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Risk Factors Associated with Prevalence of Foodborne Pathogens in Rural Households of Colorado with and without Ruminant Animals

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ABSTRACT

Ruminants are one of the reservoirs for *Listeria, Salmonella* and *Escherichia coli* O157:H7, and therefore a potential source of contamination for the household environment. Understanding consumer behavior may help in reducing infections caused by these microorganisms. This study evaluated consumer behaviors in households with/without ruminants, which may be related to increased prevalence of these pathogens. The study was completed over a three-year period, with samples collected during years 1 and 3. Rural Colorado households were recruited, and samples (food, environmental, and fecal) were collected and tested for *Listeria*, *Salmonella* and *E. coli* O157:H7 presence. Participants answered surveys regarding household cleaning habits and food/animal handling. None of the samples tested positive for *E. coli* O157:H7, while *Salmonella* was isolated only from households with ruminants. *Listeria* spp. was isolated from all types of samples with higher, but not significant ($P \geq 0.05$), prevalence in households with ruminants. *L. monocytogenes* was isolated mainly from food samples. Seven indices were developed from survey information and were statistically analyzed for relationships, with the outcome of a sample positive for *Listeria* as the dependent variable. Behavior related to handling and cooking of perishable foods affected ($P < 0.05$) the probability of households testing positive for *Listeria*, regardless of ruminant presence. Personal cleanliness habits were associated with presence of *Listeria* on shoe soles, clothes washing machines, and gloves used for farming activities. Consumer education should include proper food and animal handling practices, as well as proper cleaning of shoes and clothes, in order to reduce the prevalence of *Listeria* in the household.

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INTRODUCTION

Listeria monocytogenes has been identified as a major infectious agent causing neurological syndromes and uterine infections in bovine, sheep and goats *(20, 36)*. Animals carrying *L. monocytogenes* can directly contaminate milk as a consequence of listeric mastitis, encephalitis, or *Listeria*-related abortion *(8)*. Thus, animal feces and the farm environment may be important sources of contamination of raw milk and meat by *L. monocytogenes (20, 31)*. In addition, *L. monocytogenes* isolates found in the farm environment, and especially in environments with ruminants, have been linked to human listeriosis cases *(7)*. Other species of *Listeria* that were generally considered to be non-pathogenic to humans, such as *L. innocua*, have been identified as the cause of bacteremia and death *(34)*.

It has been reported that once pathogens that cause intestinal disease enter the domestic environment, they can be transmitted between surfaces, people and the food supply *(5, 21)*. For example, several studies have found *Listeria* in various places throughout the kitchen and the home in general, including vegetable compartments of refrigerators, kitchen sinks, dishcloths, toothbrushes, and the bathroom *(2, 6, 47)*. Duggan and Phillips *(6)* suggested that contamination with *L. monocytogenes* can be disseminated widely in kitchens. Another potential source of *L. monocytogenes* contamination in the home environment is the asymptomatic carriage of the pathogen by one or more members of the household *(41)*. Asymptomatic human carriage of *L. monocytogenes* has been reported previously *(11, 12, 26)*, and can occur not only in healthy individuals, but also among persons in high risk groups for listeriosis *(27, 28)*. Population groups at a higher risk for infection with *L. monocytogenes* include pregnant women, neonates, individuals with suppressed immune systems, and the elderly *(25)*. Since 1950, the number of persons over 65 years of age in the United States has tripled, from 12.2 million to 36 million, and it is estimated to exceed 80 million by the year 2035 *(19)*; therefore, identification of consumer behaviors that can reduce *L. monocytogenes* prevalence in the home environment is important.

Salmonella and *Escherichia coli* O157:H7 are other foodborne pathogens associated with ruminants and the farm environment that may find their way into the household environment. *Salmonella* has been isolated from different locations in the home, including vacuum cleaners, refrigerators and kitchen countertops *(15, 42)*. However, little information is available on how these pathogens are introduced into the household environment and the potential of household contamination to serve as a source of infection. Thus, the objective of this study was to evaluate, in rural households, consumer behaviors associated with house cleaning and with food and animal handling that may be associated with increased prevalence of *Listeria*, *Salmonella* and *E. coli* O157:H7 in the household environment.

