

Microbial Pathogen Risks to Dairy Groundwater Task Report 5

Project

“Long Term Risk of Groundwater and Drinking Water
Degradation from Dairies and Other Nonpoint Sources in the
San Joaquin Valley”

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Abstract

Between January 2008 and April 2009, groundwater samples were collected from commercial dairies in San Joaquin Valley for nitrate, isotopic, and microbial pathogen analysis. Four sampling events were conducted in 8 dairies with 191 water samples from monitoring wells and 23 water samples from domestic wells obtained for microbial pathogen analysis. Laboratory microbiological analysis were performed to detect indicator bacteria (generic *E. coli* and *Enterococcus* spp.) and pathogenic bacteria (*Campylobacter* spp., *E. coli* O157:H7, and *Salmonella* spp.) in groundwater. High prevalence of *Enterococcus* spp. was observed in both monitoring wells (93.2%) and domestic wells (91.3%). Generic *E. coli* was detected in 24.1% monitoring wells and 4.3% domestic wells. Average concentrations of *Enterococcus* spp. were 168.4 cfu/100 mL in positive monitoring wells and 1.6 cfu/100 mL in positive domestic wells respectively and that of generic *E. coli* were 3.2 cfu/100 mL and 0.01cfu/100 mL respectively. No confirmed *Campylobacter* spp. and *E. coli* O157:H7 were detected in either monitoring wells or domestic wells during entire project period. *Salmonella* spp. was only detected in 2 (1.0%) of monitoring wells with very low concentrations. The wide prevalence of indicator bacteria in dairy groundwater indicates the potential risks of contamination of groundwater by microbial pathogens from dairy recharges. Findings suggest the needs of developing strategies against domestic well groundwater contamination from dairy recharges.

Further information (publications, related reports, multi-media materials) is available at <http://groundwater.ucdavis.edu>.

Key words: Dairy, groundwater, *E. coli*, *Enterococcus*, *Campylobacter*, *E. coli* O157:H7, *Salmonella*

1. Introduction

Outbreaks of enteric diseases due to contaminated drinking water supplies and fresh produce have been linked to animal or human fecal waste sources in recent years (EPA 2005). This has renewed public interest in best management practices that prevent pathogen transmission to water and food supplies and highlighted the need to better understand transmission dynamics along potential transport pathways from fecal pathogen sources to humans via drinking and irrigation water supplies.

Microbial pathogens such as bacteria are prevalent in both extensive and intensive animal feeding operations. Animal feeding operations (AFO) can be a major source (nonpoint and point) of zoonotic pathogens in the environment. Dairy farms as a collection of “management units” are potential sources of contamination of water (Lewis et al., 2005). Bacterial pathogens have been documented to occur frequently in cattle feces. After excretion, fecal matters are incidentally or operationally transported throughout farm management units, resulting in a wide variety of potential sources of zoonotic pathogens in a mixed-use agricultural system (e.g., Duffy, 2003, Purdy et al., 2001). Operational transport occurs by flushing, irrigation, wastewater collection, manure spreading, or waste trucking. Incidental transport includes storm water runoff and wind-driven aerial transport. As consequences of transportation of fecal matters, pathogens are transported along with fecal matters and distributed into

critical water resources through atmospheric and hydrologic pathways and potentially by other vectors (e.g., animals, workers) throughout the farming operation and into the environment.

Dairies comprise the majority of animal feeding operations (AFOs) in California. The Central Valley houses 1.1 million milking cows, more than two-thirds of the California herd, and is experiencing a historic growth in dairy farming. Most dairies in the Central Valley occupy essentially flat land on the valley floor where little or no runoff to streams is observed. In these areas, recharge to groundwater from irrigation surplus accounts for 30%-60% of all groundwater recharge (Ruud et al., 2002). Nonpoint source pollution of groundwater is the most widespread form of groundwater contamination with significant risk to drinking water quality and human health. The overall objective of this part of the project was to monitor and enumerate the occurrence of indicator bacteria (generic *E. coli* and *Enterococcus* spp.) and pathogenic bacteria (*Campylobacter* spp., *E. coli* O157:H7 and *Salmonella* spp.) in the underlying groundwater on dairies in the Central Valley.

