Title:

Fecal indicator and pathogenic bacteria and their antibiotic resistance in alluvial groundwater of an irrigated agricultural region with dairies.

Short title:

Dairy groundwater microbiological quality

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Abbreviations:

CAFOs: Concentrated animal feeding operations
CFU: Colony-forming unit
MPN: Most probable number

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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 confined animal feeding operations (CAFOs), and 200 small (domestic and community supply district) supply wells across the entire region. *Campylobacter* was not detected in groundwater while *E. coli* O157:H7 and *Salmonella* were each detected in 2 of 190 CAFO monitoring well samples. Non-pathogenic 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 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 to well depth, seasons, 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 to any factors but concentrations of *Enterococcus* were significantly associated to 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.

Keywords: CAFOs, dairy, groundwater, bacteria, antibiotic resistance

Introduction

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 *Campylobacte*r (Dodson and LeJeune, 2005), *E. 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, etc. (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). CAFOs 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 ground-water (Embrey and Runkle, 2006). *E. coli* is able to travel long distances underground and is a useful indicator of fecal contamination of groundwater (Foppen and Schijven, 2006).

Furthermore, 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; Dues 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., 2009;

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

81 Monitoring of fecal indicator and pathogenic bacteria in groundwater is important for
82 assessing the risk of microbial contamination of groundwater, especially in regions potentially
83 influenced by CAFOs. We first conducted a pilot survey to estimate the loads of fecal indicator
84 bacteria and pathogenic bacteria in the environment of two CAFOs. We then conducted a
85 systematic survey of indicator bacteria (generic *E. coli* and *Enterococcus*), pathogenic bacteria
86 (*Campylobacter*, *E. coli* O157:H7, and *Salmonella*), and antibiotic resistance in groundwater.
87 The survey was conducted at four groundwater transport scales: a) in groundwater immediately
88 below the water table at the dairy sites; b) in production aquifers immediately below dairies, c) in
89 production aquifers within the vicinity of dairies, and d) in production aquifers away from
90 dairies.

91 The study was conducted in the unconfined alluvial aquifer system of the Central Valley of
92 California, USA, which underlies an irrigated agricultural region with a large number of dairy
93 CAFOs (Figure 1). The survey included repeated, seasonal sampling events in monitoring and
94 domestic wells of eight commercial dairies followed by a broader survey of private domestic
95 wells across the region. A subset of generic *E. coli* (gram negative) isolates and *Enterococcus*
96 (gram positive) isolates from groundwater collected in these surveys were assessed for their
97 susceptibility to antibiotics. The objective of our work was to determine the frequency and
98 magnitude of indicator and pathogenic bacteria and their antimicrobial resistance in groundwater
99 at various distances from their source, assess risk factors related to microbial contamination of
100 groundwater, and further determine antibiotic resistance characteristics of bacteria in

101 groundwater. The working hypothesis was that wells with close proximity to CAFOs are more
102 vulnerable to microbial contamination and antibiotic resistance.
103
104 **Materials and Methods**
105 **Study area**
106 The Central Valley is an area of intensive agricultural production with 3 million ha, nearly
107 two-thirds of the total land area, devoted to irrigated farming (Burow et al., 2008). Sources of
108 irrigation water include both groundwater and surface water (Faunt et al., 2009). Irrigated crops
109 on or near dairies include leafy greens used for human consumption. Rural communities and
110 households and many urban areas rely on groundwater as their sole source of drinking water,
111 with minimal or no water treatment. Microbial contamination of groundwater is therefore a
112 significant concern for food safety and human health in this region.
113 The study area was comprised of the four counties with the largest concentration of dairies in
114 the California Central Valley: Stanislaus, Merced, Tulare, and Kings Counties (Figure 1). The
115 underlying Central Valley aquifer system is formed by unconsolidated alluvial fan and fluvial
116 basin sediments of varying quaternary and late tertiary ages. These sediments comprise the upper
117 500 to 1,000 m of thicker continental and underlying, older marine sediments (DWR, 2004; Page
118 et al., 1986). Hydraulic conductivity can vary greatly depending on the particle size of
119 sediments: coarse fraction hydraulic conductivity and fine fraction hydraulic conductivity have
120 been estimated to be 1,000 m d^{-1} and less than 0.1 m d^{-1}, respectively (Faunt et al., 2009). The
121 Central Valley is broadly divided into three contiguous sub-basins, the northern Sacramento
122 Valley, the southcentral San Joaquin Valley (SJV) and the southern Tulare Lake Basin (TLB)

123 (Gronberg et al., 1998). Stanislaus and Merced Counties are within the SJV, while Tulare and
124 Kings Counties are within the TLB.

