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Xunde Li et al., *Fecal Indicator and Pathogenic Bacteria and Their Antibiotic Resistance in Alluvial Groundwater of an Irrigated Agricultural Region with Dairies*, 44 J. Envtl. Quality 1435 (2015)

Journal of Environmental Quality SPECIAL SECTION

MICROBIAL TRANSPORT AND FATE IN THE SUBSURFACE

# Fecal Indicator and Pathogenic Bacteria and Their Antibiotic Resistance in Alluvial Groundwater of an Irrigated Agricultural Region with Dairies

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## Abstract

Surveys of microbiological groundwater quality were conducted in a region with intensive animal agriculture in California, USA. The survey included monitoring and domestic wells in eight concentrated animal feeding operations (CAFOs) and 200 small (domestic and community supply district) supply wells across the region. *Campylobacter* was not detected in groundwater, whereas *Escherichia coli* O157:H7 and *Salmonella* were each detected in 2 of 190 CAFO monitoring well samples. Nonpathogenic generic *E. coli* and *Enterococcus* spp. were detected in 24.2% (46/190) and 97.4% (185/190) groundwater samples from CAFO monitoring wells and in 4.2% (1/24) and 87.5% (21/24) of CAFO domestic wells, respectively. Concentrations of both generic *E. coli* and *Enterococcus* spp. were significantly associated with well depth, season, and the type of adjacent land use in the CAFO. No pathogenic bacteria were detected in groundwater from 200 small supply wells in the extended survey. However, 4.5 to 10.3% groundwater samples were positive for generic *E. coli* and *Enterococcus*. Concentrations of generic *E. coli* were not significantly associated with any factors, but concentrations of *Enterococcus* were significantly associated with proximity to CAFOs, seasons, and concentrations of potassium in water. Among a subset of *E. coli* and *Enterococcus* isolates from both surveys, the majority of *E. coli* (63.6%) and *Enterococcus* (86.1%) isolates exhibited resistance to multiple (≥3) antibiotics. Findings confirm significant microbial and antibiotic resistance loading to CAFO groundwater. Results also demonstrate significant attenuative capacity of the unconfined alluvial aquifer system with respect to microbial transport.

## Core Ideas

- Systemic surveys of groundwater microbiological quality are performed at site and regional scales.
- Confined animal systems are chronic sources of pathogens and high enteric microbial loads.
- Pathogen loading to groundwater is effectively mitigated by alluvial aquifer system.
- Some microbial indicators are too ubiquitous to be useful as indicators.
- Antibiotic resistance from CAFOs and human sources affects the alluvial aquifer system.



J. Environ. Qual. 44:1435–1447 (2015)
doi:10.2134/jeq2015.03.0139
Freely available online through the author-supported open-access option.
Received 15 Mar. 2015.
Accepted 17 June 2015.
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Fecal-rich environments in concentrated animal feeding operations (CAFOs) are the pools and potential sources of a wide variety of zoonotic pathogens (Hoar et al., 1999; Purdy et al., 2001; Duffy, 2003; Lewis et al., 2005; Friesema et al., 2011; Won et al., 2013). For example, dairy cattle provide natural reservoirs of *Campylobacter* (Dodson and LeJeune, 2005), *Escherichia coli* O15:H7 (Shere et al., 1998; Dodson and LeJeune, 2005), *Salmonella* (Dodson and LeJeune, 2005; Cummings et al., 2010), and *Cryptosporidium* (Atwill et al., 1998). Feces with high concentrations of microbes are widely dispersed throughout CAFO environments, including flush lane, corrals, pens, excise fields, floors, and solid and liquid manure storage areas (Lewis et al., 2005; Beck et al., 2007; Edrington et al., 2009; Toth et al., 2011; Watson et al., 2012). Microbes from fecal-rich environments may reach groundwater via multiple routes, including, but not limited to, surface runoff entrainment of feces deposited on the ground, leaking of solid and liquid manure storage or storage areas, and subsurface transport (Harter et al., 2014). Concentrated animal feeding operations are of increasing concern for their impact on public health and the environment, including microbiological quality of groundwater (Kirkhorn, 2002; Bartelt-Hunt et al., 2011; Lockhart et al., 2013). Coliform bacteria are known to be widely distributed in groundwater (Embrey and Runkle, 2006). *Escherichia coli* is able to travel long distances underground and is a useful indicator of fecal contamination of groundwater (Foppen and Schijven, 2006).

The prevalence of antibiotic-resistant bacteria has been well documented in dairy animals (Fessler et al., 2012; Lindeman et al., 2013; Saini et al., 2013; Cummings et al., 2014; Duse et al., 2014; Gibbons et al., 2014; Wichmann et al., 2014). The occurrence of antibiotic-resistant bacteria in animal production systems raises the potential for promoting multiple drug–resistant bacteria (Esiobu et al., 2002; Straley et al., 2006; Wilhelm et al.,

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**Abbreviations**: bgs, below ground surface; BPW, buffered peptone water; CAFO, concentrated animal feeding operation; CFU, colony-forming units; MPN, most probable number; SJV, San Joaquin Valley; TLB, Tulare Lake Basin.

2009; Holmes and Zadoks, 2011) and the transmission of antibiotic-resistant bacteria to humans through agriculture, the food chain, and the environment (Witte, 1998; Kummerer, 2003; Ward et al., 2014; Wieler, 2014).

Monitoring of fecal indicator and pathogenic bacteria in groundwater is important for assessing the risk of microbial contamination of groundwater, especially in regions potentially influenced by CAFOs. We first conducted a pilot survey to estimate the loads of fecal indicator bacteria and pathogenic bacteria in the environment of two CAFOs. We then conducted a systematic survey of indicator bacteria (generic *E. coli* and *Enterococcus*), pathogenic bacteria (*Campylobacter*, *E. coli* O157:H7, and *Salmonella*), and antibiotic resistance in groundwater. The survey was conducted at four groundwater transport scales: (i) in groundwater immediately below the water table at the dairy sites, (ii) in production aquifers immediately below dairies, (iii) in production aquifers within the vicinity of dairies, and (iv) in production aquifers away from dairies.

The study was conducted in the unconfined alluvial aquifer system of the Central Valley of California, which underlies an irrigated agricultural region with a large number of dairy CAFOs (Fig. 1). The survey included repeated, seasonal sampling events in monitoring and domestic wells of eight commercial dairies followed by a broader survey of private domestic wells across the region. Subsets of generic *E. coli* (gram-negative) isolates and *Enterococcus* (gram-positive) isolates from groundwater collected in these surveys were assessed for their susceptibility to antibiotics. The objective of our work was to determine the frequency and magnitude of indicator and pathogenic bacteria and their antimicrobial resistance in groundwater at various distances from their source, to assess the risk factors related to microbial contamination of groundwater, and to determine antibiotic resistance characteristics of bacteria in groundwater. The working hypothesis was that wells with close proximity to CAFOs are more vulnerable to microbial contamination and antibiotic resistance.

# Materials and Methods

## Study Area

The Central Valley is an area of intensive agricultural production with 3 million ha, nearly two-thirds of the total land area, devoted to irrigated farming (Burow et al., 2008). Sources of irrigation water include groundwater and surface water (Faunt et al., 2009). Irrigated crops on or near dairies include, among others, leafy greens used for human consumption. Rural communities and households and many urban areas rely on groundwater as their sole source of drinking water, with minimal or no water treatment. Microbial contamination of groundwater is therefore a significant concern for food safety and human health in this region.

