of instances. All of the thermoduric enterococci that were assessed for growth at likely refrigeration temperatures were able to grow at both 4 and 7°C in sterile milk, with generation times of 35 to 41 h and 16 to 22 h, respectively. Thermoduric enterococci were detected in pasteurized milk stored at 4°C for 2 wk (typically 1 to 9 cells/100 mL, up to 2.82 $\log_{10}$ cfu/mL), demonstrating the potential of enterococci to survive pasteurization and contribute to milk spoilage at refrigeration temperatures. This is particularly relevant for milk that is aseptically packaged to exclude gram-negative psychrotrophic bacteria and kept above the recommended storage temperature of ≤5°C.
Key words: *Enterococcus*, raw and pasteurized milk, dairy, season, growth

INTRODUCTION

Enterococci are widespread throughout the environment (Giraffa, 2002), are inhabitants of mammalian intestines (Stiles, 1989), and have been isolated from plants and soil (Franz et al., 1999). Enterococci have been isolated from dairy cow feces (Batish and Ranganathan, 1984; Gelsomino et al., 2001) but not always from every cow tested (Kagkli et al., 2007). The survival of enterococci in feces has been shown to be longer than that of other fecal indicators (Sinton et al., 2007). Enterococci, along with *Escherichia coli*, are used as indicators of fecal contamination in watercourses (Hampson et al., 2010), as well as in food (Stiles, 1989), and can serve as indicators of process hygiene and food and drinking water quality (Halkman and Halkman, 2014). Although enterococci are recognized as conferring beneficial properties to fermented milk products, if they are present in dairy foods after manufacture, they may pose food safety risks as potential pathogens and reservoirs of antibiotic resistance and contribute to food spoilage (Giraffa et al., 1997; Ogier and Serror, 2008; Franz et al., 2011). Consequently, the importance of identifying sources of enterococcal contamination in milk supplies and risk factors for and persistence in manufactured products has received increasing interest (for example, see Buhnik-Rosenblau et al., 2013; Hammad et al., 2015).

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Enterococci can enter milk from human or animal feces, water sources, the farm environment, or from milking equipment, bulk storage tanks, and equipment used during milk harvesting or local processing (Gelsomino et al., 2001, 2002). The occurrence of enterococci in raw bovine milk has been explored in several countries, where *Enterococcus faecalis* was the predominant species found in studies from India, the Czech Republic, and Turkey (Batish and Ranganathan, 1984; Schlegelova et al., 2002; Citak et al., 2005), whereas *Enterococcus faecium* was the main raw milk species in a South African study (Wessels et al., 1988). In contrast, an Irish farmhouse study found that *Enterococcus casseliflavus* and *Enterococcus faecalis* were the most frequently isolated species in raw milk from a small herd of 27 cows (Gelsomino et al., 2001). These species were also found in human feces, cheese made from the raw milk, tap water, and milking equipment, whereas *E. faecium* dominated cow feces when isolated at all. Gelsomino et al. (2002) concluded that milking equipment was the probable source of milk contamination, where *E. casseliflavus* persisted in the machine and bulk tank on the one farm examined. The seasonality of occurrence of enterococci has not been investigated in milk and dairy products; however, a study has looked at the seasonality of bovine fecal indicator organisms, including enterococci, on New Zealand farms, over 1 yr (Sinton et al., 2007). That study found that although presumptive enterococci counts in feces were variable, the highest counts occurred in spring (Sinton et al., 2007), the peak milk harvesting season.

Although numerous studies have identified the presence of enterococci in raw and pasteurized bovine milk, no longitudinal studies on the ecology of enterococci in bovine milk have examined the seasonality of occurrence and changes of species over time. Seasonality is of particular interest to the dairy industry in Australia, as dairy production occurs in regions that vary significantly in maximum daily temperatures, climatic conditions (Australian Government, 2015), and nature of water supply. Victorian production of cow's milk is 66% of national production (9.5 million tonnes), representing ~1.5% of world production (AHDB, 2015), and occurs in 3 major dairying regions to the north, east, and west of the capital city, Melbourne (Victorian Government, 2014). The northern (north of Melbourne) and southern (East and west of Melbourne) regions experience significant differences in monthly maximum and minimum temperatures (Supplemental Figures S1 and S2; http://dx.doi.org/10.3168/jds.2015-9335) and water supply varies from rain-fed only, irrigation, and ground water supplies (Victorian Government, 2014). Given that several factors may influence the nature of the microflora in raw milk (e.g., region, climate, water supply, transport distance to manufacturing plants, and temperature of transport), a survey was undertaken to determine whether regionality, season of milk harvest (sampling over 1 yr), and location of manufacturing plants influenced the species type and prevalence of enterococci in raw milk as delivered to bulk holding facilities in manufacturing plants; to our knowledge, this is the first survey of this type in Australia. In addition, pasteurized milk from one manufacturing plant was tested over 1 yr immediately after manufacture, to determine the survival rate of enterococci after factory pasteurization (72°C, 15 s), and again after 2 wk of storage of milk at 4°C, to ascertain whether very low numbers of survivors could grow over a typical shelf-life period, and in line with the generation times measured for known thermoduric enterococci, to contribute to spoilage.

MATERIALS AND METHODS

Sample Acquisition

Raw bovine milk was sampled 12 times at approximately monthly intervals from 3 different bulk raw milk silos at each of 6 dairy factories in Victoria, Australia (n = 211). Dairy factories were located in Victoria's 3 dairying regions, east (factories A and B), north (factories C and D), and west (factories E and F) of Melbourne, the capital of Victoria. All of the factories were within a 200-km radius of Melbourne and the distance between the centers of the regions was approximately 250 km. Samples were assigned to a season based on the month they were collected (summer: December, January, February; autumn: March, April, May; winter: June, July, August; spring: September, October, November). Milk was not collected from factory C in July (winter) and only 2 milk samples were collected from factory A in December (summer) and from factory C in February (summer). Factory-pasteurized milk (72°C, 15 s) from 2 processing lines at factory B was sampled monthly over a 12-mo period (n = 24). Milk samples were placed into insulated containers with either ice packs or crushed ice, transported to the CSIRO Melbourne laboratory by overnight courier and stored at <4°C. All testing was performed on the day of sample arrival.

Enumeration of Enterococci in Raw Milk

Chromocult Enterococci broth (**CEB**; Merck, Darmstadt, Germany), prepared as single-strength (**1×CEB**) or double-strength (**2×CEB**), and CEB with 15 g/L of Bacteriological Agar No. 1 (Oxoid, Basingstoke, UK; **CEBA**) were used in the detection of enterococci in the

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