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

133 The Central Valley has a Mediterranean climate with hot, dry summers and a rainy season
134 typically lasting from November through April. Average annual precipitation in the study area is
135 310 mm. The region supports 250 including tree fruit, nuts, vineyards, vegetables, rice, cotton,
136 and forage crops (corn, sorghum, grains, and alfalfa). Approximately 1.7 million mature cows
137 plus support cattle, about three-quarters of the California dairy herd, are located on less than
138 1,500 dairy farms (United States Environmental Protection Agency, 2012), mostly in the SJV
139 and TLB portion of the Central Valley.

140 Dairies are operated as CAFOs; they house mature animals in freestalls with exercise yards
141 (freestall dairies) or in open lots (drylot dairies). Dairies collect stormwater runoff from their
142 corrals (exercise yards, open lots, other animal holding areas) and washwater from their milking
143 barn in storage lagoons. Animal waste (manure) in freestall dairies is collected in concrete lanes
144 that are frequently flushed with recycled storage lagoon water. Manure solids are mechanically
145 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, while 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) upon 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*, ×4 replicates of each weight or volume were suspended in 50-m L of buffered peptone water (BPW):10.0-g, 1.0-g, and 0.1-g of solid samples or 1.0-ml, 0.1-ml, and 0.01-ml of water samples, followed by the MPN (most probable number) 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. 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 are located in Kings County and four dairies in Tulare County, all over clayey to sandy loam soils with depth to groundwater ranging from 15 m to over 30 m. On each dairy farm, groundwater samples were collected from 5.1 cm diameter PVC monitoring wells constructed with 3 m to 6 m long well screens in the first non-clayey 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

192 available of water. In total, 190 samples were collected from 46 monitoring wells and 24 samples
193 were collected from 5 domestic on-dairy wells.
194
195 **Near-Dairy and Non-Dairy groundwater monitoring**
196 In 2010 and 2011, we extended our survey to general private domestic wells including six
197 small community service district wells across the four county regions. Domestic wells were
198 chosen based on responses from property owners to newspaper ads and to flyers mailed to rural
199 residents. In total, 200 domestic wells were enrolled (half in the SJV and half in the TLB) and
200 each well was sampled once between summer 2010 and summer 2011 (Lockhart et al., 2013).
201 Spatial analysis was used to determine the distance between a well and the nearest dairy corral or
202 lagoon. Wells located within 2.4-km from a dairy corral or dairy storage lagoon, including 12
203 domestic wells located on previously unsampled dairy properties, were classified as "near-dairy
204 wells" (132 wells), otherwise, they were classified as "non-dairy" (68 wells). Non-dairy wells
205 were considered to have low likelihood to have dairy management units within their recharge
206 source area. All wells were located in the vicinity of irrigated agricultural lands, some of which
207 may have manure applied by growers for soil amelioration (Lockhart et al., 2013).
208
209 **Groundwater sampling and filtering**
210 We developed and tested a novel approach for collecting microbial groundwater quality
211 samples from dairies. Details of the microbial field sampling protocol for monitoring wells are
212 described in Harter et al. (2014). For monitoring wells a portable, submersible, variable speed,
213 stainless steel Grundfos™ RediFlo2 pump (Enviro-Equipment, Inc.) was used. Purging volumes
214 prior to 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. 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 – 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) upon arrival at the laboratory, and processed within 24 h after collection.

For water samples collected on-dairy 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 volume filtered for turbid water samples. Water was filtered using a 10 L dispensing pressure vessel system (EMD Millipore Corporation, Billerica MA) 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, additional 100-ml was filtered for generic *E. coli* and 100-ml 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*, ×4 replicates of each volume were filtered: 2000-ml, 200-ml, and 20-ml.

