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Fecal Shedding of Zoonotic Food-Borne Pathogens by Wild Rodents in a Major Agricultural Region of the Central California Coast

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Recent outbreaks of food-borne illness associated with the consumption of produce have increased concern over wildlife reservoirs of food-borne pathogens. Wild rodents are ubiquitous, and those living close to agricultural farms may pose a food safety risk should they shed zoonotic microorganisms in their feces near or on agricultural commodities. Fecal samples from wild rodents trapped on 13 agricultural farms (9 produce, 3 cow-calf operations, and 1 beef cattle feedlot) in Monterey and San Benito Counties, CA, were screened to determine the prevalence and risk factors for shedding of several food-borne pathogens. Deer mice (*Peromyscus maniculatus*) were the most abundant rodent species trapped (72.5%). *Cryptosporidium* species (26.0%) and *Giardia* species (24.2%) were the predominant isolates from rodent feces, followed by *Salmonella enterica* serovars (2.9%) and *Escherichia coli* O157:H7 (0.2%). Rodent trap success was significantly associated with detection of *Salmonella* in rodent feces, while farm type was associated with fecal shedding of *Cryptosporidium* and *Giardia*. Seasonal shedding patterns were evident, with rodents trapped during the spring and summer months being significantly less likely to be shedding *Cryptosporidium* oocysts than those trapped during autumn. Higher rodent species diversity tended to correlate with lower fecal microbial prevalence, and most spatiotemporal pathogen clusters involved deer mice. Rodents in the study area posed a minimal risk as environmental reservoirs of *E. coli* O157:H7, but they may play a role in environmental dissemination of *Salmonella* and protozoa. Rodent control efforts that potentially reduce biodiversity may increase pathogen shedding, possibly through promotion of intraspecific microbial transmission.

Agricultural produce continues to play a substantial role in food-borne outbreaks, with changes in dietary habits, methods of produce production and processing, sources of produce, and pathogen emergence all increasing the potential for food-borne illness (1, 2). The contribution of contaminated produce to overall food-borne illness is reported to have risen, and this is attributable in part to a concomitant increase in consumption of fresh fruits and vegetables (3). Agricultural produce can get contaminated at any point in the farm-to-fork continuum, with field contamination of raw produce posing one of the largest threats to produce food safety. Several food-borne pathogens can survive and flourish on and in plants (4). Preharvest fecal and pathogen contamination may result from a variety of sources, including contamination of fields following wildlife intrusion, application of untreated manure and irrigation water to fields, and inadvertent contamination from farm workers who lack access to latrines or hand washing facilities.

The Salinas Valley is a major leafy vegetable production area in the United States that produces nearly 91% of the salad greens grown in California (5). This region has been the source of several food-borne outbreaks, including a 2006 multistate *E. coli* O157 outbreak that sickened 205 individuals and resulted in 3 deaths (6–8). Riparian areas, chaparral, annual grassland, and rangeland are all characteristic flora adjoining cropland in the region and form the essential habitats of numerous species of wild rodents. This close proximity between fields of produce and wildlife habitats can provide ample opportunity for produce contamination should infected rodents gain access, nest, forage, and/or defecate within or alongside fields of produce or contaminate irrigation water supplies and related equipment.

Wildlife have been implicated in outbreaks involving food-borne pathogens (8–10), which has prompted growers in California to implement stringent wildlife intrusion control measures, such as fencing, poison baiting, and clearing of noncrop vegetation in areas adjacent to produce fields (11). The role of various small mammal species as potential reservoirs of deleterious pathogens, such as *Salmonella* and *E. coli* O157:H7, however, still remains uncertain. Prior studies have focused on the impacts of commensal species on animal agricultural operations and the potential for pathogen interspecies transmission between domestic and wild animals (12–16). Wild rodents are ubiquitous and thrive wherever food stocks and habitats are plentiful; thus, many species can live in close proximity to humans and livestock (17). These wildlife species are competent reservoirs of a number of zoonotic diseases responsible for significant economic losses and public health problems (18–20). Despite this, there have been few studies that assess the role of wild rodents in shedding food-borne pathogens, particularly those inhabiting produce production areas.

The purpose of this study was to determine the fecal prevalence and risk factors associated with the occurrence of *E. coli* O157:H7, *Salmonella* spp., *Cryptosporidium* spp., and *Giardia* spp. in wild rodents trapped within agricultural production systems in the Salinas Valley region.