The study area was comprised of the four counties with the largest concentration of dairies in the California Central Valley: Stanislaus, Merced, Tulare, and Kings Counties (Fig. 1). The underlying Central Valley aquifer system is formed by unconsolidated alluvial fan and fluvial basin sediments of varying quaternary and late tertiary ages. These sediments comprise the upper 500 to 1000 m of thicker continental and underlying, older marine sediments (DWR, 2004; Page, 1986). Hydraulic conductivity can vary greatly depending on the particle size of

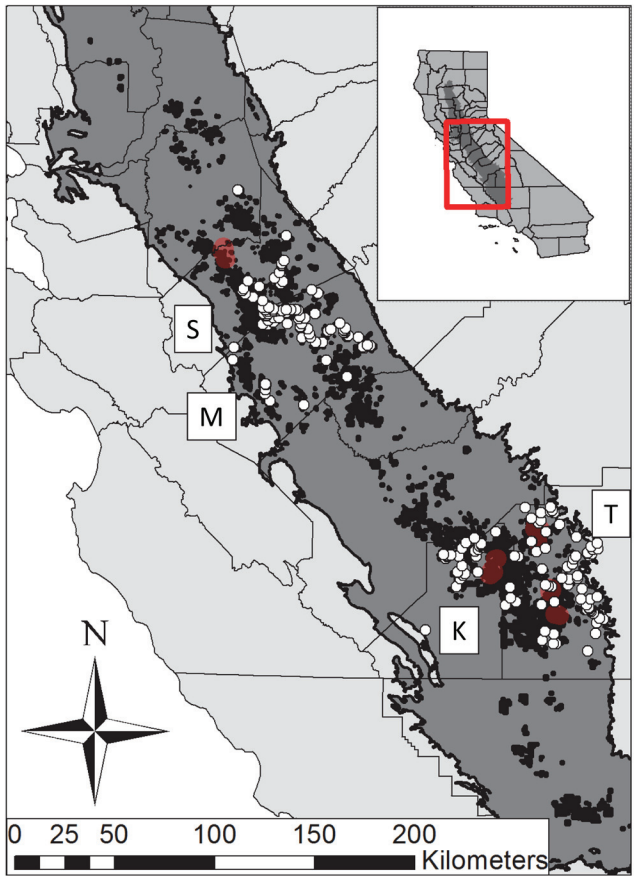


**Fig. 1. Study area in California focusing on four counties overlying the Central Valley aquifer (dark gray): Stanislaus County (S), Merced County (M), Kings County (K), and Tulare County (T). County boundaries shown as black lines. The southern and central Central Valley has a high concentration of dairy concentrated animal feeding operations and manure-treated irrigated crops (black). The study was conducted on eight dairies (red) and included a regional survey of 200 domestic wells (white).**

sediments: coarse fraction hydraulic conductivity and fine fraction hydraulic conductivity have been estimated to be 1000 and <0.1 m $d^{-1}$, respectively (Faunt et al., 2009). The Central Valley is broadly divided into three contiguous subbasins: the northern Sacramento Valley, the southcentral San Joaquin Valley (SJV), and the southern Tulare Lake Basin (TLB) (Gronberg et al., 1998). Stanislaus and Merced Counties are within the SJV, and Tulare and Kings Counties are within the TLB (Fig. 1).

Depth to the water table varies and is thought to have significant impact on microbial transport. Depth to water table near the Sierra foothills in Stanislaus and Merced Counties in spring 2010 was between 50 and 80 m below ground surface (bgs) and decreased in a southwesterly direction to between 3 and 15 m bgs near the valley axis (thalweg) formed by the San Joaquin River (DWR, 2012). Depth to unconfined and semiconfined groundwater in Tulare and Kings Counties in spring 2010 generally increased from 10 to 15 m bgs in northeastern Tulare County to over 100 m bgs in southern Tulare County and to 50 to 80 m bgs in Kings County and eastern Tulare County (DWR, 2012).

The Central Valley has a Mediterranean climate with hot, dry summers and a rainy season typically lasting from November through April. Average annual precipitation in the study area is 310 mm. The region supports 250 crops including tree fruit, nuts, vineyards, vegetables, rice, cotton, and forage crops (corn,

sorghum, grains, and alfalfa). Approximately 1.7 million mature cows plus support cattle, about three quarters of the California dairy herd, are located on less than 1500 dairy farms (USEPA, 2012), mostly in the SJV and TLB portion of the Central Valley (Fig. 1).

Dairies are operated as CAFOs; they house mature animals in freestalls with exercise yards (freestall dairies) or in open lots (drylot dairies). Dairies collect stormwater runoff from their corrals (exercise yards, open lots, other animal holding areas) and washwater from their milking barns in storage lagoons. Animal waste (manure) in freestall dairies is collected in concrete lanes that are frequently flushed with recycled storage lagoon water. Manure solids are mechanically separated from flushwater (freestall dairies) and scraped from corrals (freestall and drylot dairies). Manure solids are dried, stored, and used for animal bedding or on cropland. Wastewater from storage lagoons is typically applied to cropland via pipes and mixed with flood irrigation water. Dairies typically manage a significant amount of forage crop acreage, which is where most manure is applied. All management units of a dairy are subject to some leaching and groundwater recharge—forage fields treated with manure, lagoons, and corrals (Harter et al., 2002; Van der Schans et al., 2009). "Corrals" here include unlined freestalls, drylots, exercise yards, hospital barns, and calf and heifer housing areas.

## Fecal Indicator and Pathogenic Bacteria Occurrence at Land Surfaces in Dairies

Between 2006 and 2008, five sampling events were conducted in two commercial dairies in the Central Valley, California, for source characterization. During each sampling event, surface solid and flush water (wastewater) samples were collected from each of several management units in each dairy; solid samples were taken from manure fields, calf hutches, lactating cow freestalls, lactating cow exercise yards, hospital pens, and heifer yards. Flushwater samples were taken from storage lagoons, flush alleys in lactating cow freestalls, and a flush alley draining the calf hutch area. Water samples were collected by directly pouring into sterilized 1-L polyethylene bottles, and solid samples were collected using sterilized forceps and placed into sterilized 1-L polyethylene bottles. Within each management unit, 12 randomly distributed samples were collected, combined, and thoroughly mixed for the final composite sample at each sampling date. All samples were kept cool in an ice chest while in the field and during transportation to the laboratory, stored in a cold room (4°C) on arrival at the laboratory, and processed within 24 h after collection. One gram of solid samples or 1.0 mL of water samples were suspended in PBS in 50-mL tubes and homogenized by shaking for 15 min using a wrist action shaker. After shaking, solid particulates were removed by filtering through four-layer gauze in a funnel, and filtrates were 10-fold serially diluted. Dilutions were filtered using the membrane filtration method for detection of generic *E. coli*, *Enterococcus*, and *Campylobacter* as described below. For quantitative detection of *Salmonella*, four replicates of each weight or volume were suspended in 50 mL of buffered peptone water (BPW); 10.0, 1.0, and 0.1 g of solid samples or 1.0, 0.1, and 0.01 mL of water samples; followed by the most probable number (MPN) method described below.

## On-Dairy Groundwater Monitoring

Between 2008 and 2009, eight commercial dairy farms were enrolled for the groundwater monitoring survey based on voluntary participation ("on-dairy"). Two dairies were located in Stanislaus County in a region with highly permeable loamy sand and sandy loam soils and with a relatively shallow water table (about 3 m bgs). Two dairies were located in Kings County and four dairies were in Tulare County, all over clayey to sandy loam soils with depth to groundwater ranging from 15 to >30 m. On each dairy farm, groundwater samples were collected from 5.1-cm-diameter PVC monitoring wells constructed with 3- to 6-m-long well screens in the first nonclayey alluvial sediment unit below the water table. Monitoring wells were constructed immediately downgradient from manure-treated fields, storage lagoons, and corrals.

On-dairy groundwater samples were also obtained from domestic wells, which are typically constructed with screens that are 10 m or more below the water table (Lockhart et al., 2013). Wells were sampled seasonally, once during the coldest part of the rainy season (January 2008), twice at the end of the rainy season (April 2008, March–April 2009), and once toward the end of the hot, dry season (September 2008). Not all monitoring wells were always accessible or available of water. In total, 190 samples were collected from 46 monitoring wells, and 24 samples were collected from five domestic on-dairy wells.