For water samples collected in near-dairy and non-dairy locations between 2010 and 2011, a 50 L water sample was collected and immediately concentrated using a hollow-fiber ultrafiltration (UF) 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, Lexington, MA); 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%

238 for *E. coli* O157:H7. The retentates used for generic *E. coli* and *Enterococcus* were further split
239 into two aliquots of 5% and 95% respectively to ensure countable colonies on plates. All
240 retentates were filtered through 47-mm diameter 0.45-μm pore size nitrocellulose membrane
241 filters. For quantitative detection of *Salmonella*, ×4 replicates of each volume were filtered 50-
242 ml, 5-ml, and 0.5-ml.
243 For all groundwater samples, electrical conductivity, pH, temperature, and dissolved oxygen
244 were measured in the field using a YSI® 556Multi-Parameter Water Quality sensor. Separate
245 water samples were collected for laboratory analysis of nitrate plus nitrite and major dissolved
246 ions including potassium and sodium (APHA, 2005, US EPA 1993, 2015). Depth to water was
247 measured prior to sampling monitoring wells. Approximately 40% property owners provided
248 information of domestic well structure and water table depth (Lockhart et al., 2013).
249
250 **Detection of fecal indicator and pathogenic bacteria**
251 Immediately after filtration, filters were placed onto CHROMAgar EC plates for detection of
252 generic *E. coli*, mEI *Enterococcus* Indoxyl-β-D-Glucoside agar plates for detection of
253 *Enterococcus*, Rainbow and MacConkey agar plates for detection of *E. coli* O157:H7, and
254 Campy-Line agar (CLA) for detection of *Campylobacter*. CHROMagar EC plates were
255 incubated at 35°C for 2 h followed by incubation at 44.5°C for 24 h; mEI plates were incubated
256 at 41.0°C for 24 ~ 48 h; Rainbow and MacConkey plates were incubated at 37°C for 24 h; and
257 CLA plates were incubated in an anaerobic chamber at 42.0°C for 48h. After incubation,
258 presumptive bacterial colonies were confirmed by biochemical tests and/or molecular analysis.
259 Generic *E. coli* was confirmed by biochemical tests including Indole, Triple Sugar Iron (TSI),
260 Urea, and Simmons Citrate, and Methyl Red–Voges-Proskauer (MR-VP); *Enterococcus* was

confirmed by biochemical tests including Brain Heart Infusion agar, Brain Heart Infusion Broth (BHIB), BHIB 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 (Fermer and Engvall, 1999) to identify thermophilic campylobacters. *E. 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 number of CFU/g or ml for dairy surface solid and water samples and as number of CFU/100-ml for groundwater samples.

For enumeration of *Salmonella*, 142-mm and 47-mm filters were inserted into 20-ml or 5-ml BPW respectively and incubated at 37°C for 24 h. Following incubation 10-µL of BPW enrichment was transferred to 1-ml of RV and incubated. Five µL of the RV enrichment was plated onto XLD agar. Presumptive *Salmonella* colonies were confirmed biochemically using TSI, 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 (Curiale) and expressed as MPN/g or ml for dairy surface solid and water samples and as MPN/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 (G-) Sensititre® plate (CMV2AGNF) and a Gram

positive (G+) Sensititre® plate (CMV3AGPF) (Trek Diagnostic Systems Inc., Westlake, OH) were used for *E. coli* and *Enterococcus* respectively, according the manufactures instructions. *E. coli* strains (ATCC 25922, ATCC35218) and *Enterococcus* strain (ATCC29212) were used as quality control strains. The Minimum Inhibitory Concentration (MIC) values were the lowest concentrations of antibiotics that inhibit visible growth of bacteria. Interpretations of antibiotic resistance `were set by the criteria of the MIC breakpoints developed by the Veterinary Antimicrobial Susceptibility Testing Subcommittee of the Clinical and Laboratory Standards Institute (CLSI) (Watts, 2008). An isolate of bacteria is defined as multiple-drug resistant if the isolate is resistant to ≥3 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 non-dairy water samples. Mean concentrations of generic *E. coli* and *Enterococcus* were calculated and evaluated using one-way ANOVA (Minitab, Minitab Inc, State College, Pennsylvania) 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: less than 5-m; deep: 13-m to over 30-m) and season (winter - Jan'08, spring - Apr'08, Mar'09, and fall - Sept'08) were analyzed using Poisson regression (STATA 12 software, College Station, Texas), with $P \leq 0.05$ for inclusion in the final model. For near-dairy

and non-dairy samples, the association between bacteria concentrations and well location (near-dairy well vs. non-dairy 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, showing 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, since 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 Figure 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 above-ground matrices (liquid manure slurries and solids) ranged from hundreds of thousands to over two million CFU/100-ml slurry or CFU/g 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).