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MATERIALS AND METHODS

Study site selection. The study was carried out in 13 agricultural systems located in Monterey and San Benito Counties: 9 produce farms, 3 cow-calf rangeland operations, and 1 beef feedlot. Inclusion into the study was based on willingness to participate. Rodents were trapped from October 2009 to August 2011. During each visit, 60 Sherman live traps (HB Sherman, Tallahassee, FL) and 30 Tomahawk traps (Tomahawk live trap; Tomahawk, WI) were set 10 m apart. Sherman traps were arranged in a 10-by-10 array, while Tomahawk traps were arranged in a 5-by-5 array, establishing 3 trap grids consisting of 2 parallel transects each. Occasionally, irregularly shaped trap grids were set, so as to conform to the farm confines and topography. By using GPS coordinates and photos, attempts were made to reset traps at identical locations during sampling.

Rodent trapping and fecal sample acquisition. Smaller-sized nocturnal rodents (e.g., *Peromyscus maniculatus* and *Peromyscus californicus*) were trapped by using Sherman live traps, while California ground squirrels (*Spermophilus beecheyi*) were trapped using Tomahawk traps. Sherman traps baited with rolled oats and peanut butter were set in the evening before sunset and checked early the following morning. Cotton bedding was added to prevent hypothermia and to capture fecal pellets voided during nesting. Tomahawk traps baited with groundnuts were set during the day and checked frequently for the presence of ground squirrels. Fecal pellets (~0.025 to 0.10 g/animal) and bedding material were transported on ice to the University of California, Davis, for pathogen detection. Rodents were released unharmed at the point of capture. The location of each captured animal was recorded on a handheld GPS device (Garmin, Olathe, KS). All animals were humanely handled in accordance with an animal use protocol approved by the Institutional Animal Care and Use Committee at the University of California, Davis (protocol number 16376), U.S. Fish and Wildlife Service, and California Department of Fish and Game scientific collection permits.

Detection of *E. coli* O157:H7. An aliquot of each fecal sample (~0.025 to 0.10 g/rodent) was enriched, and pathogens were isolated by using a modification of a previously described method (6). Briefly, feces were placed in 50 ml of universal preenrichment broth (UPB; BD, Sparks, MD) and incubated at 37°C for 24 h. One milliliter of the enriched UPB was then transferred into 9 ml of Trypticase soy broth (TSB; BD, Sparks, MD), incubated at 25°C for 2 h at 100 rpm and 42°C for 8 h at 100 rpm (Multitron incubator shaker; ATR, Inc., Laurel, MD), and held overnight at 6°C. Immunomagnetic separation was performed with antibody-bound magnetic beads (Dynal; Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Fifty microliters of the resuspended beads was plated onto sorbitol-MacConkey agar (BD, Sparks, MD) containing cefixime (0.05 mg/ml) and tellurite (2.5 mg/ml) (CT-SMAC) and onto Rainbow agar (Biolog, Hayward, CA) containing novobiocin (20 mg/ml) and tellurite (0.8 mg/ml) (NT-Rainbow). Plates were incubated at 37°C overnight. Two suspect colonies were plated onto duplicate Luria base agar (LB; Fisher Scientific, Pittsburgh, PA) plates and incubated at 37°C overnight. DNA extraction was performed according to a previously described protocol (6). Briefly, 1 μl of cell growth from LB agar was resuspended in 100 μl of DNase-free water and boiled for 20 min at 100°C, followed by centrifugation at 2,600 × *g* for 10 min to remove cell debris. The supernatant was collected, without pellet disruption, and frozen at −20°C until PCR confirmation of suspect isolates. *E. coli* O157:H7 colonies were confirmed by using traditional PCR to detect the O-antigen synthesis (*rfbE*) gene (21). The PCR mixture consisted of 48.5 μl master mix and 1.5 μl DNA template. The master mix was composed of 1× buffer, 0.4 μM forward and reverse primers, 0.2 μM deoxynucleotide triphosphates, 1.5 mM $MgCl_2$, and 5 U/μl *Taq* polymerase, and the remaining volume was adjusted with DNase-free water. The PCR assays were performed using an Eppendorf thermocycler with an initial denaturation at 95°C for 1 min followed by 30 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 1 min. The final extension was carried out at 72°C for 1 min, and the samples were held at 4°C until removed from the thermocycler. The PCR product was visualized on an ethidium bromide-stained 2% agarose gel with UV transillumination.

Detection of *Salmonella*. An aliquot of each fecal sample (~0.025 to 0.10 g/rodent) was preenriched in 50 ml buffered peptone water (BPW; BD, Franklin Lakes, NJ) for 20 h at 37°C. Ten microliters of preenrichment broth was transferred to 1 ml of Rappaport Vassiliadis medium (RV; BD, Franklin Lakes, NJ) for 48 h at 42°C. A loopful of broth was streaked for isolation onto xylose lysine deoxycholate agar (XLD; BD, Franklin Lakes, NJ). A pure isolated colony was biochemically confirmed by using lysine (BD, Franklin Lakes, NJ), citrate (BD, Franklin Lakes, NJ), triple sugar iron (TSI; BD, Franklin Lakes, NJ), and urea (BD, Franklin Lakes, NJ).