MATERIALS AND METHODS

Recruiting of participants and behavioral data collection

The study protocol was approved by the Human Research Committee of Colorado State University (CSU). Rural households with and without ruminant animals were recruited from the Fort Collins, CO surrounding area by researchers in the Department of Food Science and Human Nutrition. Recruitment methods included letters and fliers sent by email to local 4-H families, veterinarians and Future Farmers of America (FFA) chapters for further distribution. The recruiting flier was also distributed within the CSU campus and Veterinary Teaching Hospital and posted on the CSU Today Web site. Interested families contacted researchers directly by telephone to sign up as participants in the study. Each participant household received a monetary compensation of $65 in years 1 and 3 for their time and samples collected.

To qualify for the study, participants needed to have their household in a rural environment (outside city limits), have children in the household under age 18 and be willing to participate over a 3-year time period. Each household also needed to be willing to participate in an audio-taped interview, complete additional surveys, allow the research assistant to conduct household environmental and food samplings, and provide human stool samples for microbiological analysis. Households were classified into those with and without ruminant animals (cattle, sheep, goats, llamas and/or alpacas) on their premises. Households without ruminants were required to have no contact with ruminant animals during the sample collection period.

Each household was visited four times, at 2–4 week intervals, between February and July. The primary household food preparer was asked to complete a Household Survey (47 and 42 questions for households with and without ruminants, respectively), and a Food Handling and Eating Preferences Questionnaire (29 questions). Households with ruminants were also asked to complete a Farmer/Rancher Survey (19 questions). Questions included in these instruments had been previously tested and validated for reliability *(22)*. These instruments were mailed in advance to participants and gathered by a researcher during the first household visit. During that visit, the researcher placed a calibrated commercial instant-read digital thermometer (Taylor Precision Products, Las Cruces, NM) in the middle of the middle shelf of the refrigerator, then conducted an audio-taped structured interview with the primary food preparer (70 questions). Following the interview (approximately 1 hour), visual assessments of the cleanliness of the kitchen and refrigerator (scales of 1 = not clean to 5 = very clean) were made, and the temperature of the refrigerator was recorded. Interview questions were developed by the project team to assess awareness and knowledge of foodborne pathogens, food shopping, preparation and storage practices, and kitchen cleaning procedures; pilot tested in two prospective households, and then revised as needed. Survey responses were entered onto the interview form, then rechecked using the audio-taped recording. Food and environmental samples were also collected (procedure follows below), follow-up visits were scheduled, and the participant was provided with a bathroom commode specimen collection system (Cardinal Manufacturers Inc., Streetboro, OH) along with instructions for stool sample collection at follow-up visits. Follow-up visits (visits 2, 3 and 4) involved only sample collection for microbiological analysis. The complete

TABLE 1. Demographic characteristics of participating households

Characteristic	Ruminant households n = 28		Non-ruminant households n = 26	
	Number	%	Number	%
Highest level of education completed by any adult household member:				
High school graduate	1	3.6	0	0.0
Some college/technical school	6	21.4	4	15.4
4-year college degree	14	50.0	3	11.5
Post-graduate studies	7	25.0	19	73.1
Age of house:				
<5 years	3	10.7	4	15.4
5–14 years	7	25.0	5	19.2
15–24 years	5	17.9	3	11.5
>25 years	13	46.4	14	53.9

protocol for behavioral data and sample collection was completed in its entirety during year 1 and year 3 of the study.

Sample collection

During each visit, 3 food samples, 5 environmental samples and, in the case of farm households, a ruminant fecal sample were collected. In addition, during visits 2, 3 and 4 of year 1, a stool sample from any member of the household was also collected. Food samples included leftovers (preferably from a home-made meal), dairy products (preferably from non-pasteurized milk), deli meats and cut fruit and/or vegetables. Environmental samples were taken from the refrigerator (handles and one shelf, preferably the meat drawer), kitchen sink (faucet and drain), clothes washing machine (rim), shoe soles and the floor underneath the shoes (if this was not carpet), kitchen countertop or utility sink (faucet and drain) next to the clothes washer, and/or gloves used for farming activities. Food samples were collected with a sterilized metal spoon and placed in a sterile Whirl-Pak® bag (15 by 23 cm; Nasco, Modesto, CA). Environmental samples were collected with a moist sponge (10 ml buffered peptone water; HydraSponge™, 3M Microbiology, St. Paul, MN) by swabbing. All food and environmental samples were collected by the participants, after proper instruction to ensure uniform collection methods. Stool samples from any household member (one sample per visit for visits 2, 3 and 4 of year 1 of the study) were collected from each participant household in the commode specimen collection system. Ruminant fecal samples were collected from the ground with a sterilized tongue depressor and transferred to a Whirl-Pak bag. All samples were transported to the laboratory in coolers with ice packs, and analyzed within 24 h of collection.