329 The primary environmental load of *Campylobacter* appeared to be liquid manure slurries and
330 not the large amount of surface manure solids present on the dairies: *Campylobacter* was
331 detected in slurries at concentrations typically between 10^2 and 10^4 CFU/100-ml while it was
332 detected in only a single sample of surface solids. *Salmonella* counts in liquid manure samples
333 were generally lower, compared to *Campylobacter* and also appeared to have high temporal
334 variability between sampling events. In contrast to *Campylobacter*, *Salmonella* was detected
335 more frequently, if only at low levels, in surface solids on the dairy, particularly in the shaded
336 hospital pen and freestall structures. With the exception of *Salmonella* in April '07, no pathogens
337 were detected at any time in control fields.
338
339 **On-Dairy monitoring of fecal indicator and pathogenic bacteria in groundwater**
340 In on-dairy groundwater samples, *Campylobacter* was neither detected in groundwater
341 immediately below the water table (monitoring wells), nor in domestic wells, which tap
342 groundwater at several tens to over one hundred meter below the water table. In contrast
343 *Salmonella* and *E. coli* O157:H7, while not present in domestic well water, each occurred in 1%
344 (2/190) of monitoring well samples.
345 The two *Salmonella* detections occurred during the winter sampling, in January 2008. One
346 monitoring well, with a low concentration of 0.04 MPN/100-ml, was located downgradient of a
347 typical manure-treated field with sandy loam soil and relatively shallow 5 m depth to water table
348 on a dairy located in the SJV. Nitrate and salinity show significant influence from manure
349 applications, but are not as high as in other wells located downgradient from manure-treated
350 fields on this or nearby dairies described in Harter et al. (2002). Hence, the well does not appear
351 exceptionally vulnerable to manure leaching. The other monitoring well, with a concentration of

0.02 MPN/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 m 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 months 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 3-5 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 4 sampling events from on-dairy domestic wells, only 1 was positive for generic *E. coli* with a concentration of 0.01 CFU/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 which 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%-27.5% between different seasons. Generic *E. coli* was not detected in monitoring wells at two relatively new (<10 year 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 CFU/100 ml to 35.01 CFU/100 ml.

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/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-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 both 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). *E. coli* did not occur in domestic wells at sufficient rates to be included in the statistical model. In order 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 (Figure 3). The highest frequencies of detection of both generic *E. coli* and *Enterococcus* were associated with monitoring wells immediately downgradient of manure-treated fields and corrals. 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 whether the domestic well was nearby or further away from a dairy ('near-dairy' vs. 'non-dairy'). However, 4.5% and 7.5% of near-dairy wells were positive for generic *E. coli* and *Enterococcus*. Similarly, 5.9% and 10.3% of non-dairy 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). But *Enterococcus* results were significantly different between near-dairy wells and non-dairy wells, 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 multi-drug resistance (resistant 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. non-dairy) of the well from which samples were collected and used for isolating *E. coli* and *Enterococcus* (Table 6). Among the near-dairy domestic well, one generic *E. coli* and three *Enterococcus* isolates came from domestic wells on dairy facilities not studied in 2006-2009. Like others, these isolates exhibited multi-resistant properties. We found that generic *E. coli* were most often resistant to azithromycin, chloramphenicol, trimethoprim/sulfamethoxazole, and tetracycline and *Enterococcus* were most often resistant to tigecycline, quinupristin/dalfopristin, linezolid, chloramphenicol, erythromycin, iprofloxacin, and tetracycline.