Detection of *Cryptosporidium* oocysts and *Giardia* cysts. When a sufficient amount of fecal mass was present (≥0.050 g/rodent), the samples were also tested for the presence of *Cryptosporidium* and *Giardia* (n = 285 rodents). *Cryptosporidium* oocysts and *Giardia* cysts were detected from fecal materials by using a direct immunofluorescence antibody kit (Waterborne, New Orleans, LA) (22). Fecal samples were resuspended with an equal volume of phosphate-buffered saline and homogenized. Ten microliters of homogenized fecal suspensions was overlaid on a well of a pretreated glass slide from the kit. Slides were dried at room temperature and labeled with immunofluorescent anti-*Cryptosporidium* and anti-*Giardia* antibodies (Waterborne, New Orleans, LA). Then, slides were screened at 400× magnification for the presence of *Cryptosporidium* oocysts and *Giardia* cysts by using a fluorescence microscope (Olympus BX 60). This procedure has been effective in detection of *Cryptosporidium* oocysts in feces from California ground squirrels (*Spermophilus beecheyi*) (19, 23).

Demographic and environmental factors. We examined demographic and environmental factors potentially associated with the shedding of bacterial and protozoan pathogens in the feces of captured rodents. We recorded the sex, age, and weight, and took various morphometric measurements to assist in species identification, including body length, tail length, hind foot length, and ear length. Age was determined by molt pattern and pelage color, and captured rodents were classified as juveniles or adults. Temperature and humidity were determined from the University of California online integrated pest management program (www.ipm.ucdavis.edu/WEATHER/index.html). The maximum daily temperature was divided into 2 categories: 11 to 22°C and 23 to 35°C. The number of days after the new moon (moon phase) was determined from a moon phase calendar available from MoonConnection and divided into 3 levels: 0 to 10 days, 11 to 20 days, and ≥21 days. Moonlight has been found to influence nocturnal rodent behavior, with reduced rodent activity reported during periods of bright moonlight (24). Trap success was calculated by dividing the number of rodents captured by the number of trap nights (number of traps set multiplied by the number of nights) and assigned to 3 categories: 0 to 10%, 11 to 20%, and ≥21%. Farm information, including farm type (produce, cow-calf, feedlot) and irrigation system (drip, overhead, and sprinkler), was determined from site visits. To assess rodent diversity, a Shannon-Wiener index was calculated for each farm.

Statistical analyses. The associations between potential risk factors and the probability of pathogen shedding were tested using exact logistic regression within SAS version 9.3. When the maximum conditional likelihood estimates for the odds ratio (OR) did not exist (e.g., estimate of 0 in one of the cells), the median unbiased point estimate of the odds ratio was used (25). The variables were first assessed singly, and those significant at a P level of <0.20 were selected for inclusion in a multivariable model. Different models were compared using the likelihood ratio test. Rodent species diversity for each farm was calculated using the Shannon-Wiener index: $-\Sigma(p_i)(\log_2 p_i)$, where p_i is the proportion of rodents on each farm belonging to the ith species (26). Pathogen spatiotemporal clustering on each farm was determined using SatScan version 9.1.1 (http://www.satscan.org). A Bernoulli model was used to evaluate clustering. The SatScan test was run using 9,999 Monte Carlo replications, with a circular

scanning window used to assess the presence of clustering over the study area. Maximum likelihood estimation was then used to identify the most significant clusters.

RESULTS

In total, 1,071 wild rodents were trapped during 8,113 trap-nights (including rodents released without sampling and those captured more than once), for an overall trap success of 13.2%. Samples were collected from wild rodents belonging to 4 taxonomic families: *Cricetidae* (including *Peromyscus boylii*, *Peromyscus maniculatus*, *Reithrodontomys megalotis*, *Microtus californicus*, *Peromyscus californicus*, and *Neotoma fuscipes*), *Heteromyidae* (including *Chaetodipus californicus* and *Dipodomys heermani*), *Sciuridae* (including *Spermophilus beecheyi*), and *Muridae* (including *Mus musculus* and *Rattus norvegicus*). Two (0.2%) of 1,043 fecal specimens tested were positive for *E. coli* O157: H7. Both of these samples were isolated from rodents trapped next to produce production fields. Thirty (2.9%) of 1,043 fecal specimens were positive for *Salmonella* organisms, while 74 (26.0%) and 69 (24.2%) of 285 fecal specimens were positive for *Cryptosporidium* oocysts and *Giardia* cysts, respectively. Deer mice (*Peromyscus maniculatus*) were the most abundant species, accounting for 72.5% of all trapped rodents (Table 1). Twenty-five (83.3%) of the 30 positive *Salmonella* samples were obtained from this species. Sixty-five (87.8%) of 74 and 54 (78.3%) of 69 positive *Cryptosporidium* and *Giardia* fecal samples, respectively, were recovered from deer mice.

