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# Detection and Prevalence of *Cryptosporidium* spp. and *Giardia* spp. from Wild Rodents Adjacent to Produce Production Fields in California

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**ABSTRACT:** Between 2009 and 2011, fecal samples were collected from ten species of wild rodents in locations adjacent to leafy green blocks in a major produce production region of California. Samples were screened for *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts using immunofluorescent microscopy. Five and seven species of wild rodents carried *Cryptosporidium* spp. and *Giardia* spp., respectively. In general, 26.0% and 24.2% of the trapped wild rodents were positive for *Cryptosporidium* spp. and *Giardia* spp., respectively. Deer mice (*Peromyscus maniculatus*) were the primary trapped species, with 30.3% (63/208) positive for *Cryptosporidium* spp. and 25.5% (53/208) positive for *Giardia* spp.

**KEY WORDS:** California, *Cryptosporidium*, deer mouse, disease, food safety, *Giardia*, leafy green crops, pathogens, *Peromyscus* spp., protozoan parasites, rodents

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## INTRODUCTION

Wildlife intrusion into produce production fields and fecal deposition are of increasing concern for produce contamination in California (Jay et al. 2007). Wild rodents are abundant in North America and can be found in many types of habitats. For example, the deer mouse (*Peromyscus maniculatus*) and California ground squirrel (*Spermophilus beecheyi*) are ubiquitous in grasslands, meadow complexes, agricultural regions, and lower-elevation woodlands (Hanney 1975). Wild rodents may serve as reservoirs of zoonotic pathogens including bacteria, parasites, and viruses (Easterbrook et al. 2007). Previously, we found that overall 12% California of ground squirrels shed *Cryptosporidium* spp. at concentrations of >40,000 oocysts per gram of feces (Atwill et al. 2004). *Cryptosporidium* and *Giardia* are two major protozoan parasites causing intestinal illnesses and are transmitted through contaminated food or water (Slifko et al. 2000). Endemic infection among wild rodent populations can greatly promote the transmission of *Cryptosporidium* spp. and *Giardia* spp. in the environment and among other populations of susceptible host species. Accumulated environmental loads of *Cryptosporidium* spp. and *Giardia* spp. can potentially contaminate preharvest produce by direct exposure or through contamination of irrigation water. The objective of the present work was to identify major wild rodent species and determine the prevalence

of *Cryptosporidium* spp. and *Giardia* spp. in wild rodent populations adjacent to fields of produce in a major production region of central coastal California.

## METHODS

### Wild Rodent Trapping and Sampling

Between October 2009 and August 2011, 6 commercial fields for growing conventional leafy green in Monterey County, 3 fields for growing organic leafy green in San Benito County, and 3 commercial cattle ranches in Monterey County and 1 in San Benito County were enrolled as sites for trapping of wild rodents. All personnel involved in trapping were subject to medical surveillance procedures in accordance with Federal and state laws and regulations and University policies. Tomahawk traps (Tomahawk Live Trap, Model 202 - collapsible trap, Hazelhurst, WI) and Sherman traps (H. B. Sherman Traps - Model XLK, Tallahassee, FL) were used for trapping and set at the trapper's discretion, forming clusters in different places on farms. Thirty Tomahawk traps were baited with shelled peanuts and corn, placed in the morning and checked twice a day, and 60 Sherman traps were baited with rolled oats and peanut butter and provisioned with cotton balls for bedding, set in the evening and checked in the morning. In leafy green fields, traps were set as close as possible to the production blocks. Traps were decontaminated prior to and between uses as described

**Table 1. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in wild rodents in produce production area, California (2009-2011).**

Rodent Species	Prevalence (positive/n) for <i>Cryptosporidium</i> spp.	Prevalence (positive/n) for <i>Giardia</i> spp.
Brush mouse ( <i>Peromyscus boylii</i> )	0% (0/3)	0% (0/3)
California ground squirrel ( <i>Spermophilus beecheyi</i> )	50% (1/2)	50% (1/2)
California parasitic mouse ( <i>Peromyscus californicus</i> )	10.5% (4/38)	13.2% (5/38)
California pocket mouse ( <i>Chaetodipus californicus</i> )	0% (0/4)	25% (1/4)
Deer mouse ( <i>Peromyscus maniculatus</i> )	30.3% (63/208)	25.5% (53/208)
Dusky-footed wood rat ( <i>Neotoma fuscipes</i> )	16.7% (1/6)	16.7% (1/6)
Harvest mouse ( <i>Reithrodontomys megalotis</i> )	0% (0/1)	0% (0/1)
House mouse ( <i>Mus musculus</i> )	0% (0/3)	66.7% (2/3)
Kangaroo rat ( <i>Dipodomys deserti</i> )	33.3% (1/3)	33.3% (1/3)
Meadow vole ( <i>Microtus californicus</i> )	0% (0/3)	0% (0/3)
Undetermined species	28.6% (4/14)	35.7% (5/14)
<b>Total</b>	<b>26.0% (74/285)</b>	<b>24.2% (69/285)</b>