Discussion

The high level 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

421 50% on a wet weight basis in many samples. High occurrence rates of *E. coli* and *Enterococcus*
422 have also been found on dairies in the northeastern U.S. dairies (Pradhan et al., 2009).
423 *Enterococcus* has been found in surface water and groundwater impacted by a concentrated
424 swine feeding operation in the Mid-Atlantic United States (Sapkota et al., 2007). Similarly,
425 pathogenic bacteria including *Campylobacter*, *E. coli* O157:H7 and *Salmonella* have been
426 commonly detected in dairy environment elsewhere but at significantly lower concentration than
427 indicator bacteria (Murinda et al., 2004; Toth et al., 2013; Ravva and Sarreal, 2014). In our
428 survey, the primary source of *Campylobacter* among the various dairy management units is
429 difficult to discern – solids samples did not yield significant information, while freestall and
430 lagoon water, which consistently yield significant *Campylobacter* may originate, with the
431 exception of manure-treated fields, from any of the dairy management units shown in Figure 2.
432 Calf hutch flush water originates from tap water. Hence, the consistent occurrence of both
433 *Campylobacter* and *Salmonella* indicates that calf hutches are at least one of the contributing
434 sources of pathogens. *Salmonella* was also most common in liquid slurries, but also occurred in
435 the surface solids that had little exposure to direct sunlight (hospital barn and freestall lots). The
436 lack of pathogens on other surface solids is consistent with earlier findings (Nicholson et al.,
437 2005) probably due to inactivation after exposure to ambient conditions including higher
438 temperatures (Hoar et al. 1999; Sinton et al., 2007; Mariarty et al., 2011). Survival of pathogens
439 in the dairy environment depends on numerous complex environmental factors (Toth et al., 2011;
440 Ravva and Sarreal, 2014), reflected here by the lack of strong seasonal signatures, despite the
441 high contrast between hot, dry summers and moist, cool winters. The lower frequencies and
442 concentrations of *Campylobacter* and *Salmonella* in liquid samples, when compared to fecal

443 indicator concentrations may largely be due to those being shed only by infected animals, which
444 may represent only a fraction of the herd.

445 Indicator bacteria and pathogens occurring on dairy CAFOs may be subject to transport into
446 the environment surrounding dairies through surface runoff to streams, and through incidental or
447 intentional infiltration into and transport through unsaturated porous medium to groundwater
448 (Joy et al., 1998; Searcy et al., 2005; Park et al., 2012; Unc and Goss, 2014). Unc et al. (2012)
449 found at least three orders of magnitude reduction in *Enterococcus* concentration across the 3 m
450 unsaturated zone profile on one of the two SJV dairies. Li et al (2014) estimated attenuation rates
451 for generic *E. coli* ranging from 3 to 7 orders of magnitude using 2006-2008 surface samples
452 reported here and a limited number of groundwater samples collected concurrently with surface
453 samples (not included in this study).

454 There are distinct differences in generic *E. coli* and in *Enterococcus* detection frequencies
455 between monitoring wells located immediately upgradient of dairies, which are comparable to
456 those in domestic wells, and detection frequencies in monitoring wells located within dairies
457 (Figure 3). Monitoring wells downgradient of corrals and manure-treated fields have much
458 higher detection frequencies than those downgradient of lagoons. The difference may be due to
459 more attenuation of microorganisms by the fine-grained sludge layer commonly found on the
460 bed of storage lagoons than in the fractured and mechanically impacted corral surface. Due to
461 mechanical preparation (ploughing etc.), fields provide a more open surface with significantly
462 higher infiltration rates than either corrals or lagoon beds, and thus less filtration of colloidal
463 microorganisms. Similarly, the already low risk of pathogenic contamination may actually be
464 lowest in the vicinity of storage lagoons relative to other dairy management units.
465 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, while 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 TDS) 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). As mentioned above, 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 0.00013-140 MPN/ml or g. Given the 3 to 7 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 to generic E. coli and Enterococcus (Figure 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. *E. coli* O157:H7 and *Salmonella* are each detected in 2 of 51 on-dairy monitoring wells across 8 dairies, but in only 1 of 4 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 non-dairy domestic wells. This suggest that 3 m to 30 m of unsaturated alluvial sediments with silty sand, loamy sand, fine sand, and sandy loam or finer materials provide significant protection from pathogenic transport to the water table. Assuming 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 liters per day, using water from monitoring wells as a source of drinking water or using other on-dairy sources for municipal purposes may pose an unacceptable risk of waterborne transmission if not treated. Hence, groundwater supplies for drinking water, typically obtained tens to over 100 meters below the water table are also 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 (Horn et al., 2009) recognized