Trap success, type of farm, and season were associated with the detection of one or more microorganisms (Table 2). Farms with higher rodent trap success (which is a proxy for rodent density) were associated with a higher prevalence of *Salmonella* in the sampled rodents (Fig. 1). Of the variables that were included in the multivariable model, trap success was significantly associated with fecal shedding of *Salmonella enterica* serovars in wild rodents (Table 3). Rodents from locations or sessions with more than 21% trap success were more likely to be shedding *Salmonella* than were rodents from locations or sessions with 0 to 10% trap success (OR, 5.6; 95% confidence interval [CI], 1.3 to 23.6). Farm type was positively associated with shedding of *Cryptosporidium* and *Giardia*. Rodents trapped within a feedlot production system were more likely to be shedding *Cryptosporidium* oocysts (OR, 7.1; 95% CI, 1.2 to 42.7) and *Giardia* cysts (OR, 8.1; 95% CI, 1.5 to 43.1) than were rodents trapped on produce farm environments (Table 3). Interestingly, there was no significant difference in (oo)cyst shedding between rodents trapped on produce farms compared to cow-calf or stocker operations ($P > 0.05$). Peak seasonal shedding in autumn was evident for *Cryptosporidium* (Fig. 2), with the odds of oocyst shedding in wild rodents being 3 to 5 times higher in autumn than in spring (OR, 0.3; 95% CI, 0.1 to 0.8) or summer (OR, 0.2; 95% CI, 0.1 to 0.4) (Table 3).

Rodent species diversity as determined by the Shannon-Wiener index was negatively correlated with aggregate microbial prevalence at the farm level (R^2, 0.348; $P = 0.034$). Several spatiotemporal microbial clusters were identified in the study (Table 4). All microbial clusters detected involved at least one deer mouse. Farm 6, a produce farm, had 1 and 2 clusters of *Cryptosporidium* and *Salmonella*, respectively. This farm also had the highest rodent trap success (Fig. 1).

DISCUSSION

We found that wild rodents inhabiting agricultural production systems in central coastal California shed *E. coli* O157:H7, *Salmonella*, *Cryptosporidium* oocysts, and *Giardia* cysts in their feces. Consequently, rodents with access to produce fields can defecate and potentially transfer microbes onto agricultural commodities. The majority of the rodents trapped and found to be shedding potentially human-infective microbes belonged to the rodent family *Cricetidae*, which comprises a variety of New World rats and mice (27). This finding suggests that the efficiency of on-farm pest management measures designed to reduce the risk for potential rodent-borne preharvest contamination could be improved by targeting this group of rodents.

The fecal prevalence of *E. coli* O157:H7 fell within previously reported ranges of the pathogen in rodents. The majority of prior studies focused on the potential for interspecies pathogen transmission between synanthropic rodents and domesticated farm animals. Less than 1% prevalence has been reported for *E. coli* O157:H7 (13, 28, 29), suggesting that wild rodents may not serve as major reservoirs of *E. coli* O157:H7. However, substantial biases can occur in estimating fecal prevalences of microbial shedding when fecal amounts per assay are less than 0.10 g and shedding intensity is low, producing an artificial downward bias for the prevalence by well over 50% (30). In our study, the typical fecal mass collected from a trapped rodent ranged from 0.025 to 0.10 g, with about one-third of the mass used per assay (*E. coli* O157:H7, *Salmonella*, protozoan parasites). This suggests that our reported prevalences for these four potential zoonotic pathogens may be underestimated. In addition, rodent mobility and survival of the pathogen in rodent feces may make rodents competent vectors when they have access to fields of produce and especially when they have direct access to the harvestable portions of produce. Experimentally, *E. coli* O157 in rat feces was found to survive for up to 9 months with no apparent clinical symptoms in infected rats (31), a considerably longer survival period than found in pathogen survival studies using ruminant feces (32).

Fecal prevalence of *Salmonella* in the current study also fell within previously reported ranges in wild rodents (33). In one study, none of the specimens from wild mice trapped in a major produce production region in California was positive for *Salmonella* (34). Similarly, none of the rodents sampled following traceback studies carried out after an almond-associated *Salmonella* outbreak in California was positive for the pathogen (35), underscoring the potentially low occurrence of the organism in rodents in this region of California.