## Near-Dairy and Nondairy Groundwater Monitoring

In 2010 and 2011, we extended our survey to general private domestic wells, including six small community service district wells across the four-county region. Domestic wells were chosen based on responses from property owners to newspaper ads and to flyers mailed to rural residents. In total, 200 domestic wells were enrolled (half in the SJV and half in the TLB), and each well was sampled once between summer 2010 and summer 2011 (Lockhart et al., 2013). Spatial analysis was used to determine the distance between a well and the nearest dairy corral or lagoon. Wells located within 2.4 km from a dairy corral or dairy storage lagoon, including 12 domestic wells located on previously unsampled dairy properties, were classified as "near-dairy wells" (132 wells). Otherwise, they were classified as "nondairy" (68 wells). Nondairy wells were considered to have low likelihood to have dairy management units within their recharge source area. All wells were located in the vicinity of irrigated agricultural lands, some of which may have manure applied by growers for soil amelioration (Lockhart et al., 2013).

## Groundwater Sampling and Filtering

We developed and tested a novel approach for collecting microbial groundwater quality samples from dairies. Details of the microbial field sampling protocol for monitoring wells are described in Harter et al. (2014). For monitoring wells, a portable, submersible, variable-speed, stainless steel Grundfos RediFlo2 pump (Enviro-Equipment, Inc.) was used. Purging volumes before sampling ranged from 13 to 18 well volumes (about 190 L). At domestic or small community service district wells, samples were collected with a closed, air-tight sampling system to avoid exposure to atmospheric cross-contamination. Samples were collected from spigots before the storage tank

when possible or at the closest accessible spigot to the wellhead. Purging volumes ranged from 60 to 400 L. Water samples were kept cool in an ice chest while in the field and during transportation to the laboratory, stored in a cold room (4°C) on arrival at the laboratory, and processed within 24 h after collection.

For water samples collected from on-dairy wells between 2008 and 2009, the default volume of water filtered for generic *E. coli*, *Enterococcus*, *E. coli* O157:H7, and *Campylobacter* was 10 L for each microbial analyte (40 L total), with occasionally smaller volumes filtered for turbid water samples. Water was filtered using a 10-L dispensing pressure vessel system (EMD Millipore Corp.) through 142-m-diameter, 0.45-μm pore size nitrocellulose membrane filters as previously described (Li et al., 2014). To ensure numbers of colonies on plates were countable for samples with high concentrations, an additional 100 mL was filtered for generic *E. coli*, and 100 and 1 mL were filtered for *Enterococcus* through 47-mm-diameter, 0.45-μm pore size nitrocellulose membrane filters using a membrane filtration method. For quantitative detection of *Salmonella*, four replicates of each volume were filtered 2000, 200, and 20 mL.

For water samples collected in near-dairy and nondairy locations between 2010 and 2011, a 50-L water sample was collected and immediately concentrated using a hollow-fiber ultrafiltration technique (also called tangential flow) that has been reported to be effective for recovering a diverse array of microbes from water (Hill et al., 2005). The ultrafiltration was conducted using single-use F200NR dialysis filters (Fresenius Medical Care); samples were concentrated to ~1000 mL (retentate). Each retentate was split to 5% for *Enterococcus*, 15% for generic *E. coli*, 25% for *Salmonella*, 25% for *Campylobacter*, and 30% for *E. coli* O157:H7. The retentates used for generic *E. coli* and *Enterococcus* were further split into two aliquots of 5 and 95%, respectively, to ensure countable colonies on plates. All retentates were filtered through 47-mm-diameter, 0.45-μm pore size nitrocellulose membrane filters. For quantitative detection of *Salmonella*, four replicates of each volume were filtered at 50, 5, and 0.5 mL.

For all groundwater samples, electrical conductivity, pH, temperature, and dissolved oxygen were measured in the field using a YSI 556Multi-Parameter Water Quality sensor. Separate water samples were collected for laboratory analysis of nitrate plus nitrite and major dissolved ions, including potassium and sodium (APHA, 2005; USEPA 1993). Depth to water was measured before sampling monitoring wells. Approximately 40% property owners provided information of domestic well structure and water table depth (Lockhart et al., 2013).

## Detection of Fecal Indicator and Pathogenic Bacteria

Immediately after filtration, filters were placed onto CHROMAgar EC plates for detection of generic *E. coli*, mEI *Enterococcus* Indoxyl-β-D-Glucoside agar plates for detection of *Enterococcus*, Rainbow and MacConkey agar plates for detection of *E. coli* O157:H7, and Campy-Line agar for detection of *Campylobacter*. CHROMagar EC plates were incubated at 35°C for 2 h followed by incubation at 44.5°C for 24 h, mEI plates were incubated at 41.0°C for 24 to approximately 48 h, Rainbow and MacConkey plates were incubated at 37°C for 24 h, and Campy-Line agar plates were incubated in an anaerobic chamber at 42.0°C for 48 h. After incubation, presumptive bacterial colonies were confirmed by biochemical tests and/or molecular analysis. Generic *E. coli* was confirmed by biochemical tests including Indole, triple sugar iron, Urea, and Simmons Citrate, and Methyl Red–Voges–Proskauer; *Enterococcus* was confirmed by biochemical tests including Brain Heart Infusion agar, Brain Heart Infusion Broth, Brain Heart Infusion Broth with 6.5% NaCl, and Bile Esculin reactions. Confirmation of *Campylobacter* was done by biochemical tests and gram stain morphology for dairy samples collected between 2006 and 2007 and by biochemical tests and molecular analysis for water samples collected in subsequent years. The biochemical tests for *Campylobacter* included Hippuric acid, Oxidase, and Catalase reactions. For molecular analysis, we used a specific PCR described previously (Fermér and Engvall, 1999) to identify thermophilic campylobacters. *Escherichia coli* O157:H7 was confirmed by PCR using primers and PCR conditions described by Paton and Paton (2003). Concentrations of confirmed bacteria for each sample were calculated and expressed as colony-forming units (CFU) $g^{-1}$ or mL for dairy surface solid and water samples and as number of CFU per 100 mL for groundwater samples.

For enumeration of *Salmonella*, 142- and 47-mm filters were inserted into 20- or 5-mL BPWs, respectively, and incubated at 37°C for 24 h. After incubation, 10 μL of BPW enrichment was transferred to 1 mL of Rappaport–Vassiliadis broth and incubated. Five microliters of the Rappaport–Vassiliadis enrichment was plated onto xylose lysine deoxycholate agar. Presumptive *Salmonella* colonies were confirmed biochemically using triple sugar iron, urea, and lysine iron agar. The numbers of confirmed positive reactions of each filtration (volume and replicate) were used for calculating *Salmonella* concentrations using a MPN calculator (i2workout.com/mcuriale/mpn/index.html) and expressed as MPN $g^{-1}$ or mL for dairy surface solid and water samples and as MPN per 100 mL for each groundwater sample.

## Antibiotic Resistance Assay of Indicator Bacteria

Antibiotic-resistant profiles were determined for a subset of generic *E. coli* and *Enterococcus* obtained from groundwater. A gram-negative Sensititre plate (CMV2AGNF) and a gram-positive Sensititre plate (CMV3AGPF) (Trek Diagnostic Systems Inc.) were used for *E. coli* and *Enterococcus*, respectively, according the manufacturer's instructions. *Escherichia coli* strains ATCC25922 and ATCC35218 and *Enterococcus* strain ATCC29212 were used as quality control strains. The minimum inhibitory concentration values were the lowest concentrations of antibiotics that inhibit visible growth of bacteria. Interpretations of antibiotic resistance were set by the criteria of the minimum inhibitory concentration breakpoints developed by the Veterinary Antimicrobial Susceptibility Testing Subcommittee of the Clinical and Laboratory Standards Institute (Watts et al., 2008). An isolate of bacteria is defined as multiple-drug resistant if the isolate is resistant to at least three antibiotics.