Microbiological analyses of samples

All samples were analyzed for presence of *Listeria* by use of the procedure outlined in the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) Microbiology Laboratory Guidebook *(44)*, with the following modifications. For environmental samples, 90 ml of Universal Preenrichment Broth (UPB, Difco, Becton Dickinson, Sparks, MD) was added to each pre-moistened sponge in its bag, and for food and fecal samples, 225 ml of UPB was added to 25 g of sample in a Whirl-Pak bag (15 by 23 cm). Samples were then homogenized for 2 min (Masticator, IUL Instruments, Barcelona, Spain) and incubated at 35°C for 22–24 h. Then, 1 ml of the UPB enrichment was transferred to 9 ml of Fraser Broth (FB, Difco) and incubated at 35°C for 22–24 h. After incubation, tubes of FB showing signs of darkening were streak-plated onto PALCAM agar (Difco) plates and incubated at 30°C for 48 ± 2 h. Colonies on PALCAM agar plates with morphologies typical of *Listeria* were isolated and purified for further biochemical analyses for differentiation between *L. monocytogenes* and other *Listeria* spp. *(44, 46)*. Suspect colonies (up to five per sample) were confirmed as *Listeria* based on Gram Stain, motility, catalase activity and oxidase activity. *L. monocytogenes* was differentiated from other *Listeria* spp. by hemolysis of sheep blood agar and fermentation of rhamnose, xylose and mannitol *(46)*. Isolates identified as *L. monocytogenes* on the basis of their biochemical reactions were sent to the Ohio Agricultural Research and Development Center (The Ohio State University, Wooster, OH) for serotyping, using a previously described *(49)* multiplex PCR assay.

Environmental samples (i.e., sponge swabs from refrigerators, kitchen and utility sinks, kitchen countertops,

TABLE 2. Samples positive for *Listeria* in Colorado rural households with ruminant animals

			Samples positive for (description of sample [serotype]):	
ID	Year	Visit	*Listeria* spp.	*Listeria monocytogenes*
1	3	4	animal feces (cows)	
2	1	1	animal feces (cows)	
		3	animal feces (cows)	
	3	1	animal feces (cows), shoe soles, washing machine	
		2	animal feces (cows)	
		3	animal feces (cows)	
		4	animal feces (cows)	
3	1	1	food (chipped beef, cottage cheese), kitchen sink	food (chipped beef [4b and other[a]], cottage cheese [1/2a and 4b]), kitchen sink [4b]
4	1	2	animal feces (goats)	animal feces (goats [4b])
		4	animal feces (goats)	
5	1	1	food (cheddar cheese), kitchen sink, washing machine	
		2	refrigerator	
		3	kitchen sink	
	3	1	kitchen sink	
		2	kitchen sink	
		3	kitchen sink	
		4	kitchen sink, shoe soles	
6	1	1	kitchen sink	
		2	animal feces (cows)	
7	3	1	animal feces (sheep)	
8	1	2	food (lunch meat)	food (lunch meat [atypical[b]])
9	3	4	kitchen sink	kitchen sink [atypical[b]]
10	1	3	food (turkey)	
	3	2	food (deli chicken breast)	
		3	shoe soles	
11	3	3	shoe soles	
12	1	3	animal feces (cows), refrigerator	
	3	1	food (round steak)	
		2	shoe soles, animal feces (cows), food (pork sausage)	animal feces (cows [1/2a]), food (pork sausage [1/2a])
		3	animal feces (cows), refrigerator	
		4	shoe soles	
13	1	1	animal feces (sheep)	
		2	animal feces (sheep)	
		3	animal feces (sheep)	
	3	1	shoe soles	
		2	animal feces (sheep)	
		3	animal feces (sheep)	
		4	animal feces (sheep), shoe soles, refrigerator	animal feces (sheep [1/2a])
14	3	1	farming gloves	
15	3	1	shoe soles	
16	3	1	shoe soles	
		2	animal feces (cows)	

