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Prevalence and characterization of foodborne pathogens from Australian dairy farm environments

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ABSTRACT

The ability of foodborne pathogens to gain entry into food supply systems remains an ongoing concern. In dairy products, raw milk acts as a major vehicle for this transfer; however, the sources of pathogenic bacteria that contaminate raw milk are often not clear, and environmental sources of contamination or the animals themselves may contribute to the transfer. This survey examined the occurrence of 9 foodborne pathogens in raw milk and environments of 7 dairy farms (3 bovine, 3 caprine, and 1 ovine farm) in summer and autumn, in Victoria, Australia. A total of 120 samples were taken from sampling points common to dairy farms, including pasture, soil, feed, water sources, animal feces, raw milk, and milk filters. The prevalence of the *Bacillus cereus* group, *Campylobacter*, *Clostridium perfringens*, *Cronobacter*, Shiga-toxigenic *Escherichia coli*, *Listeria*, *Salmonella*, coagulase-positive staphylococci (CPS), and *Yersinia enterocolitica* across the farms was investigated. The 2 most prevalent bacteria, which were detected on all farms, were the *B. cereus* group, isolated from 41% of samples, followed by *Cl. perfringens*, which was isolated from 38% of samples. The highest occurrence of any pathogen was the *B. cereus* group in soil, present in 93% of samples tested. Fecal samples showed the highest diversity of pathogens, containing 7 of the 9 pathogens tested. *Salmonella* was isolated from 1 bovine farm, although it was found in multiple samples on both visits. Out of the 14 occurrences where any pathogen was detected in milk filters, only 5 (36%) of the corresponding raw milk samples collected at the same time were positive for the same pathogen. All of the CPS were *Staphylococcus aureus*, and were found in raw milk or milk filter samples from 6 of the 7 farms, but not in other sample types. Pathogenic *Listeria* species were detected on 3 of the 7 farms, and included 4 *L. ivanovii*-positive samples, and 1 *L. monocytogenes*-positive water sample. Shiga-toxigenic *Escherichia coli* were identified in fecal samples from 3 of the 7 farms and in a single raw milk sample. *Cronobacter* species were identified on 4 of the 7 farms, predominantly in feed samples. No *Y. enterocolitica* was detected. Results of this study demonstrate high standards of pathogen safety across the 7 farms, with a low incidence of pathogens detected in raw milk samples. Monitoring feed contamination levels may help control the spread of bacterial species such as *Cl. perfringens* and *B. cereus* through the farm environment, which is a natural reservoir for these organisms.

Key words: dairy farm, pathogen, prevalence

INTRODUCTION

The dairy farm is a dynamic environment and it has a complex associated microbial ecology. A wide variety of bacteria have natural reservoirs among components of this ecosystem. The farm environment, however, represents a possible entry point into the food chain, primarily in terms of milk and associated dairy products that may be produced there. The occurrence of various pathogenic microorganisms on farms is therefore a concern if they are able to contaminate raw milk, which then provides entry into the food supply (Oliver et al., 2005). As such, understanding transmission routes of bacterial pathogens and spoilage organisms into raw milk is an important component in implementing an effective control strategy.

The entry route of foodborne pathogens from the dairy farm environment into raw milk may come from several different vectors, and includes the animal itself, feces, contaminated crops and feed, bedding, housing, and water (Oliver et al., 2005; Quigley et al., 2013). Effective control of this cross-contamination thus includes many considerations; for example, udder hygiene, feed contamination, hide contamination, gut colonization and fecal carriage, and control of movements of other animals, including birds and rodents, through the farm environment.

The means by which pathogenic microorganisms in raw milk enter products and are consumed was out-

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lined effectively in a review by Oliver et al. (2005). Many foodborne outbreaks described were traced back to both raw and pasteurized milk. Some members of society have access to and consume raw milk, and similarly some cheeses are made from raw milk. Without a thermal inactivation step, producing pathogen-free raw milk is critical to preventing illness due to consumption of contaminated product. Pasteurization of raw milk, however, does not preclude the milk or dairy products from contamination by pathogens, which can contaminate products postprocessing at dairy processing plants. Indeed, not all pathogens are eliminated by pasteurization, and pathogens may also survive in milk that has had improper pasteurization.