2. Methods

Sample collection and filtration:

Groundwater was collected from monitoring wells (MW) and domestic wells (DW) at the depths of 10 to 30 feet below grounds. Groundwater was directly pumped into sterilized 10 L plastic carboys using a portable and submersible stainless steel Grundfos™ pump. The exterior wall of hose and pump head were wiped with sterile cloth and the interior of the system (the pump and the tubing) was washed by pumping 50 L of deionized water before use and between wells, a procedure avoiding cross contamination confirmed by laboratory testing (data not shown). Samples were maintained on ice during transportation, stored in a cold room (4°C) upon arrival at laboratory, and processed for bacteria isolation within 24 h after sampling. Water samples were filtered using a positive pressure vessel filter system and 142mm diameter membrane filters. Pore sizes of membrane filters were 0.45µm *E. coli*, *E. coli* O157:H7, *Enterococcus* spp. and *Salmonella* spp., and 0.22µm for *Campylobacter* spp.

Analytical methods for detecting generic *E. coli*, *Enterococcus* spp., and *Campylobacter* spp:

Direct plating methods were used for detecting generic *E. coli*, *Enterococcus* spp., and *Campylobacter* spp. For each water sample, 10 L were filtered for each of the three organisms respectively. After filtration, membrane filters were placed onto respective media, ChromAgar EC agar for *E. coli*, Modified membrane *Enterococcus* agar (mEI) for *Enterococcus*, and *Campylobacter* Line Agar (CLA) for *Campylobacter*. *E. coli* plates were incubated at 37°C for 2h followed by 22h cultured at 44.5°C; *Enterococcus* plates were incubated at 41.0°C for 24h; and *Campylobacter* plates were incubated at 42°C in an anaerobic chamber. After incubation, numbers of colonies on each plate were counted and concentrations of bacteria were expressed CFU (colony forming units) per 100 milliliters ((cfu/100 mL) = (no. of colonies/mL of water filtered) × 100)). Presumptive positive *Campylobacter* colonies were confirmed by biochemical tests (Catalase, TSI, Oxidase and Hippurric).

Analytical methods for detecting *Salmonella* spp.

An enrichment and Most Probable Number (MPN) method were used for quantitative detection of *Salmonella* spp. For each sample, water was filtered at 2000 mL (×4 replicates), 200 mL (×4 replicates)

and 20 mL (×4 replicates). Membrane filters were folded and placed into plastic bags contain 10 mL buffered peptone water (BPW) and incubated at 37°C for 24 h. Ten microliter of the BPW enrichment solution was transferred into a well of 96-well plate contains 1.0 mL of Rappaport-Vassiliadis (RV) broth and incubated at 42°C for 24 h. Five microliters of the RV enrichment solution from each well was streaked as a lane on Xylose Lysine Deoxycholate (XLD) agar plates and incubated at 37°C for 24 h. Lanes with black colonies were presumptive positive *Salmonella* reaction which was confirmed by biochemical tests (Triple Sugar Iron Agar, Urea Agar, and Lysine Iron Agar). The numbers of positive reaction lanes of each volume tested were used for calculating *Salmonella* spp. concentration using computer software based MPN calculator (Mike Curiale). Concentrations of *Salmonella* spp. were calculated as no. of MPN per 100 mL of water. We have found this procedure is typically capable of detecting as few as 0.33 CFU per g of sample with over 90% certainty.

Analytical methods for detecting *E. coli* O157:H7

A previously described Enrichment and ImmunoMagnetic Separation (IMS) method (Paton and Paton, 2003) were used for the detecting *E. coli* O157:H7. Samples were screened for presence of *E. coli* O157 with a qualitative (yes/no) procedure by filtering 10 L water of each sample. Membrane filter was placed in 100 mL Tryptic Soy Broth (TSB) and incubated in a Multitron programmable shaking incubator for 2 h at 25°C followed by 8 h at 42°C and hold at 6°C overnight. After incubation, Immunomagnetic separation (IMS) was performed using anti-*E. coli* O157 beads (Invitrogen, Carlsbad, CA) with a Dynal Bead Retriever (Thermo, Finland) per manufacturer's instructions. One milliliter of the enrichment solution was used for the IMS and 100 µl final solutions were obtained from the IMS. Fifty µl of the final solutions were streaked onto Rainbow agar (Biolog, Hayward, CA) with novobiocin (20 mg/L) and tellurite (0.8 mg/L) (MP Biomedicals, Solon, OH). The remaining 50 µl was streaked onto Sorbitol MacConkey Agar (BD Becton, Sparks, MD) with cefixime (0.05 mg/L) (USP, Rockville, MD) and tellurite (2.5 mg/L). Both plates were incubated at 37°C for 24 h. Presumptive positive colonies on Sorbitol MacConkey agar plate (Figure 2. A) or Rainbow agar plate were confirmed by specific PCR using forward primer: 3' CGG ACA TCC ATG TGA TAT GG 5' and reverse primer: 5' TTG CCT ATG TAC AGC TAA TCC 3'. The sensitivity of this method determined in our laboratory is as low as 1 CFU per liter of water with 90% certainty. PCR confirmed *E. coli* O157 isolates (if any) are sent to the *E. coli* Reference Center at Pennsylvania State University for H7 serotyping.