TABLE 2. Samples positive for *Listeria* in Colorado rural households with ruminant animals (*Continued*)

			Samples positive for (description of sample [serotype]):	
ID	Year	Visit	*Listeria* spp.	*Listeria monocytogenes*
17	1	2	food (queso fresco, lettuce)	food (queso fresco [1/2a], lettuce [1/2a])
		3	refrigerator	refrigerator [1/2a]
18	1	1	refrigerator, food (sliced ham)	food (sliced ham [other[a]])
		3	food (ham)	
	3	4	animal feces (sheep)	animal feces (sheep [4b and other[a]])
19	3	3	shoe soles	
		4	shoe soles	

[a]Other serotype different from 1/2a and 4b

[b]A 350 bp band was amplified from *inlB* instead of the 500 bp band expected for *L. monocytogenes*

washing machines, shoes and gloves) were also tested for *Salmonella* and *E. coli* O157:H7 presence. For *Salmonella* testing, the USDA-FSIS Microbiology Laboratory Guidebook *(45)* protocol was followed, with the following modifications. One ml of the UPB enrichment was transferred to 9 ml of Tetrathionate Broth (TTB, Difco) and incubated at 35°C for 22–24 h. After incubation, a loopful of the TTB enrichment was streak-plated onto Brilliant Green Sulfa agar (Difco) and Xylose Lysine Tergitol™ 4 agar (Difco) plates. Plates were incubated at 35°C and were first examined at 18–24 h and later after 48 h for *Salmonella* suspect colonies. Up to five suspect colonies per sample were selected for biochemical confirmation with API 20E strips (bioMérieux sa, Marcy-l'Etoile, France). Serotyping of the isolates was performed by the Veterinary Diagnostic Laboratory, Veterinary Teaching Hospital, Colorado State University (Fort Collins, CO).

To test for the presence of *E. coli* O157:H7 in the environmental samples, the USDA-FSIS protocol *(43)* was followed, with the following modifications. One ml of the UPB enrichment was transferred to 9 ml of modified *E. coli* broth (mEC, Difco), and after incubation (35°C, 22–24 h) was streak-plated onto sorbitol MacConkey agar (Difco) supplemented with cefixime and potassium tellurite (Invitrogen Dynal, Oslo, Norway) (35°C, 22–24 h). Suspect colonies (up to five per sample) were tested for the O157 antigen, using the RIM™ *E. coli* O157:H7 Latex Test (Remel, Lenexa, KS). Agglutination-positive isolates were further tested with API 20E strips, and subjected to PCR analysis *(17)* for confirmation.

Statistical analysis

Answers from surveys and interview questionnaires were coded on a scale of 0 to 5, with 0 being the least desirable behavior/response and 5 being the most desirable behavior/response. Refrigerator temperatures were also coded on a scale of 0 to 5, with 0, 3 and 5 assigned to temperatures ≥ 50°F (10°C), 41 to 49°F (5 to 9.4°C) and ≤ 40°F (4.4°C), respectively. All data were uploaded into Microsoft Excel® files and imported into SAS/STAT® *(40)*. Seven indices were developed by grouping related questions from the behavioral data collection instruments. The PROC CORR function of SAS/STAT® *(40)* was used to calculate Cronbach's alpha coefficients to test for internal reliability of each index *(3, 4)*. A Cronbach's alpha coefficient of at least 0.5 was considered acceptable for relatedness of the questions *(3)*. The indices included Perishable Food Handling and Cooking Index (PFHCI), Pathogen Awareness Index (PAI), Personal Cleanliness Index (PCI), Kitchen and Household Cleanliness Index (KHCI), Inside Cross-contamination Index (ICCI), Outside Cross-contamination Index (OCCI), and Risky Foods Preferences Index (RFPI). Logistic regression analysis with the GLIMMIX® procedure of SAS/STAT® *(40)* was used to determine the potential relationship between indices and prevalence of *Listeria* in the households. Prevalence of *Listeria* was divided into Overall Prevalence (OP), which included all samples within a household except for animal and human fecal samples; Food Prevalence (FP), which included all food samples collected; Kitchen Environment Prevalence (KEP), which included all samples from refrigerators, kitchen sinks and kitchen countertops; and Non-kitchen Environment Prevalence (NKEP), which included samples from shoes, utility sink, farming gloves and clothes washing machine. Differences were considered statistically significant at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Household demographics