In this study, 26% and 24.2% of the rodents sampled were shedding *Cryptosporidium* oocysts and *Giardia* cysts, respectively. Of these, 1 of 2 California ground squirrels sampled for these protozoan parasites was found to be positive for *Cryptosporidium* and *Giardia*. In a prior study conducted in California, 49 (16%) of 309 fecal samples collected from California ground squirrels dispatched by expert marksmen were found to be positive for *Cryptosporidium parvum* oocysts (23). We analyzed comparably fewer samples because of the low trap success associated with live trapping of California ground squirrels (36). Prevalence rates as high as 62% and 74% for *Cryptosporidium* and *Giardia* have been recorded for voles (37). In an agricultural site in the United Kingdom, *Cryptosporidium* oocysts were isolated from wild rodents with a characteristic autumnal peak prevalence that paralleled our findings (17).

TABLE 1 Prevalence of fecal shedding of *E. coli* O157:H7, *Salmonella*, *Cryptosporidium*, and *Giardia* by wild rodents, stratified by potential risk factors

Variable	No. of rodents (%)[a]	Prevalence (%)[b] of:			
		E. coli O157:H7	*Salmonella*	*Cryptosporidium*	*Giardia*
Age					
Juvenile	217/1,071 (20.3)	0/211	7/211 (3.3)	12/44 (27.3)	8/44 (18.2)
Adult	760/1,071 (71)	1/730 (0.1)	20/730 (2.7)	60/232 (25.9)	57/232 (24.6)
Unidentified	94/1,071 (8.8)	1/85 (1.2)	3/85 (3.5)	2/9 (22.2)	4/9 (44.4)
Sex					
Male	592/1,071 (55.3)	1/574 (0.2)	18/574 (3.1)	36/157 (22.9)	32/157 (20.4)
Female	368/1,071 (34.4)	0/352	9/352 (2.6)	34/113 (30.1)	31/113 (27.4)
Unidentified	111/1,071 (10.4)	1/100 (1)	3/100 (3)	4/15 (26.7)	6/15 (40)
County					
Monterey ($n = 9$)	996/1,071(93)	2/967 (0.2)	30/967 (3.1)	73/274 (26.6)	68/274 (24.8)
San Benito ($n = 4$)	75/1,071 (7)	0/76	0/76	1/11 (9.1)	1/11 (9.1)
Farm type					
Produce ($n = 9$)	882/1,071 (82.4)	2/859 (0.2)	29/859 (3.4)	68/247 (27.5)	58/247 (23.5)
Rangeland ($n = 3$)	127/1,071 (11.9)	0/127	1/127 (0.8)	3/31 (9.7)	6/31 (19.4)
Feedlot ($n = 1$)	62/1,071 (5.8)	0/57	0/57	3/7 (42.9)	5/7 (71.4)
Taxonomic family					
Cricetidae	927/1,071 (86.6)	1/890 (0.1)	28/890 (3.1)	70/265 (26.4)	60/265 (22.6)
Heteromyidae	57/1,071 (5.3)	1/53 (1.9)	0/53	1/7 (14.3)	2/7 (28.6)
Sciuridae	22/1,071 (2.1)	0/21	0/21	1/2 (50)	1/2 (50)
Muridae	37/1,071 (3.5)	0/36	0/36	0/3	2/3 (66.7)
Unidentified species[c]	28/1,071 (2.6)	0/43	2/43 (4.7)	2/8 (25)	4/8 (50)
P. manicultus	777/1,071 (72.5)	1/747 (0.1)	25/747 (3.3)	65/214 (30.4)	54/214 (25.2)
P. californicus	87/1,071 (8.1)	0/85	2/85 (2.4)	4/38 (10.5)	5/38 (13.2)
D. heermani	47/1,071 (4.4)	1/43 (2.3)	0/43	1/3 (33.3)	1/3 (33.3)
S. beecheyi	22/1,071 (2.1)	0/21	0/21	1/2 (50)	1/2 (50)
Season					
Autumn	226/1,071 (21.1)	1/213 (0.5)	9/213 (4.2)	41/98 (41.8)	25/98 (25.5)
Winter	256/1,071 (23.9)	0/238	2/238 (0.8)	10/43 (23.3)	11/43 (25.6)
Spring	275/1,071 (25.7)	1/283 (0.4)	6/283 (2.1)	9/45 (20)	11/45 (24.4)
Summer	314/1,071 (29.3)	0/309	13/309 (4.2)	14/99 (14.1)	22/99 (22.2)
Trap success					
0–10%	245/1,071 (22.9)	0/244	2/244 (0.8)	10/46 (21.7)	13/46 (28.3)
11–20%	234/1,071 (21.8)	0/229	3/229 (1.3)	5/34 (14.7)	9/34 (26.5)
≥21%	592/1,071 (55.3)	2/570 (0.4)	25/570 (4.4)	59/205 (28.8)	47/205 (22.9)
Max. daily temp (°C)					
11–22	703/1,071 (65.6)	1/674 (0.1)	16/674 (2.4)	53/158 (33.5)	38/158 (24.1)
23–35	368/1,071 (34.4)	1/369 (0.3)	14/369 (3.8)	21/127 (16.5)	31/127 (24.4)
Moon phase (days)					
0–10	451/1,071 (42.1)	1/429 (0.2)	16/429 (3.7)	48/141 (34)	35/141 (24.8)
11–20	323/1,071 (30.2)	1/322 (0.3)	8/322 (2.5)	11/73 (15.1)	17/73 (23.3)
21–31	297/1,071 (27.7)	0/292	6/292 (2.1)	15/71 (21.1)	17/71 (23.9)
Irrigation system (produce farms)					
Drip	8/1,071 (0.7)	0/13	0/13	2/6 (33.3)	1/6 (16.7)
Overhead	249/1,071 (23.2)	1/237 (0.4)	10/237 (4.2)	37/90 (41.1)	20/90 (22.2)
Sprinkler	570/1,071 (53.2)	1/553 (0.2)	19/553 (3.4)	29/151 (19.2)	37/151 (24.5)
Unidentified	244/1,071 (22.8)	0/240	1/240 (0.4)	6/38 (15.8)	11/38 (28.9)
Total	1,071	2/1,043 (0.2)	30/1,043 (2.9)	74/285 (26)	69/285 (24.2)