## Statistical Analysis

Because *Campylobacter* was not detected and *Salmonella* and *E. coli* O157:H7 were each only detected in two samples, statistical analyses were conducted on generic *E. coli* and *Enterococcus* for on-dairy, near-dairy, and nondairy water samples. Mean concentrations of generic *E. coli* and *Enterococcus* were calculated and evaluated using one-way ANOVA (Minitab, Minitab Inc.)

tests to determine statistical differences between bacterial concentrations within well types and designations. Significance was set at $P \leq 0.05$ for each test. For on-dairy samples, the association between bacteria concentrations and well types (domestic vs. monitoring well), the primary dairy facility component in upgradient proximity to wells relative to groundwater flow (lagoon, corral, manure-treated field), water table depth (shallow: <5 m deep; deep: 13 to >30 m deep) and season (winter [January 2008], spring [April 2008 and March 2009], and fall [September 2008]), was analyzed using Poisson regression (STATA 12 software), with $P \leq 0.05$ required for inclusion in the final model. For near-dairy and nondairy samples, the association between bacteria concentrations and well location (near-dairy well vs. nondairy well), the distance to the nearest dairy corral or lagoon (disregarding groundwater gradients), field temperature, pH, salinity, solute concentrations, and sampling event season were analyzed using the same Poisson regression. Two wells were excluded as outliers because they showed extreme differences in bacterial counts relative to the overall dataset. We did not consider groundwater flow direction in the determination of domestic well distance to dairy because gradients are highly variable, transient, and often controlled by seasonal irrigation wells that pump at rates exceeding 15 $m^3$ $min^{-1}$.

# Results

## Survey of Fecal Indicator and Pathogenic Bacteria at Land Surfaces on Dairies

Surveys of fecal indicator and pathogenic bacteria concentrations on land surfaces in CAFO dairies are shown in Fig. 2. Figure 2 also illustrates two typical variations of the spatial layouts of Central Valley freestall dairy management units, albeit without the complete layout of manure-treated fields surrounding these dairies. The two fecal indicator bacteria, generic *E. coli* and *Enterococcus*, were widely distributed in all solid and water samples of the various surface environments in the two CAFOs. Typical concentrations of these two bacterial indicators in the aboveground matrices (liquid manure slurries and solids) ranged from hundreds of thousands to over two million CFU per 100-mL slurry or CFU $g^{-1}$ solids. Despite their ubiquitous occurrence through dairy management units in contact with manure, much lower concentrations of indicator bacteria were found in the control fields next to each dairy that were not treated with manure (2 to >3 orders of magnitude less).

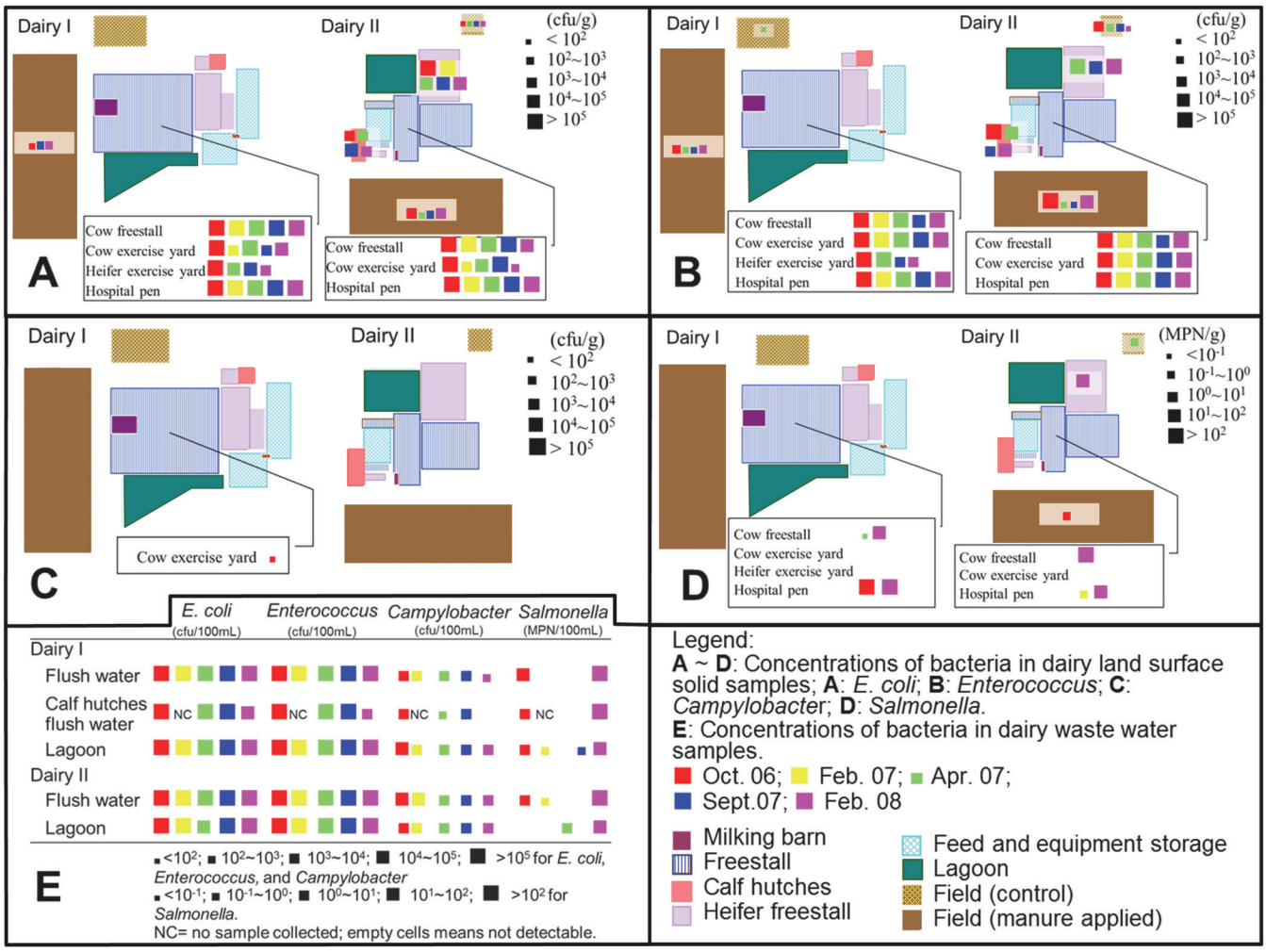


**Fig. 2. Concentration of generic *Escherichia coli* (A), *Enterococcus* (B), *Campylobacter* spp. (C), and *Salmonella* spp. (D) in surface solids of various management units on two San Joaquin Valley dairies in the Stanislaus and Merced County portion of our study area. These schematics represent a scaled version of the dairy management units. (E) Concentrations of these bacteria in flush water, in calf hutches flush water, and in storage lagoons.**

The primary environmental load of *Campylobacter* appeared to be liquid manure slurries and not the large amount of surface manure solids present on the dairies: *Campylobacter* was detected in slurries at concentrations typically between $10^2$ and $10^4$ CFU per 100 mL, whereas it was detected in only a single sample of surface solids. *Salmonella* counts in liquid manure samples were generally lower compared with *Campylobacter* and appeared to have high temporal variability between sampling events. In contrast to *Campylobacter*, *Salmonella* was detected more frequently, if only at low levels, in surface solids on the dairy, particularly in the shaded hospital pen and freestall structures. With the exception of *Salmonella* in April 2007, no pathogens were detected at any time in control fields.

## On-Dairy Monitoring of Fecal Indicator and Pathogenic Bacteria in Groundwater

In on-dairy groundwater samples, *Campylobacter* was not detected in groundwater immediately below the water table (monitoring wells) or in domestic wells, which tap groundwater at several tens to over 100 m below the water table. In contrast, although *Salmonella* and *E. coli* O157:H7 were not present in domestic well water, each occurred in 1% (2/190) of monitoring well samples.

The two *Salmonella* detections occurred during the winter sampling in January 2008. One monitoring well, with a low concentration of 0.04 MPN per 100 mL, was located downgradient of a typical manure-treated field with sandy loam soil and relatively shallow 5-m depth to water table on a dairy located in the SJV. Nitrate and salinity show significant influence from manure applications but are not as high as in other wells located downgradient from manure-treated fields on this or nearby dairies described in Harter et al. (2002). Hence, the well does not appear exceptionally vulnerable to manure leaching. The other monitoring well, with a concentration of 0.02 MPN per 100 mL, was located adjacent to a corral on a TLB dairy overlying 27 m of unsaturated, highly heterogeneous, sandy, loamy, and clayey alluvial sediments. The monitoring well is screened from the water table at 27 to 35 m. Total nitrogen (7 mg $L^{-1}$) and salinity are lower than at nearby wells and do not indicate strong manure influence but may be influenced by recharge from a nearby (150-m) unlined irrigation canal.