3. Results and Discussion

Occurrence of bacteria in Monitoring wells

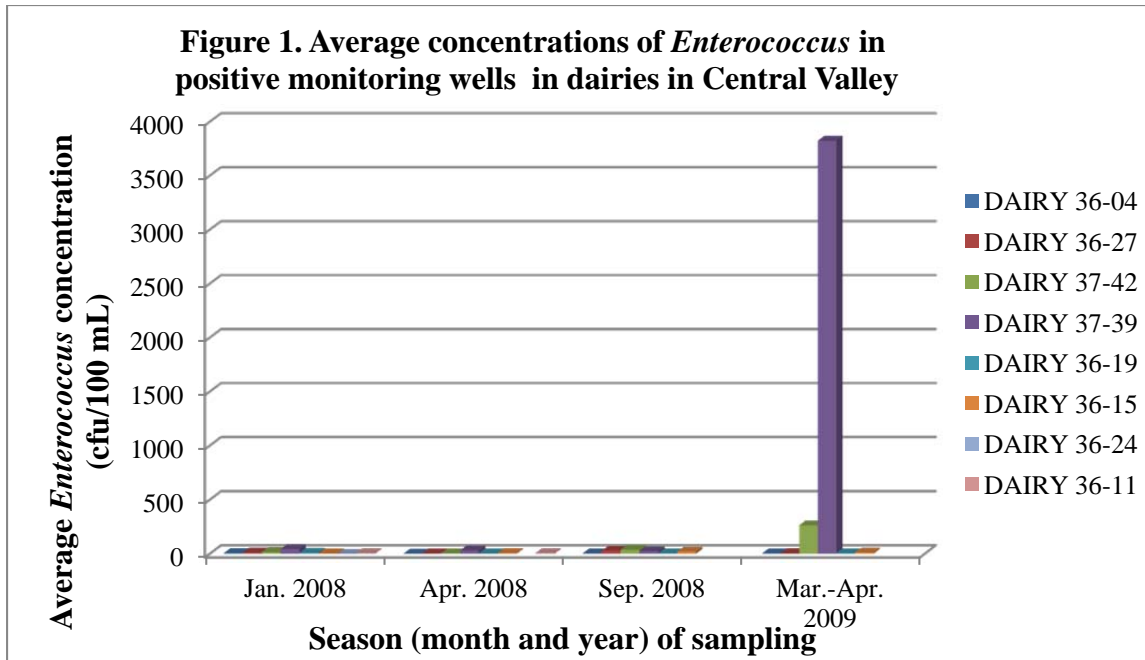
Water samples from 191 monitoring wells were received for microbiological analysis. *Enterococcus* spp., an indicator bacterium was widely distributed in water with an average of 93.2% samples were positive year round. Another indicator bacterium, generic *E. coli* was found in 24.1% water samples (Table 1).

Table 1. Seasonal occurrence of bacteria in monitoring wells (MW) in dairies in Central Valley