[a] Prevalence values are the number of rodents trapped in each category divided by total number of rodents trapped. Not all rodents provided fecal samples.
[b] Prevalence values are the number of positive samples divided by the number tested for each category. Fecal samples recovered from traps that didn't trigger and trap rodents were also analyzed. Overall, 1,043 fecal samples were tested for *E. coli* O157:H7 and *Salmonella*, with a subset of 285 fecal samples tested for *Cryptosporidium* and *Giardia* (when a sufficient amount of fecal pellets was present).
[c] Four of the 11 most common rodent species trapped in the study.

TABLE 2 Univariate analysis of factors significantly associated with detection of one or more food-borne pathogens in wild rodent fecal specimens[a]

Risk factor	*Salmonella*		*Cryptosporidium*		*Giardia*	
	OR[b]	*P* value	OR[b]	*P* value	OR[b]	*P* value
Season						
Autumn	1.0		1.0		1.0	
Winter	0.2	**0.04**	0.5	**0.04**	1.0	1.0
Spring	0.5	0.2	0.4	**0.01**	0.9	0.9
Summer	1.0	1.0	0.3	**<0.01**	0.8	0.6
Max. daily temp (°C)						
11–22	1.0		1.0		1.0	
23–35	1.6	0.2	0.4	**0.01**	1.0	0.9
Trap success (%)						
0–10	1.0		1.0		1.0	
11–20	1.6	0.6	0.6	0.4	0.9	0.9
≥21	5.6	**0.02**	1.5	0.3	0.8	0.4
Farm type						
Produce	1.0		1.0		1.0	
Rangeland cattle	0.2	0.2	0.3	**0.04**	0.8	0.6
Feedlot	0.4	0.3	1.9	0.4	8.1	**0.01**
Moon phase (days)						
0–10	1.0		1.0		1.0	
11–20	0.7	0.3	0.4	**<0.01**	0.9	0.8
21–31	0.5	0.2	0.5	**0.05**	1.0	0.9

[a] *P* values for variables statistically significantly different from the reference category are shown in boldface ($P \leq 0.05$).
[b] Referent categories for the odds of shedding were assigned a value of 1.0.

Rodents trapped on a typical feedlot production system had higher odds of shedding *Cryptosporidium* and *Giardia* in their feces than rodents trapped on produce farms. Rodents have been found to be susceptible to infection with the same assemblages of *Giardia* and the same species of *Cryptosporidium* as cattle (38), and they are thought to be an important reservoir of infection of *Cryptosporidium* oocysts for livestock (39). In contrast, there was no significant difference in the odds of (oo)cyst shedding between rodents trapped on produce farms and rodents trapped on commercial cow-calf and stocker operations ($P > 0.05$). In contrast to the high density of cattle in feedlots and associated large amounts of fecal loading, we speculate that the low stocking density of rangeland beef cattle for this region of California did not create the necessary conditions for detectable levels of interspecies transmission of *Cryptosporidium* from infected cattle to susceptible rodents.