The two *E. coli* O157:H7 detections occurred during sampling in March 2009. One sample came from the same well that was positive for *Salmonella* 14 mo earlier. The second detection was in a well located adjacent to a freestall flush lane in a nearby SJV dairy. At both locations, the water table is relatively shallow, at 3 to 5 m below ground surface.

Table 1 shows the survey results of generic *E. coli* in groundwater in CAFOs. Among the 24 samples collected over the four sampling events from on-dairy domestic wells, only one sample was positive for generic *E. coli*, with a concentration of 0.01 CFU per 100 mL. This sample, from a dairy in the SJV study area, was obtained from a well where depth to ground water varies (3–5 m) and that had an unknown screened interval. In contrast, among on-dairy monitoring wells, 24.2% (46/190) of the water samples were positive for generic *E. coli*, with a range of 15.2 to 27.5% between different seasons. Generic *E. coli* was not detected in monitoring wells at two relatively new (<10 yr old) dairy farms with depth to groundwater exceeding 20 m (however, one of these was only sampled in January 2008). Depending on season and farm, mean concentrations of generic *E. coli* in monitoring wells ranged from 0.01 to 35.01 CFU per 100 mL.

**Table 1. Survey of generic *Escherichia coli* in groundwater from concentrated animal feeding operations wells located in the Central Valley, California (2008–2009).**

| Dairy ID | Jan. 2008 | | Apr. 2008 | | Sept. 2008 | | Mar.–Apr. 2009 | |
|---|---|---|---|---|---|---|---|---|
| | % (positive/ sampled wells) | Mean concentration† (±SD) | % (positive/ sampled wells) | Mean concentration† (±SD) | % (positive/ sampled wells) | Mean concentration† (±SD) | % (positive/ sampled wells) | Mean concentration† (±SD) |
| **Monitoring wells (*n* = 190)** | | | | | | | | |
| 36–04 | 0 (0/3) | NA‡ | 0 (0/3) | NA | 0 (0/4) | NA | 0 (0/4) | NA |
| 36–11 | 0 (0/3) | NA | 33.3 (1/3) | 0.02 | ND§ | NA | 0 (0/1) | NA |
| 36–15 | 0 (0/10) | NA | 20.0 (2/10) | 0.03 (0.007) | 21.4 (3/14) | 0.02 (0.006) | 14.3 (2/14) | 0.01(0) |
| 36–19 | 9.1 (1/11) | 5.45 | 36.4 (4/11) | 0.04 (0.06) | 23.1 (3/13) | 0.01 (0.005) | 8.3 (1/12) | 0.05 |
| 36–24 | 0 (0/2) | NA | ND | NA | ND | NA | ND | NA |
| 36–27 | 0 (0/3) | NA | 66.7 (2/3) | 0.70 (0.98) | 0 (0/3) | NA | 0 (0/3) | NA |
| 37–39 | 60.0 (3/5) | 35.01 (30.30) | 33.3 (2/6) | 0.48 (0.65) | 100 (6/6) | 1.58 (3.64) | 87.5 (7/8) | 3.24 (8.40) |
| 37–42 | 33.3 (3/9) | 2.24 (3.78) | 12.5 (1/8) | 0.04 | 11.1 (1/9) | 4.30 | 44.4 (4/9) | 0.75 (1.47) |
| Overall | 15.2 (7/46) | 16.74 (24.58) | 27.3 (12/44) | 0.22 (0.45) | 26.5 (13/49) | 1.06 (2.66) | 27.5 (14/51) | 1.84 (5.93) |
| **Domestic wells¶ (*n* = 24)** | | | | | | | | |
| 36–04 | ND | NA | ND | NA | 0 (0/1) | NA | 0 (0/1) | NA |
| 36–15 | ND | NA | ND | NA | 0 (0/1) | NA | 0 (0/1) | NA |
| 36–19 | 0 (0/1) | NA | 0 (0/1) | NA | 0 (0/1) | NA | 0 (0/1) | NA |
| 36–27 | 0 (0/1) | NA | 0 (0/1) | NA | 0 (0/1) | NA | 0 (0/1) | NA |
| 37–39 | 33.3 (1/3) | 0.01 | 0 (0/3) | NA | 0 (0/3) | NA | 0 (0/3) | NA |
| Overall | 20.0 (1/5) | 0.01 | 0 (0/5) | NA | 0 (0/7) | NA | 0 (0/7) | NA |

† Concentrations were expressed as CFU per 100 mL.

‡ Not applicable.

§ Not done because the well was not accessible or no water was available.

¶ No domestic wells were sampled on dairies 36–11, 36–24, and 37–42.

*Enterococcus* was detected in 97.4% (185/190) of water samples from monitoring wells (Table 2). Despite their ubiquitous presence, concentrations mostly did not exceed 100 CFU per 100 mL. Some extremely high concentrations were detected in monitoring wells at the two SJV dairies with the shallow (<10 m) water table in March and April of 2009. In on-dairy domestic wells, 87.5% (21/24) of water samples tested positive for *Enterococcus* but with overall lower concentrations than in monitoring wells (Table 2).

The concentrations of generic *E. coli* and *Enterococcus* were significantly associated with the type of dairy land use immediately upgradient of monitoring wells, with the depth to water table, and with season. Well type (domestic vs. monitoring) and, thus, depth of well screen below the water table (immediately below the water table vs. production level groundwater) was also a statistically significant factor (Table 3). *Escherichia coli* did not occur in domestic wells at sufficient rates to be included in the statistical model. To assess the association between dairy management units and the occurrence of indicator bacteria, the distribution of types of land use with proximity to wells and the frequency of detection of generic *E. coli* and *Enterococcus* in water from monitoring wells were compared (Fig. 3). The highest frequencies of detection of both generic *E. coli* and *Enterococcus* were associated with monitoring wells immediately downgradient of corrals and manure-treated fields. Monitoring wells downgradient of lagoons had lower concentrations than others but were higher than those of the (deeper screened) on-dairy domestic wells.

## Monitoring of Fecal Indicator and Pathogenic Bacteria in Drinking Water Supply Wells

We detected no pathogenic bacteria in any water samples from the 200 domestic wells sampled in the 2010–2011 campaign regardless of whether the domestic well was nearby or further away from a dairy (near-dairy vs. nondairy). However, 4.5 and 7.5% of near-dairy wells were positive for generic *E. coli* and *Enterococcus*, respectively. Similarly, 5.9 and 10.3% of nondairy wells were positive for generic *E. coli* and *Enterococcus*, respectively (Table 4). Concentrations of generic *E. coli* were not significantly related to the distance from the nearest corral or lagoon, water quality parameters, or seasons (statistical data not shown). However, *Enterococcus* results were significantly different between near-dairy wells and nondairy wells and between seasons and were negatively correlated to potassium concentration (Table 5). Microbial indicators were not significantly associated with other dissolved solutes or water quality parameters in groundwater, including total dissolved solids concentration.