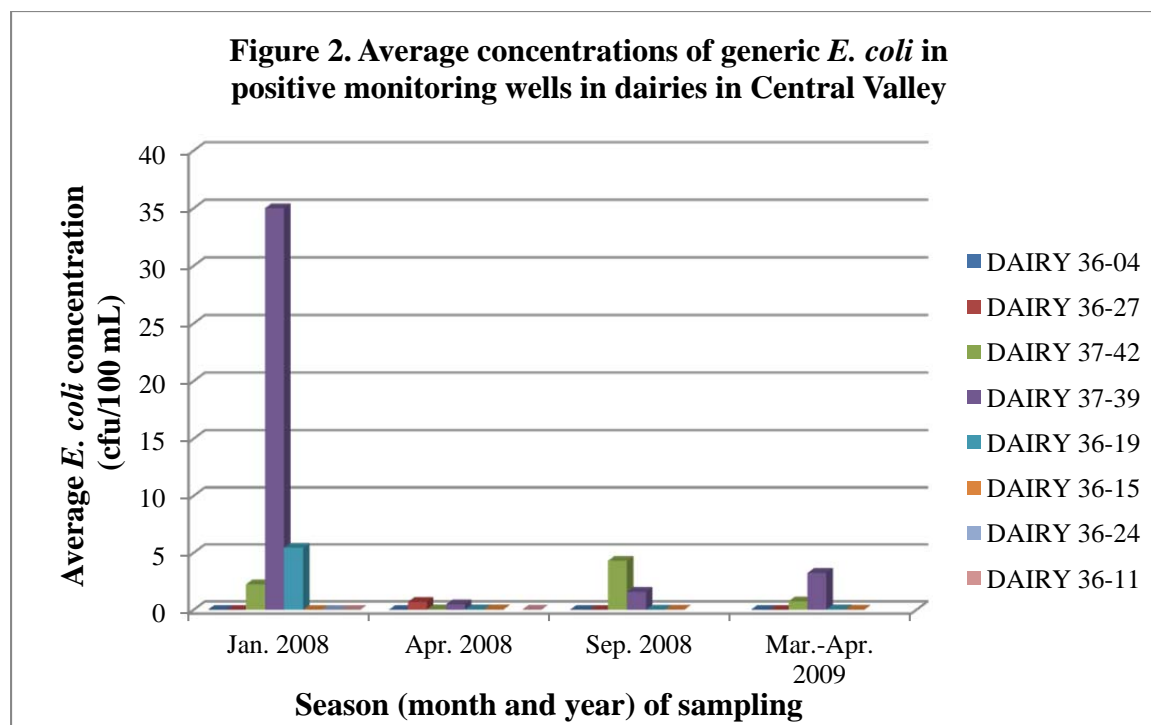
| Season and Year | No. of wells | No. (%) of positive wells by organisms* | | | | |
|------------------|--------------|---|---------------------|----------------------|------------------------|-------------------|
| | | Generic <i>E. coli</i> | <i>Enterococcus</i> | <i>Campylobacter</i> | <i>E. coli</i> O157:H7 | <i>Salmonella</i> |
| January 2008 | 46 | 7 (15.0) | 44(95.7) | 0(0) | 0 (0) | 2(4.3) |
| April 2008 | 44 | 12 (27.3) | 39 (93.0) | 0(0) | 0(0) | 0(0) |
| September 2008 | 50 | 13 (26.0) | 44(88.0) | 0(0) | 0(0) | 0(0) |
| March-April 2009 | 51 | 14 (27.5) | 51 (100) | 0(0) | 0(0) | 0(0) |
| Total | 191 | 46(24.1) | 178 (93.2) | 0(0) | 0(0) | 2 (1.0) |

* Generic *E. coli* and *Enterococcus* spp. are indicator organisms and *Campylobacter* spp., *E. coli* O157:H7 and *Salmonella* spp. are pathogenic organisms

Average concentrations of *Enterococcus* spp. in positive wells in each dairy ranged from less than 1 cfu to approximately 40 cfu per 100 mL of water, with exceptional higher concentrations of 263 cfu/100 mL and 3822 cfu/100 mL in dairy 37-42 and 37-49 respectively during the season of March-April 2009. The genera average concentration of *Enterococcus* spp. in positive monitoring wells from all dairies and entire project period was 168.4 cfu/100 mL of water (Figure 1). Average concentrations of *E. coli* in positive wells was 2 magnitude lower, with typical ranges between <1 cfu to >2 cfu per 100 mL of water. Only one higher average concentration (35 cfu/100 mL) was detected in dairy 37-39 in January 2008. The genera average concentration of *E. coli* in positive domestic wells from all dairies and entire project period was 3.2 cfu/100 mL (Figure 2).



Although high prevalence of indicator bacteria especially *Enterococcus* spp. was observed in water from monitoring wells, pathogenic bacteria were not or hardly detected. For instances, no confirmed *Campylobacter* spp. and *E. coli* O157:H7 were detected during the entire project period. *Salmonella* spp. was detected only in one well in dairy 37-42 and one well in dairy 36-15 in January 2008 (Table 1). In addition, concentrations of *Salmonella* spp. in the two positive wells were very low (0.023 and 0.039 MPN/100 mL respectively; data not shown).