Table 1 presents the demographic characteristics of the households recruited. A total of 54 rural households were initially recruited, 28 with and 26 without ruminant animals. Ruminant animals on the household premises included (numbers in parentheses are households) cattle (12), goats (13), sheep (9), llamas (5) and/or alpacas (1). Some households, including those classified as non-ruminant households, may have had other animals such as cats, dogs, horses, pigs, and chickens as well as other birds. Two households with ruminants decided not to participate in the second sample collection period (year 3), but their data from the first sample collection period (year 1) were used in the analysis. Two households classified as non-ruminant households in year 1 acquired animals

TABLE 3. Samples positive for *Listeria* in Colorado rural households without ruminant animals

ID	Year	Visit	Samples positive for (description of sample [serotype]): *Listeria* spp.	*Listeria monocytogenes*
A	3	1	food (bacon)	food (bacon [1/2a])
B	1	1	food (sliced cheese)	food (sliced cheese [other[a]])
		4	kitchen countertop	kitchen countertop [4b]
C	1	2	food (roast beef)	food (roast beef [other[a]])
D	1	2	shoe soles	
E	1	4	food (lettuce)	food (lettuce [4b])
	3	3	food (bacon)	
F	1	1	refrigerator	
	3	2	kitchen sink	
		3	kitchen sink, shoe soles	kitchen sink [4b]
		4	kitchen sink	kitchen sink [4b]
G	1	4	washing machine	washing machine [4b]
H	1	4	refrigerator	refrigerator [other[a]]
	3	1	food (salmon spread)	food (salmon spread [1/2a])
I	1	3	shoe soles	shoe soles [4b]
J	1	4	food (taco)	food (taco [1/2a and other[a]])
K	3	2	food (mushrooms)	
L	1	3	refrigerator	refrigerator [4b and other[a]]

[a]Other serotype different from 1/2a and 4b

after the first sampling period ended, and so were considered ruminant households for the year 3 sample collection period. One household classified as a ruminant household in year 1 sold all their animals after the first sample collection period was completed, and so was considered a non-ruminant household in year 3.

Overall *Listeria* prevalence

Listeria spp. was recovered from all types of samples collected, except from human stools and swabs from utility sinks (Tables 2–4). For reporting purposes, *Listeria*-positive households with ruminant animals were reassigned a number (1 through 19) for identification, and *Listeria*-positive households without ruminants were reassigned a letter (A through L) for identification (Tables 2 and 3). Overall, *L. monocytogenes* prevalence was very low (0 to 3.1% for various types of samples tested; Table 4). Of note, however, was that about half of the samples that tested positive for the pathogen were from foods (7 of 14 positive samples in households with ruminants, and 6 of 13 positive samples in households without ruminants; Table 4). The majority of *L. monocytogenes* isolates belonged to serotypes 1/2a and 4b (Tables 2 and 3), which, along with serotype 1/2b, are responsible for 95% of human cases of listeriosis infection *(10)*. Most of the *L. monocytogenes*-positive food samples were cheeses, meats, and meat products, which are known vehicles for the pathogen. This indicates that food purchase behavior may have an effect on the prevalence of *L. monocytogenes* in the household environment *(48)*.

Because of the low prevalence of *Listeria* spp. and *L. monocytogenes* in the different types of samples, prevalence data were grouped according to the origin of the sample within the household, and the results by household type and collection year for *L. monocytogenes* and other *Listeria* spp. are presented in Table 5. There was no effect ($P \geq 0.05$) of ruminant presence or collection year on the grouped prevalence of *Listeria* (Table 5). However, there was a clear trend for the grouped prevalence of *Listeria* to be numerically higher in ruminant households than in households without ruminants (Table 5), potentially indicating a higher exposure of these households to *Listeria*. The lack of statistical significance may have been due to the relatively small sample size of households and the very small number of samples that were positive for *Listeria.*