## Antibiotic Resistance Assay of a Subset of Indicator Bacteria

Although only small subsets of bacteria were tested, all isolates of generic *E. coli* and *Enterococcus* demonstrated resistance to at least one antibiotic. Moreover, the majority of generic *E. coli* isolates (63.6%) and *Enterococcus* isolates (86.1%) exhibited multidrug resistance (resistance to three or more drugs) regardless of well type (monitoring vs. domestic wells on CAFOs of the on-dairy survey) or distance from a dairy (near-dairy vs. nondairy) of the well from which samples were collected and used for isolating *E. coli* and *Enterococcus* (Table 6). Among the near-dairy domestic wells, one generic *E. coli* and three *Enterococcus* isolates came from domestic wells on dairy facilities not studied from 2006 to 2009. Like the others, these isolates exhibited multiresistant properties. We found that generic *E. coli* were most often resistant to azithromycin, chloramphenicol, trimethoprim/sulfamethoxazole, and tetracycline, whereas *Enterococcus* species

**Table 2. Survey of *Enterococcus* spp. in groundwater from concentrated animal feeding operations wells in the Central Valley, California (2008–2009).**

| Dairy ID | Jan. 2008 | | Apr. 2008 | | Sept. 2008 | | Mar.–Apr. 2009 | |
|---|---|---|---|---|---|---|---|---|
| | % (positive/ sampled wells) | Mean concentration† (±SD) | % (positive/ sampled wells) | Mean concentration† (±SD) | % (positive/ sampled wells) | Mean concentration† (±SD) | % (positive/ sampled wells) | Mean concentration† (±SD) |
| **Monitoring wells (*n* = 190)** | | | | | | | | |
| 36–04 | 100 (3/3) | 6.71 ± 6.09 | 100 (3/3) | 0.37 (0.34) | 100 (4/4) | 4.75 (6.05) | 100 (4/4) | 3.58 (4.48) |
| 36–11 | 100 (3/3) | 7.08 ± 6.36 | 100 (3/3) | 5.11 (3.62) | ND‡ | NA§ | 100 (1/1) | 13.60 |
| 36–15 | 100 (10/10) | 2.83 ± 3.67 | 100 (10/10) | 5.52 (8.65) | 85.7 (12/14) | 19.49 (48.13) | 100 (14/14) | 11.99 (11.34) |
| 36–19 | 100 (11/11) | 8.87 ± 7.08 | 100 (11/11) | 3.22 (3.73) | 100 (13/13) | 4.87 (5.72) | 100 (12/12) | 5.11 (4.77) |
| 36–24 | 0 (0/2) | NA | ND | NA | ND | NA | ND | NA |
| 36–27 | 100 (3/3) | 11.82 ± 11.20 | 66.7 (2/3) | 3.14 (4.33) | 100 (3/3) | 26.56 (21.05) | 100 (3/3) | 5.57 (4.12) |
| 37–39 | 100 (5/5) | 43.19 ± 56.28 | 100 (6/6) | 30.14 (43.93) | 100 (6/6) | 22.15 (10.60) | 100 (8/8) | 3822.88 (7181.30) |
| 37–42 | 100 (9/9) | 15.21 ± 21.06 | 87.5 (7/8) | 4.58 (6.02) | 88.9 (8/9) | 36.92 (90.24) | 100 (9/9) | 263.59 (726.44) |
| Overall | 100 (46/46) | 12.63 ± 23.27 | 95.5 (42/44) | 7.29 (17.21) | 93.9 (46/49) | 17.33 (45.38) | 100 (51/51) | 651.55 (3036.82) |
| **Domestic wells¶ (*n* = 24)** | | | | | | | | |
| 36–04 | ND | NA | ND | NA | 100 (1/1) | 0.38 | 100 (1/1) | 0.30 |
| 36–15 | ND | NA | ND | NA | 100 (1/1) | 0.35 | 100 (1/1) | 0.60 |
| 36–19 | 100 (1/1) | 0.80 | 100 (1/1) | 3.66 | 100 (1/1) | 0.28 | 100 (1/1) | 1.30 |
| 36–27 | 100 (1/1) | 4.59 | 100 (1/1) | 0.27 | 100 (1/1) | 3.12 | 100 (1/1) | 0.40 |
| 37–39 | 66.7 (2/3) | 2.39 (0.26) | 66.7 (2/3) | 0.10 (0.05) | 66.7 (2/3) | 0.42 | 100 (3/3) | 7.0 (6.0) |
| Overall | 80.0 (4/5) | 2.54 (1.56) | 80.0 (4/5) | 1.03 (1.78) | 85.7 (6/7) | 0.91 (1.24) | 100 (7/7) | 3.37 (4.86) |

† Concentrations were expressed as CFU per 100 mL.

‡ Not done because the well was not accessible or no water was available.

§ Not applicable.

¶ No domestic wells were sampled on dairies 36–11, 36–24, and 37–42.

**Table 3. Factors associated to the concentrations of generic *Escherichia coli* and *Enterococcus* spp. in groundwater in confined animal feeding operations in the Central Valley, California (2008–2009).**

| Bacteria | Factor | Coefficient | SE | *P* value | 95% confidence interval |
|---|---|---|---|---|---|
| Generic *E. coli* | land use | | | | |
| | corral† | 0 | – | – | – |
| | field | −2.824 | 0.331 | 0.000 | −3.473 to −2.175 |
| | lagoon | −2.008 | 0.484 | 0.000 | −2.958 to −1.058 |
| | upgradient | −6.098 | 2.887 | 0.035 | −11.758 to −0.438 |
| | water table depth | | | | |
| | deep† | 0 | – | – | – |
| | shallow | 3.825 | 0.381 | 0.000 | 3.077 to 4.572 |
| | season‡ | | | | |
| | spring† | 0 | – | – | – |
| | fall | 0.436 | 0.360 | 0.226 | −0.269 to 1.141 |
| | winter | 2.734 | 0.255 | 0.000 | 2.233 to 3.235 |
| *Enterococcus* | well type | | | | |
| | monitoring well† | 0 | – | – | – |
| | domestic well | −3.536 | 0.155 | 0.000 | −3.839 to −3.233 |
| | land use | | | | |
| | corral† | 0 | – | – | – |
| | field | −1.239 | 0.021 | 0.000 | −1.280 to −1.197 |
| | lagoon | 1.836 | 0.011 | 0.000 | 1.812 to 1.859 |
| | upgradient | −3.322 | 0.090 | 0.000 | −3.500 to −3.145 |
| | water table depth | | | | |
| | deep† | 0 | – | – | – |
| | shallow | 4.109 | 0.032 | 0.000 | 4.046 to 4.172 |
| | season‡ | | | | |
| | spring† | 0 | – | – | – |
| | fall | −2.828 | 0.036 | 0.000 | −2.899 to −2.757 |
| | winter | −5.874 | 0.056 | 0.000 | −5.984 to −5.764 |

† Referent category for categorical variable.

‡ Spring: Apr. 2008 and Mar./Apr. 2009; fall: Sept. 2008; winter: Jan. 2008.

were most often resistant to tigecycline, quinupristin/dalfopristin, linezolid, chloramphenicol, erythromycin, iprofloxacin, and tetracycline.

# Discussion

The high levels of fecal indicator bacteria in CAFO surface samples are consistent with what we would expect given the large fraction of fecal solids mixed in with these samples, exceeding 50% on a wet weight basis in many samples. High occurrence rates of *E. coli* and *Enterococcus* have also been found on dairies in the northeastern United States (Pradhan et al., 2009). *Enterococcus* has been found in surface water and groundwater affected by a concentrated swine feeding operation in the mid-Atlantic United States (Sapkota et al., 2007). Similarly, pathogenic bacteria, including *Campylobacter*, *E. coli* O157:H7 and *Salmonella*, have been commonly detected in dairy environments elsewhere but at significantly lower concentrations than indicator bacteria (Murinda et al., 2004; Toth et al., 2013; Ravva and Sarreal, 2014). In our survey, the primary source of *Campylobacter* among the various dairy management units is difficult to discern; solids samples did not yield significant information, whereas freestall and lagoon water, which consistently yield significant *Campylobacter*, may originate, with the exception of manure-treated fields, from any of the dairy management units shown in Fig. 2.