Occurrence of bacteria in domestic wells

Water samples from 23 monitoring wells were received for microbiological analysis. Similarly to monitoring wells, high prevalence (91.3%) of *Enterococcus* spp. was detected in domestic wells. In contrast, generic *E. coli* was detected in only one (4.3%) well and no confirmed *Campylobacter* spp., *E. coli* o157:H7 and *Salmonella* spp. was detected (Table 2).

Table 2. Seasonal occurrence of bacteria in domestic wells (DW) in dairies in Central Valley

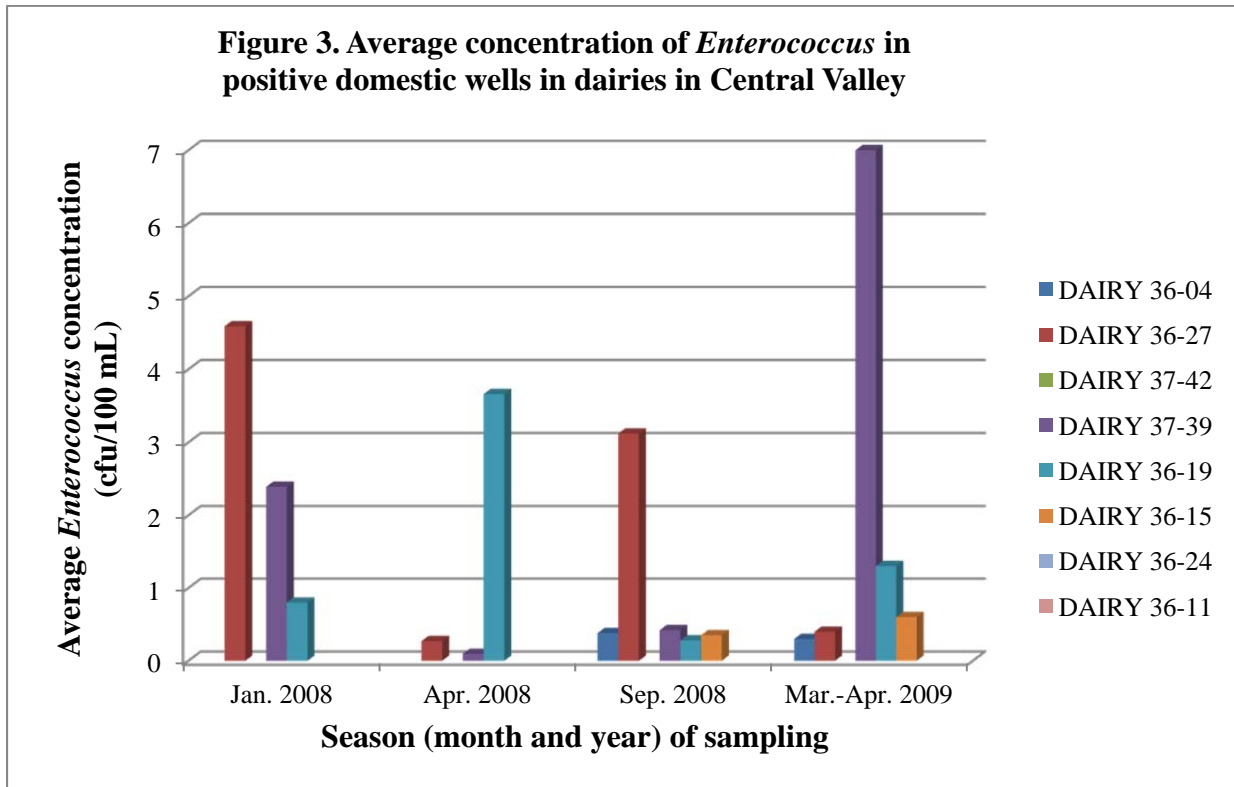
| Season and Year | No. of wells | No. (%) of positive wells by organisms* | | | | |
|-----------------|--------------|---|---------------------|----------------------|------------------------|-------------------|
| | | Generic <i>E. coli</i> | <i>Enterococcus</i> | <i>Campylobacter</i> | <i>E. coli</i> O157:H7 | <i>Salmonella</i> |
| January 2008 | 5 | 1 (20.0) | 4(80.0) | 0(0) | 0 (0) | 0(0) |
| April 2008 | 5 | 0 (0) | 4(80.0) | 0(0) | 0(0) | 0(0) |
| September | 6 | 0 (0) | 6(100.0) | 0(0) | 0(0) | 0(0) |