The current study investigated the prevalence of *Bacillus cereus, Campylobacter*, *Clostridium perfringens*, *Cronobacter*, Shiga-toxigenic *Escherichia coli* (**STEC**), *Listeria*, *Salmonella*, coagulase-positive staphylococci (**CPS**), and *Yersinia enterocolitica* in a range of environments common to most dairy farms, including pasture, soil, feed, and water sources, as well as animal feces, raw milk, and milk filters. The natural sources of the microorganisms investigated in this survey can be environmental, enteric, or a combination of both. *Campylobacter*, *Salmonella*, STEC, and *Y. enterocolitica* are all enteric foodborne pathogens (Milnes et al., 2008), of which *Salmonella* and STEC can survive in soil and the environment for long periods (Jay et al., 2003; Farrokh et al., 2013). *Clostridium perfringens* is also widespread both enterically and in soil (Bates and Bodnaruk, 2003). Similarly, soil is the ecological niche for *B. cereus* and *Listeria* (Jenson and Moir, 2003; Sutherland et al., 2003). Plants are thought to be the natural habitat for *Cronobacter* (Schmid et al., 2009). *Staphylococcus aureus* is found on the skin of humans and animals as well as being distributed in human environments (Stewart, 2003). Milk is not always the primary source of these microorganisms in the food supply, as is the case for *Cronobacter*, which has been more frequently isolated from animal feed and cereals (Molloy et al., 2009). Nonetheless, all of these microorganisms have been found in raw milk (Friedemann, 2007; Claeys et al., 2013; Quigley et al., 2013) and have potential to gain access to the food supply via this route.

Previous work investigating prevalence of pathogens in the dairy farm environment has typically focused on only a select few pathogens at a time, most commonly from bovine dairy farms (Cortés et al., 2006; Fox et al., 2009; Molloy et al., 2009; Bernardino-Varo et al., 2013; Bianchini et al., 2014). This survey looks at the broad occurrence of 9 foodborne pathogens from the raw milk and environments of 7 bovine, ovine, and caprine dairy farms in the summer and autumn in Victoria, Australia.

MATERIALS AND METHODS

Farms and Sampling

A total of 7 farms located throughout Victoria were included in this survey. These comprised 3 bovine farms (farms A, B, and C), and 3 caprine farms and 1 ovine farm (farms D, E, F, G; grouped together to maintain anonymity). Bovine farms were medium to large, whereas caprine and ovine farms were small to medium in size. All farms produced raw milk for further processing into milk, dairy products, or both. Farms observed a hazard analysis and critical control point (HACCP) approach to preventing contamination of the milk and milking area, including measures such as hand-wash stations, pest control measures, and sanitization. Between 7 and 11 samples were taken from each farm on each visit. A total of 120 samples were obtained over the 2 visits to each farm (as indicated in Table 1). The first visit was in early summer (December 2013) and the second visit was in early autumn (March 2014). Samples obtained comprised raw milk, milk filters, feces, soil, water, feed, and grass from pastures. The range of sample types tested depended on the target microorganism and where it was expected to be found; thus, not all 120 samples were tested for all pathogens. The raw milk samples were obtained from the bulk milk tanks except for the first visit to farm F and the second visit to farm G, where the milk was obtained directly from the animals. Raw milk was additionally obtained from the bulk tank on the second visit to farm G. Milk filters were not always available at each farm; milk filters were obtained from farms A, B, E, and G on both visits and from farm F on the second visit only. Milk filters were not available from farms C and D on either visit. Water samples were sourced from troughs on all farms as well as from a combination of dams, streams, and rainwater. All water samples were taken from surface water. Feed samples included grain, moist greenhouse grass, pellets, hay, and silage. Where multiple feeds were in use, these were included in the sample set taken when available. For bovine farm C, an additional fecal sample was taken from the animal walkway leading to the milking parlor.

Sample Analysis

A total of 9 bacterial species or genera were tested for, using ISO, Australian standard methods, or validated in-house methods, as listed in Table 2. These comprised the *Bacillus cereus* group (*B. cereus*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus thuringiensis*, *Bacillus weihenstephanensis*, *Bacillus anthracis*, and *Bacillus cytotoxicus*), *Campylobacter* species, *Cl. perfringens*,