Calf hutch flush water originates from tap water. Hence, the consistent occurrence of both *Campylobacter* and *Salmonella*

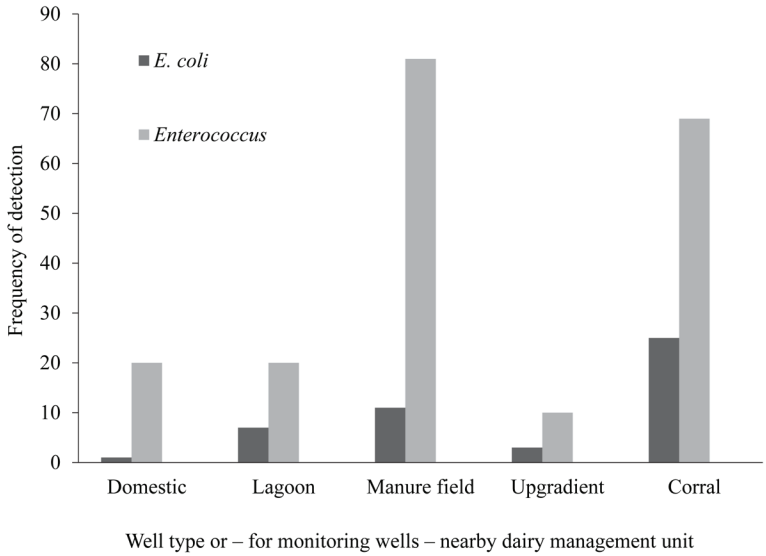


**Fig. 3. Frequencies of detection of generic *Escherichia coli* and *Enterococcus* spp. in groundwater from monitoring wells and proximity to different types of land on concentrated animal feeding operations in the Central Valley, California (2008–2009).**

**Table 4. Survey of indicator and pathogenic bacteria in groundwater from domestic wells with and without likely dairy influence in the Central Valley, California (2010–2011).**

| Well types† | Bacteria | Positive wells | Concentration‡ |
|---|---|---|---|
| | | *n* (%) | CFU per 100 mL |
| Near-dairy wells (*n* = 132) | | | |
| Within dairy facility (*n* = 12) | generic *E. coli* | 1 (8.3) | 0.72 |
| | *Enterococcus* spp. | 3 (25.0) | 0.13 (0.15) |
| | *Campylobacter* spp. | 0 (0) | NA§ |
| | *Salmonella* spp. | 0 (0) | NA |
| | *E. coli* O157:H7 | 0 (0) | NA |
| Outside of dairy facilities (*n* = 120) | generic *E. coli* | 5 (4.2) | 0.26 (0.39) |
| | *Enterococcus* spp. | 7 (5.8) | 16.93 (43.33) |
| | *Campylobacter* spp. | 0 (0) | NA |
| | *Salmonella* spp. | 0 (0) | NA |
| | *E. coli* O157:H7 | 0 (0) | NA |
| Nondairy wells (without dairy influence) (*n* = 68) | | | |
| | generic *E. coli* | 4 (5.9) | 1.93 (3.33) |
| | *Enterococcus* spp. | 7 (10.3) | 15.03 (37.15) |
| | *Campylobacter* spp. | 0 (0) | NA |
| | *Salmonella* spp. | 0 (0) | NA |
| | *E. coli* O157:H7 | 0 (0) | NA |

† Wells are defined as "near-dairy" if the distance between a well and a dairy lagoon or corral is ≤2.4 km and as "nondairy" if the distance between a well and a dairy lagoon or corral is >2.4 km.

‡ Values for concentration are means with SD in parentheses.

§ Not applicable.

indicates that calf hutches are at least one of the contributing sources of pathogens. *Salmonella* was also most common in liquid slurries but also occurred in the surface solids that had little exposure to direct sunlight (hospital barn and freestall lots). The lack of pathogens on other surface solids is consistent with earlier findings (Nicholson et al., 2005), probably due to inactivation after exposure to ambient conditions, including higher temperatures (Hoar et al., 1999; Sinton et al., 2007; Moriarty et al., 2011). Survival of pathogens in the dairy environment depends on numerous complex environmental factors (Toth et al., 2011; Ravva and Sarreal, 2014), reflected here by the lack of strong seasonal signatures despite the high contrast between hot, dry summers and moist, cool winters. The lower frequencies and concentrations of *Campylobacter* and *Salmonella* in liquid samples, when compared with fecal indicator concentrations, may largely be due to those being shed only by infected animals, which may represent only a fraction of the herd.

Indicator bacteria and pathogens occurring on dairy CAFOs may be subject to transport into the environment and surrounding dairies through surface runoff to streams and through incidental or intentional infiltration into and transport through unsaturated porous medium to groundwater (Joy et al., 1998; Unc and Goss, 2004; Searcy et al., 2005; Park et al., 2012). Unc et al. (2012) found at least three orders of magnitude reduction in *Enterococcus* concentration across the 3-m unsaturated zone profile on one of the two SJV dairies. Li et al. (2014) estimated attenuation rates for generic *E. coli* ranging from three to seven orders of magnitude using 2006–2008 surface samples reported here and a limited number of groundwater samples collected concurrently with surface samples (not included in this study).

There are distinct differences in generic *E. coli* and in *Enterococcus* detection frequencies between monitoring wells located immediately upgradient of dairies, which are comparable to those in domestic wells, and detection frequencies in monitoring wells located within dairies (Fig. 3). Monitoring wells downgradient of corrals and manure-treated fields have much higher detection frequencies than those downgradient of lagoons. The difference may be due to more attenuation of microorganisms

**Table 5. Factors associated to the concentrations of *Enterococcus* spp. in groundwater from domestic wells in the Central Valley, California (2010–2011).**

| Factor | Coefficient | SE | *P* value | 95% CI† |
|---|---|---|---|---|
| Well designation | | | | |
| Nondairy well‡ | 0 | – | – | – |
| Near-dairy well | 1.900 | 0.761 | 0.013 | 0.408 to 3.393 |
| Potassium | −29.955 | 11.284 | 0.008 | −52.071 to −7.839 |
| Season | | | | |
| Spring‡ | 0 | – | – | – |
| Summer | −2.120 | 4.935 | 0.667 | −11.793 to 7.552 |
| Fall | 1.922 | 0.853 | 0.024 | 0.251 to 3.593 |
| Winter | 0.967 | 1.043 | 0.354 | −1.077 to 3.010 |

† Confidence interval.

‡ Referent category for categorical variable.

**Table 6. Number of antibiotic resistant isolates of generic *Escherichia coli* and *Enterococcus* spp. isolated from groundwater.**

| Bacteria | Campaign | Type of well | Isolates tested | Isolates† resistant to ≥1 antibiotics | Isolates† resistant to ≥3 antibiotics |
|---|---|---|---|---|---|
| *Escherichia coli* | on-dairy | monitoring wells | 2 | 2 | 2 |
| | | domestic wells | 0 | NA‡ | NA |
| | 2010–2011 | near-dairy wells§ | 5 | 5 | 3 |
| | | nondairy wells | 4 | 4 | 2 |
| | total | | 11 | 11 | 7 |
| *Enterococcus* | on-dairy | monitoring wells | 18 | 18 | 16 |
| | | domestic wells | 4 | 4 | 2 |
| | 2010–2011 | near-dairy wells | 8 | 8 | 8 |
| | | nondairy wells | 6 | 6 | 5 |
| | total | | 36 | 36 | 31 |

† Each tested isolate was from different wells.

‡ Not applicable.

§ Near-dairy wells are located at a distance not exceeding 2.4 km from the nearest dairy lagoon or corral; wells are otherwise defined as "nondairy."

by the fine-grained sludge layer commonly found on the bed of storage lagoons than in the fractured and mechanically impacted corral surface. Due to mechanical preparation (plowing etc.), fields provide a more open surface with significantly higher infiltration rates than either corrals or lagoon beds, and thus less filtration of colloidal microorganisms. Similarly, the already low risk of pathogenic contamination may be lowest in the vicinity of storage lagoons relative to other dairy management units. Coincidentally, the two *Salmonella* occurrences were not associated with lagoon leakage.

In agreement with previous reports, we find that microbial groundwater contamination generally decreases with increased well depth (Goss et al., 1998; Pitkänen et a., 2011). Monitoring wells are in closer proximity to animal production areas and waste storage facilities, whereas domestic wells are screened at some depth below the water table. Concentrations of the most commonly found bacteria in both types of wells, *Enterococcus*, is therefore not surprisingly significantly less in domestic wells on dairies than in their monitoring wells (Fig. 1). On the other hand, similar to the survey conducted in private wells used for drinking water in northeastern Ohio (Won et al., 2013), no significant correlation was found between *E. coli* concentrations and potential pollution factors in our domestic wells survey.

Concentrations of *Enterococcus* were significantly associated with potassium concentration in groundwater. The electrochemical properties of soil can alter the transportation of bacteria (Unc and Goss, 2004), which may explain the relationship of *Enterococcus* and the concentrations of potassium in groundwater. Ionic strength (as indicated by total dissolved solids) was not a significant factor. The association may also be explained by the fact that highest potassium concentrations are often found in the anaerobic shallow ammonium plumes emanating from older storage lagoons overlying shallow groundwater (Harter et al., 2002). The lagoon bed may be a significant filter of microbial contaminants, thus becoming a source of relatively low indicator bacteria counts while also being a source of high potassium concentrations.