2008

| | | | | | | |
|------------------|----|---------|-----------|------|------|-------|
| March-April 2009 | 7 | 0 (0) | 7 (100.0) | 0(0) | 0(0) | 0(0) |
| Total | 23 | 1 (4.3) | 21 (91.3) | 0(0) | 0(0) | 0 (0) |

* Generic *E. coli* and *Enterococcus* spp. are indicator organisms and *Campylobacter* spp., *E. coli* O157:H7 and *Salmonella* spp. are pathogenic organisms

Average concentrations of *Enterococcus* spp. in positive domestic wells were mostly <1cfu/100 mL of water and were 2 magnitude lower than that in monitoring wells. Highest concentration was 7 cfu/100 mL which was detected in dairy 37-39 in March-April 2009. The genera average concentration of *Enterococcus* spp. in positive domestic wells from all dairies and entire project period was 3.2 cfu/100 mL of water (Figure 3), also 2 magnitude lower than that in monitoring wells. The sole generic *E. coli* positive domestic well was detected in dairy 37-39 in January 2008 and the concentration was only 0.01cfu/100 mL (data not shown).



Various studies worldwide report prevalence rates for verocytotoxigenic *E. coli* that are typically in the range of 2% to 20% in dairy cattle with seasonal peaks occurring in spring and summer (Elder et al., 2000; Duffy, 2003, Kuhnert et al., 2005). Similar prevalence rates have been reported for *Salmonella* (Wray et al., 1988). *Campylobacter* spp. frequently occurs in the feces of mammals and birds. It can be present in cattle feces at concentrations ranging from 2 to 3 log₁₀ MPN (most probable number) per gram of feces (Stanley and Jones, 2003). However, survival of bacteria in the environment is associated with many environmental factors among which temperature is a critical one. A review of numerous studies has indicated average inactivation rates for coliform bacteria, enterococci, and *Salmonella* to be on the order of 0.07-0.1 log₁₀ day⁻¹ at typical groundwater temperatures (John and Rose, 2005). In addition, underground transportation can significantly reduce loads of microorganisms. For example, data from laboratory column experiments have demonstrated that *Salmonella* and *Cryptosporidium* loads significantly declined after transportation through porous media (Dowd and Pillai, 1997; Searcy et al., 2005).

The most common indicators for the presence of fecal pathogens are generic *E. coli*, fecal coliforms, and total coliform bacteria and *Enterococcus* spp. *Enterococcus* spp. is typically in high concentration in mammalian fecal matters comparable to generic *E. coli*. In addition, *Enterococcus* spp. exhibits longer survival time in the environment compared to *E. coli* (Edberg et al., 1997; Kinzelman et al., 2003). It is not surprising that in the present work we detected ~ 90% of *Enterococcus* spp. and ~ 20% *E. coli* in monitoring wells. Despite the ubiquitous presence of indicator *Enterococcus* spp. in groundwater, *Campylobacter* spp. and *E. coli* O157:H7 were not detected and *Salmonella* spp. was only detected in 1% of water samples from monitoring wells. Similarly, in previous studies fecal coliform and fecal streptococci were detected in as much as 10-40% of rural well samples with no *Salmonella* or *Campylobacter* spp. present (Korhonen et al., 1996, Diergaardt et al., 2004). Nevertheless, we cannot ignore the potential risks of groundwater contamination by pathogens since microbiological groundwater contamination has been documented as the source of waterborne outbreaks of disease (Fong et al., 2007).

4. Conclusion

High prevalence of *Enterococcus* spp., an indicator bacterium was observed in both monitoring wells and affiliated domestic wells on dairies in Central Valley. Concentrations of *Enterococcus* spp. and *E. coli* in domestic wells were significantly lower than that in monitoring wells. Despite the fact that no pathogenic bacteria (except for *Salmonella* spp. in 1% samples) were detected, the wide prevalence of indicator bacteria in dairy groundwater indicates the potential risks of contamination of groundwater by microbial pathogens from dairy recharges. Findings suggest the needs of developing strategies against domestic well groundwater contamination from dairy operations.

5. Acknowledgements

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