In this study, shedding of *Salmonella* was positively associated with trap success, a measure that has been used as a proxy for population density (40). Trap happiness and trap shyness are common behavioral responses of small rodents that frequently encounter traps (41). Rodents that exhibit trap happiness in response to baiting are more likely to be captured between trap sessions. However, the short life span of rodents in the wild and the fact that we visited each farm on average once every 3 months make it unlikely that these behaviors greatly influenced our results. With increasing trap success, there was a concurrent increase in the odds of shedding of *Salmonella*, suggesting that a possible density-dependent horizontal transmission mechanism occurs between rodents. This finding is supported by studies that have reported an increased risk of shedding with increased rodent density (33, 42). High rates of rodent dispersal have also been associated with increased density (43), which may in turn facilitate emigration of infected rodents into new areas.

Seasonal variation in shedding of food-borne pathogens by domestic animals has been widely reported (44–46). However, few studies have investigated this phenomenon in wild animals. In this study, rodents were less likely to be shedding *Cryptosporidium* during spring and summer compared to autumn. Similar peak

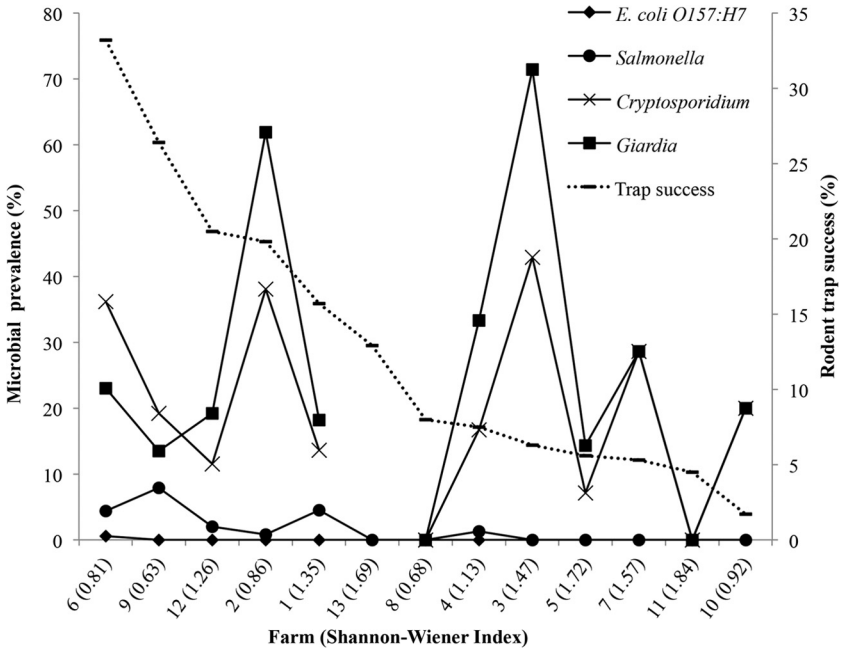

FIG 1 Associations between microbial prevalence, trap success, and rodent diversity. Shannon-Weiner values are presented in parentheses, following the farm numbers, listed on the *x* axis: produce farms (1, 2, 6, 7, 8, 9, 10, 12, and 13), cattle rangeland farms (4, 5, and 11), and cattle feedlots (3).

TABLE 3 Adjusted odds ratios for risk factors associated with shedding of *Salmonella*, *Cryptosporidium*, and *Giardia* by wild rodents[a]

Factor	*Salmonella*		*Cryptosporidium*		*Giardia*	
	OR (CI)[b]	*P* value	OR (CI)[b]	*P* value	OR (CI)[b]	*P* value
Farm type						
Produce			1.0		1.0	
Rangeland			0.4 (0.1–1.4)	0.2	0.8 (0.3–2.0)	0.6
Feedlot			7.1 (1.2–42.7)	**0.03**	8.1 (1.5–43.1)	**0.01**
Trap success						
0–10%	1.0					
11–20%	1.6 (0.3–9.7)	0.6				
≥21%	5.6 (1.3–23.6)	**0.02**				
Season						
Autumn			1.0			
Winter			1.0 (0.3–3.4)	1.0		
Spring			0.3 (0.1–0.8)	**0.01**		
Summer			0.2 (0.1–0.4)	**<0.01**		

[a] *P* values for variables statistically significantly different from the reference category are shown in boldface ($P \leq 0.05$).
[b] Referent categories for the odds of shedding were assigned a value of 1.0.

autumn prevalences have been reported by others (17). Ziegler et al. suggested that higher prevalences in autumn may be linked to breeding cycles of small mammal species, since the majority give birth during warmer months of the year and begin to disperse in autumn (43, 47).

Intraspecific transmission of microbes may act as a mechanism for maintaining food-borne pathogens within rodent populations. We identified clustering of pathogens that predominantly involved deer mice. This may be attributable to a social behavior by which close contact may facilitate fecal-oral transmission. Grooming habits of mice, such as frequent licking of extremities, could also contribute to transmission and maintenance of infection (42). Most microbial clusters were detected in produce farms, raising questions as to the possible sources of rodent infection. Food-borne pathogens have been isolated from soil, water, and sediment on produce farm environments (34, 48, 49); however, no links have been made to rodents in these studies.