The main reason for the low detection rate of pathogens in monitoring wells and their absence in domestic wells appears to be the strong attenuation in the unsaturated zone combined with physical limits of detection: assuming there is no inhibition within the assay, as few as 1 CFU per volume filtered can be detected with membrane or pressure vessel filtration direct plating methods. For the various *Salmonella* MPN methods used for this work, the detection limit varies from 0.00013 to 140 MPN $mL^{-1}$ or g. Given the three-to-seven order of magnitude attenuation estimated from highly prevalent indicator organisms at our dairy sites (Li et al., 2014), we would expect pathogen concentrations to follow a similar trend in reduction. Hence, given the lower starting concentrations in manure slurries compared with generic *E. coli* and *Enterococcus* (Fig. 2), pathogen concentrations would be expected to be mostly below detection limits of these water assays. This is confirmed by the fact that even the shallow-most groundwater samples below dairies did not yield any *Campylobacter* occurrence. *Escherichia coli* O157:H7 and *Salmonella* are each detected in 2 of 51 on-dairy monitoring wells across eight dairies but in only one of four sampling campaigns. Also consistent with attenuation rates estimated from indicator organisms, we detected no pathogenic bacteria in the survey of on-dairy, near-dairy, or nondairy domestic wells. This suggests that 3 to 30 m of unsaturated alluvial sediments with silty sand, loamy sand, fine sand, and sandy loam or finer materials provides significant protection from pathogenic transport to the water table. Assuming that human water consumption on the dairy is limited to domestic wells, these data suggest that the risk of human waterborne illness from consumption of domestic well water is very low. Given that normal water consumption patterns of children and adults can range from 1 to 3 L $d^{-1}$, using water from monitoring wells as a source of drinking water or using other shallow on-dairy sources for municipal purposes may pose an unacceptable risk of waterborne transmission if not treated. Groundwater supplies for drinking water, typically obtained tens to over 100 m below the water table, are well protected in these landscape settings.

The high pathogenic loading at the land surface of dairy CAFOs may pose a significant risk to groundwater in other hydrogeologic and well settings: Horn and Harter (2009) recognized that poor well seal construction may be a significant risk factor for groundwater contamination. Compromised wells allow for rapid transport through the gravel filter of a domestic well.

Soils with significant macropores (e.g., fractured clay or till) or of much less thickness than 3 m overlying more vulnerable sand and gravel aquifers or highly fractured rock aquifers may be at significant risk near similarly managed CAFOs. We note

that most of the surveyed domestic wells are in the vicinity of a private onsite wastewater treatment system (septic system) that may serve as a source for enteric indicator bacteria and pathogens (Bremer and Harter, 2012). This and the use of manure as soil amendment may explain the occurrence of indicator bacteria at significant distances from dairies.

Generic *E. coli* and *Enterococcus* are among the commonly used indicator organisms for monitoring microbiological quality of water (Edberg et al., 1997). It is generally assumed that indicator microbial pollution poses a significant risk of pathogen occurrence due to similar transport mechanisms (Goss et al., 2002). In the investigated alluvial systems, it appears that the significant difference in concentration of indicator versus pathogenic bacteria at dairy surfaces leads to the significant occurrence of indicator organisms, whereas the actual risk of pathogenic bacteria occurrence is very low. On the other hand, the absence of indicator organisms is not a guarantee of clean water. In the two cases of pathogen detection immediately below the water table, samples were negative for generic *E. coli* and had average *Enterococcus* concentrations (data not shown). Other studies have also reported the lack of correlation between fecal indicator bacteria and pathogens in groundwater (Ferguson et al., 2012).

High spatial attenuation rates of *E. coli* and other fecal indicator bacteria through sandy aquifers have been found by other studies (Pang, 2009; Knappett et al., 2012). Although we did not conduct microbial source tracking studies to determine the sources of generic *E. coli* and *Enterococcus* spp. in groundwater, the resistance to multiple veterinary or medical drugs among a subset of these bacteria points to human- or animal-waste–derived sources of antibiotic-resistant organisms. The presence of antibiotic-resistant bacteria within groundwater from CAFO-specific monitoring wells suggests these are animal derived and from fecal-rich environments within CAFOs. Several studies have documented antibiotic-resistant bacteria in groundwater under the influence of concentrated swine operations (Chee-Sanford et al., 2001; Anderson and Sobsey, 2006; Mackie et al., 2006; Koike et al., 2007; Sapkota et al., 2007).

A previous study, which collected information about antibiotics use on two participating CAFOs (Watanabe et al., 2010), also sampled surface solids for antibiotics within the same 2006–2008 campaign. They detected varied antibiotic residues, such as tetracycline, lincomycin, trimethoprim, sulfadimethoxine, and sulfamethazine. According to a survey conducted by the USDA Animal and Plant Health Inspection Service (APHIS), sulfonamide and tetracycline are among the most common antibiotics used in dairies in the United States (APHIS, 2015). In the present study, we found that genetic *E. coli* and *Enterococcus* isolates were resistant to many of these antibiotics that are commonly deployed and used in the United States. Additionally, we found that the antibiotic resistance patterns of generic *E. coli* within groundwater samples were consistent with or similar to generic *E. coli* within surface water samples from dairy CAFOs (Gibson and Schwab, 2011a,b; Li et al., 2014). Our findings indicate that there is significant potential risk of groundwater contamination with antibiotic-resistant bacteria derived from CAFOs even if the subsurface environment is not suitable to transmit pathogenic bacteria.

An earlier study documented the public health implications regarding multiple antibiotic-resistant gram-negative bacteria in rural groundwater supplies used as a drinking water source (McKeon et al., 1995). It remains unclear to which degree onsite wastewater treatment systems contribute to antibiotic resistance found in groundwater samples of domestic wells, especially in areas further than 2.4 km away from dairies. Manure amendments are commonly used in irrigated agriculture throughout the region. This suggests an alternative source of antibiotic-resistant bacteria outside the direct zone of influence from dairy facilities. For future work, we propose surveying antibiotic use across dairies and assessing antibiotic resistance within both gram-positive and gram-negative bacteria from dairy environments. This could include groundwater and surface water with commonly used, site-specific antibiotics.

## Conclusion

In groundwater immediately below the water table and in groundwater at production depth in this irrigated agricultural region overlying an alluvial aquifer, we detected *E. coli* in 15 to 27% and *Enterococcus* in 80 to 100% of groundwater in dairy CAFOs. Both indicator bacteria were detected at much lower rates (≤10%) in groundwater at near-dairy and nondairy domestic wells of the same region. The prevalence of *Enterococcus* was significantly associated with the influence of dairy operations. We did not detect pathogenic bacteria within domestic wells, on-dairy, near-dairy, or in nondairy areas through use of filtrate from 10-L water samples and enrichment; however, most isolates of *E. coli* and *Enterococcus* from production depth groundwater exhibited multidrug antibiotic resistance. These findings outline the broad reach of antibiotic-resistant bacteria in the groundwater of this region. Applying good agricultural practices on CAFOs and improving well maintenance practices such as well seals (Rudolph et al., 1998) are among several possible measures to prevent bacteria at CAFO surfaces from entering groundwater. From a public health perspective, continuous and effective groundwater monitoring is important for protection from the residual microbiological risks associated with contaminated groundwater. Further work is needed to better understand the sources, occurrence, and public health implications of antibiotic resistance in enterically derived and/or environmental bacteria within groundwater environments.

## Acknowledgments

Funding for our research projects was provided by the California State Water Resources Control Board Contracts 03-244-555-01, 04-184-555-0, and 11-168-150. We gratefully acknowledge the support and collaboration of the eight dairy landowners and of the many domestic well owners who allowed us to sample wells on their property. We also acknowledge the project support from JohnFranco Saraceno and Dana Erickson at the USGS. Comments from two anonymous reviewers greatly improved the manuscript.

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