512 that poor well seal construction may be a significant risk factor groundwater contamination.
513 Compromised wells allow for rapid transport though the gravel filter of a domestic well.
514 Also, soils with significant macropores (e.g., fractured clay, till) or of much less thickness
515 than 3 m overlying more vulnerable sand and gravel aquifers or highly fractured rock aquifers
516 may be at significant risk near similarly managed CAFOs. We note that most of the surveyed
517 domestic wells are also in the vicinity of a private onsite wastewater treatment system (septic
518 system) that may serve as a source for enteric indicator bacteria and pathogens (Bremer et al,
519 2012). This and the use of manure as soil amendment may explain the occurrence of indicator
520 bacteria at significant distances from dairies.
521 Generic *E. coli* and *Enterococcus* are among the commonly used indicator organisms for
522 monitoring microbiological quality of water (Edberg et al., 1997). It is generally assumed that
523 indicator microbial pollution poses a significant risk of pathogen occurrence due to similar
524 transport mechanisms (Goss et al., 2002). In the investigated alluvial systems, it appears that the
525 significant difference in concentration of indicator vs. pathogenic bacteria at dairy surfaces lead
526 to significant occurrence of indicator organisms, while the actual risk of pathogenic bacteria
527 occurrence is very low. On the other hand, the absence of indicator organisms is not a guarantee
528 of clean water. In the two cases of pathogen detection immediately below the water table,
529 samples were negative for generic *E. coli* and had average *Enterococcus* concentrations (data not
530 shown). Other studies have also reported the lack of correlation between fecal indicator bacteria
531 and pathogens in groundwater (Ferguson et al., 2012).
532 High spatial attenuation rates of *E. coli* and other fecal indicator bacteria through sandy
533 aquifers have been found by other studies (Knappett et al., 2012; Pang, 2009). Although we did
534 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. Presence of antibiotic-resistant bacteria within groundwater from CAFO-specific monitoring wells suggests these are animal-derived, and from fecal-rich environment 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 U.S. (retrieved info sheet from APHIS). In the present study, we found that genetic *E. coli* and *Enterococcus* isolates were resistant to many of these commonly-deployed and used antibiotics of the US industry. Additionally, we found 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 suggest and 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 and pointed out public health implications regarding multiple antibiotic resistance gram negative bacteria in rural groundwater supplies used as 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 G+ and G- 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% - 27% and *Enterococcus* in 80%-100% of groundwater in dairy CAFOs. Both indicator bacteria were detected at much lower rates ($\leq$ 10%) in groundwater at near-dairy and non-dairy domestic wells of the same region. The prevalence of *Enterococcus* was significantly associated to the influence of dairy operations. We did not detect pathogenic bacteria within domestic wells, on-dairy, near-dairy, or in non-dairy 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 multi-drug antibiotic resistance. These findings outline the broad reach of antibiotic resistant bacteria in groundwater of this region. Applying Good Agricultural Practices (GAPs) on CAFOs and improving well maintenance practices such as well seals (Rudolph et al., 1996) 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

581 for protection from residual microbiological risks associated with groundwater. Further work is
582 needed to better understand the sources, occurrence and public health implications of antibiotic
583 resistance in enterically-derived and/or environmental bacteria within groundwater
584 environments.
585
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593
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Table 1. Survey of generic *E. coli* in groundwater from CAFOs wells located in the Central Valley, California (2008-2009).

Dairy ID	January 2008		April 2008		September 2008		March-April 2009	
	% (positive /sampled wells)	Mean concen.† (±SD)	% (positive /sampled wells)	Mean concen.† (±SD)	% (positive /sampled wells)	Mean concen.† (±SD)	% (positive /sampled wells)	Mean concen.† (±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/100-ml; ‡ No domestic wells were sampled on dairies 36-11, 36-24 and 37-42; NA=Not applicable; ND=Not done (due to the well was not accessible or no water available).

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Table 2. Survey of *Enterococcus* spp. in groundwater from CAFO wells in the Central Valley, California (2008-2009).