**Table 2. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in deer mouse (*Peromyscus maniculatus*).**

Stratification	Percent of positive mice (# positive / # sampled)	
	<i>Cryptosporidium</i> spp.	<i>Giardia</i> spp.
<b>Age</b>		
Adults	30.2% (51/169)	26.6% (45/169)
Juvenile	30.8% (12/39)	20.5% (8/39)
<b>Sex</b>		
Male	27.3% (33/121)	21.5% (26/121)
Female	34.5% (30/87)	31.0% (27/87)
<b>Age and sex</b>		
Male adults	30.2% (29/96)	24.0% (23/96)
Male juveniles	16.0% (4/25)	12.0% (3/25)
Female adults	31.5% (23/73)	30.1% (22/73)
Female juveniles	50.0% (7/14)	35.7% (5/14)
<b>Total</b>	<b>30.3% (63/208)</b>	<b>25.5% (53/208)</b>

in Mills et al. (1995). For each trapped rodent, species was identified and morphometric measures (total length, tail length, back foot length, ear length, and body weight) were measured, and sex and age class (juvenile, adult) were determined. Fresh defecated feces were collected using sterilized materials during the retaining period. Fecal samples were immediately placed into pre-weighed tubes and placed in a cooler with ice. Rodents were released after sampling.

#### Detection of *Cryptosporidium* spp. and *Giardia* spp.

Upon arrival at the laboratory, samples were immediately placed in a refrigerator (4°C) until processed. Sample tubes were weighed and fecal weight of each sample was determined. *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts were detected from fecal materials using a direct immunofluorescence antibody kit (Waterborne, New Orleans, LA). Fecal materials were resuspended with

equal volume of PBS 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 immunofluorescence anti-*Cryptosporidium* and anti-*Giardia* antibodies. Then, slides were screened at ×400 magnification for presence of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts using a fluorescent microscope (Olympus BX 60). This procedure has been effective in detection of *Cryptosporidium* oocysts in feces from California ground squirrels (*Spermophilus beecheyi*) (Atwill et al. 2001, 2004).

#### RESULTS AND DISCUSSION

Approximately 85% of rodents were trapped adjacent to produce farms and 15% were trapped proximate to cattle ranches. Results of wild rodent sampling and prevalence of *Cryptosporidium* spp. and *Giardia* spp. are shown in Table 1.

In total, 285 fecal samples were obtained; among these, 271 were from 10 identified rodent species and 14 were from unidentified rodent species. Among the total 285 samples, 74 (26.0%) were positive for *Cryptosporidium* spp. and 69 (24.2%) were positive for *Giardia* spp.

Deer mice (*Peromyscus maniculatus*) are the most common *Peromyscus* species in the U.S. (Hanney 1975). The wide range of geographic distribution and habitats of deer mice facilitates the species to be the dominant rodent species in the sampled region. As shown in Table 2, we found that 30.3% (63/208) and 25.5% (53/208) deer mice were positive of *Cryptosporidium* spp. and *Giardia* spp., respectively. Similar percentages of *Cryptosporidium* and *Giardia*-shedding animals were observed in age (adult and juvenile) and sex (male and female) groups. Because active infection with *Cryptosporidium* spp. and *Giardia* spp. occurs in all age and sex classifications of deer mice, there is the potential that the two parasites maintain infection in

the deer mouse population year round.

Fresh fruit and vegetable contamination by protozoan parasites including *Cryptosporidium* spp. and *Giardia* spp. has been documented in Alberta, Canada (Bohaychuk et al. 2009), Costa Rica (Monge and Arias 1996, Calvo et al. 2004), and Norway (Robertson and Gjerde 2001), which demonstrates the importance of determining the parasite host sources of on-farm produce contamination. In the current work, we found relatively high prevalence of *Cryptosporidium* spp. (30%) and *Giardia* spp. (25%) in deer mice adjacent to blocks of produce production or proximate to cattle ranches. Findings from the present work indicate that wild rodents, especially deer mice, are a potential source of *Cryptosporidium* and *Giardia* contamination in fields. Currently, we are estimating the daily environmental loading of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts, which can be calculated by mean shedding intensity, mean daily fecal output, and animal population density as described previously (Atwill et al. 2001). Once the calculation is completed, we will be able to determine how many *Cryptosporidium* oocysts and *Giardia* cysts are shed by deer mouse populations per acre of produce production area.

Contaminated irrigation water has been identified as sources of produce contamination of *Cryptosporidium* and *Giardia* in production fields (Amorós et al. 2010, Mota et al. 2009). Given that *Cryptosporidium* oocysts are capable of survival in water for weeks to months under favorable temperatures (Li et al. 2010), these parasites from deer mouse feces could be one of the sources of environmental water and irrigation water contamination. In addition to direct contamination by contaminated irrigation water, fecal scats shed by deer mice may also contaminate produce through spray irrigation, based on field experiments showing increased probability of produce contamination from fecal scats following spray irrigation (E. R. Atwill and M. Jay-Russell, pers. commun.).

In summary, this is the first report of detection and prevalence of *Cryptosporidium* spp. and *Giardia* spp. in wild rodent populations adjacent to produce production fields. Depending on the host specificity of *Cryptosporidium* and *Giardia* carried by deer mice, these species may serve as a mechanism of environmental dissemination of protozoan parasites within and between wild and domestic animals, and in addition, to produce crops.

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