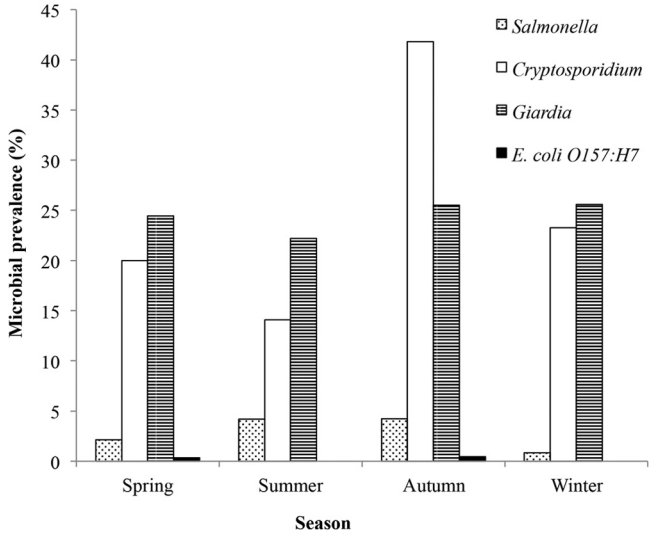

FIG 2 Seasonal fecal prevalence of bacterial and protozoan microbes shed by wild rodents trapped on 13 agricultural production systems in 2 counties along the California central coast.

The dilution effect is a phenomenon characterized by the suppressive effect of high species diversity on disease risk (50). In this study, higher rodent diversity tended to correlate with lower pathogen prevalence. Similar patterns involving *Bartonella* spp. and hantavirus pulmonary syndrome in rodents have been reported (51). To our knowledge, this is the first report of this phenomenon involving food-borne pathogens and rodents. Ostfeld and Keesing suggested that high diversity may reduce rates of intraspecific contact either by direct reduction of population density of primary reservoirs or by facilitating interspecific contact at the expense of intraspecific contact (51). Further research is necessary to elucidate the interaction between biodiversity and disease risk in agricultural systems and the implications for rodent control.

Our results should be carefully interpreted, because they might be biased by certain features of the study. Inclusion into the study was based on a convenient sample of farms. It is possible that those who elected to participate differed from the population of the ranchers and growers in the region. We also managed to include 13 farms in the study, constituting a small percentage of the total farming acreage in the Salinas Valley region. However, the farms included in the study were located along an approximately 120-km distance of the Salinas Valley, ranging from Marina to San Ardo, CA. In order to honor confidentiality agreements with the farmers, exact farm locations are not reported.

In conclusion, rodents in the region posed a minimal risk as fecal shedders of *E. coli* O157:H7 and a moderate risk for *Salmonella*, yet play a greater role in disseminating protozoan pathogens such as *Cryptosporidium* spp. in these agricultural systems. Control efforts that potentially reduce biodiversity, such as noncrop vegetation clearing and indiscriminate poison baiting, may increase fecal shedding potentially through promotion of intraspecific transmission of pathogens. In a study carried out by Stuart, 88.9% of crop growers in the Salinas Valley region that were interviewed indicated that they had taken at least one measure to actively discourage or eliminate wildlife, with bare ground buffers and poisoned stations being used by more than one-half of the

TABLE 4 Spatiotemporal clusters of food-borne pathogens recovered from wild rodents trapped on 4 farms located in Monterey County, CA[a]

Microbe	Cluster[a]	Farm no.	Cluster radius (m)	Cluster period (wks)	O(E)[b]	RR[c]	*P* value[d]	Rodent species involved
Salmonella	1	9	54	76	6 (0.36)	20.69	<0.01	*P. manicultus*, *P. californicus*
	2	6	11	8	4 (0.15)	30.77	<0.01	*P. manicultus*, *N. fuscipes*
	3	6	110	3	5 (0.36)	16.55	0.04	*P. manicultus*
Cryptosporidium	1	6	60	4	20 (6.2)	4.12	<0.01	*P. manicultus*, *P. californicus*
Giardia	1	2	32	4	6 (1.44)	4.49	0.04	*P. manicultus*
	2	2, 3	910	21	9 (2.67)	3.8	0.05	*P. manicultus*, *M. musculus*

[a] Category 1, the primary cluster was least likely due to chance; categories 2 and 3, secondary clusters.
[b] O(E), observed (expected) number of cases.
[c] RR, relative risk.
[d] Only clusters with statistically significant *P* values (≤0.05) are displayed.

respondents (11). The challenge still remains as to how we can enhance preharvest food safety while promoting environmentally sustainable farm practices.

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