Dairy ID	January 2008		April 2008		September 2008		March-April 2009	
	% (positive /sampled wells)	Mean concen.† (±SD)	% (positive /sampled wells)	Mean concen.† (±SD)	% (positive /sampled wells)	Mean concen.† (±SD)	% (positive /sampled wells)	Mean concen.† (±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/100-ml; ‡ No domestic wells were sampled on dairies 36-11, 36-24 and 37-42; NA=Not applicable; ND=Not done (due to the well was not accessible or no water available).

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Table 3. Factors associated to the concentrations of generic *E. coli* and *Enterococcus* spp. in groundwater in CAFOs in the Central Valley, California (2008-2009).

Bacteria	Factor	Coef.	Std. Err.	*P* value	95% CI
Generic *E. coli*	Land use				
	Corral†	0	--	--	--
	Field	-2.824	0.331	0.000	(-3.473, -2.175)
	Lagoon	-2.008	0.484	0.000	(-2.958, -1.058)
	Upgradient	-6.098	2.887	0.035	(-11.758, -0.438)
	Water table depth				
	Deep†	0	--	--	--
	Shallow	3.825	0.381	0.000	(3.077, 4.572)
	Season‡				
	Spring†	0	--	--	--
	Fall	0.436	0.360	0.226	(-0.269, 1.141)
	Winter	2.734	0.255	0.000	(2.233, 3.235)
Enterococcus	Well type				
	Monitoring well†	0	--	--	--
	Domestic well	-3.536	0.155	0.000	(-3.839, -3.233)
	Land use				
	Corral†	0	--	--	--
	Field	-1.239	0.021	0.000	(-1.280, -1.197)
	Lagoon	1.836	0.011	0.000	(1.812, 1.859)
	Upgradient	-3.322	0.090	0.000	(-3.500, -3.145)
	Water table depth				
	Deep†	0	--	--	--
	Shallow	4.109	0.032	0.000	(4.046, 4.172)
	Season‡				
	Spring†	0	--	--	--
	Fall	-2.828	0.036	0.000	(-2.899, -2.757)
	Winter	-5.874	0.056	0.000	(-5.984, -5.764)

† Referent category for categorical variable.
‡ Spring: April 2008 and March/April 2009; Fall: September 2008; Winter: January 2008.

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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	No. (%) of positive wells	Concentration (CFU/100ml) Mean (±SD)
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
Non-Dairy 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 2.4km or less; wells are defined as "non-dairy" if the distance between a well and a dairy lagoon or corral is greater than 2.4km; NA=Not applicable.

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

Factor	Coef.	Std. Err.	P value	95% CI
Well designation				
Non-Dairy well[†]	0	--	--	--
Near-Dairy well	1.900	0.761	0.013	(0.408, 3.393)
Potassium	-29.955	11.284	0.008	(-52.071, -7.839)
Season				
Spring[†]	0	--	--	--
Summer	-2.120	4.935	0.667	(-11.793, 7.552)
Fall	1.922	0.853	0.024	(0.251, 3.593)
Winter	0.967	1.043	0.354	(-1.077, 3.010)

[†] Referent category for categorical variable

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

Bacteria	Campaign	Type of wells	No. isolates tested	No. isolates† resistant to ≥1 antibiotics	No. isolates† resistant to ≥3 antibiotics
E. coli	On-dairy	Monitoring wells	2	2	2
		Domestic wells	0	NA	NA
	2010-2011	Near-dairy wells‡	5	5	3
		Non-dairy 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
		Non-dairy wells‡	6	6	5
	Total		36	36	31

†Each tested isolate was from different wells; ‡ 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 "non-dairy"; NA=Not applicable.

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881 **Figure Captions**

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883 **Figure 1.** Study area in California, USA focusing on four counties overlying the Central Valley
884 aquifer (dark grey): Stanislaus County (S), Merced County (M), Kings County (K),
885 and Tulare County (T). The southern and central Central Valley has a high
886 concentration of dairy CAFOs and manure-treated irrigated crops (black). The study
887 was conducted on eight dairies (red) and also included a regional survey of 200
888 domestic wells (white).

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

895 **Figure 3.** Frequencies of detection of generic *E. coli* and *Enterococcus* spp. in groundwater from
896 monitoring wells proximity to different types of land on CAFOs in the Central Valley,
897 California (2008-2009).