Listeria prevalence in human stools

Because none of the stool samples tested positive during year 1 of sample collection, this collection was discontinued in year 3 (Table 4). Household

TABLE 4. Number of samples positive for *Listeria* (%) in households by type of sample, collection year, and presence of ruminants

Type of sample	Year 1						Year 3						Total (Year 1 + Year 3)					
	Ruminants			No ruminants			Ruminants			No ruminants			Ruminants			No ruminants		
	n	*L.* spp.	*L. m.*	*n*	*L.* spp.	*L. m.*	*n*	*L.* spp.	*L. m.*	*n*	*L.* spp.	*L. m.*	*n.*	*L.* spp.	*L.m.*	*n*	*L.* spp.	*L.m.*
Food	336	9 (2.7)	6 (1.8)	309	4 (1.3)	4 (1.3)	322	3 (0.9)	1 (0.3)	304	4 (1.3)	2 (0.7)	658	12 (1.8)	7 (1.1)	613	8 (1.3)	6 (1.0)
Refrigerator	112	4 (3.6)	1 (0.9)	103	3 (2.9)	2 (1.9)	106	2 (1.9)	0 (0.0)	100	0 (0.0)	0 (0.0)	218	6 (2.8)	1 (0.5)	203	3 (1.5)	2 (1.0)
Kitchen sink	112	4 (3.6)	1 (0.9)	103	0 (0.0)	0 (0.0)	106	4 (3.8)	1 (0.0)	100	4 (4.0)	3 (1.3)	218	8 (3.7)	2 (0.5)	203	4 (2.0)	2 (1.5)
Kitchen countertop	60	0 (0.0)	0 (0.0)	87	1 (1.1)	1 (1.1)	53	0 (0.0)	0 (0.0)	74	0 (0.0)	0 (0.0)	113	0 (0.0)	0 (0.0)	161	1 (0.6)	1 (0.6)
Washing machine	112	1 (0.9)	0 (0.0)	103	1 (1.0)	1 (1.0)	106	1 (0.9)	0 (0.0)	98	0 (0.0)	0 (0.0)	218	2 (0.9)	0 (0.0)	201	1 (0.5)	1 (0.5)
Shoe soles	112	0 (0.0)	0 (0.0)	103	2 (1.9)	1 (1.0)	103	12 (11.7)	0 (0.0)	98	1 (1.0)	0 (0.0)	215	12 (5.6)	0 (0.0)	201	3 (1.5)	1 (0.5)
Utility sink	37	0 (0.0)	0 (0.0)	15	0 (0.0)	0 (0.0)	38	0 (0.0)	0 (0.0)	17	0 (0.0)	0 (0.0)	75	0 (0.0)	0 (0.0)	32	0 (0.0)	0 (0.0)
Farming gloves	16	0 (0.0)	0 (0.0)	*	*	*	14	1 (7.1)	0 (0.0)	7	0 (0.0)	0 (0.0)	30	1 (3.3)	0 (0.0)	4	0 (0.0)	0 (0.0)
Human stools	77	0 (0.0)	0 (0.0)	73	0 (0.0)	0 (0.0)	*	*	*	*	*	*	77	0 (0.0)	0 (0.0)	73	0 (0.0)	0 (0.0)
Ruminant feces	107	9 (8.4)	1 (0.9)	n/a	n/a	n/a	97	13 (13.4)	3 (3.1)	n/a	n/a	n/a	204	22 (10.8)	4 (2.0)	n/a	n/a	n/a

n: number of samples collected

L. spp.: *Listeria* spp.

L. m.: *Listeria monocytogenes*

*: samples of this type were not collected for this sampling period

n/a: not applicable

members who provided a stool sample were between 11 months and 69 years of age. *Listeria* was not recovered from any of the samples, probably because of the small number of samples collected, long sample collection intervals (2–4 weeks), different individuals within the same household providing the samples, and the short length of *Listeria* fecal shedding periods in humans, which have been reported to last no more than 4 days *(1, 12)*. It has been reported that the prevalence of *Listeria* in human stools is low (<1 to 3.4%) among healthy individuals *(11, 12, 23, 26, 39)*.

Listeria prevalence in feces of ruminants

Listeria spp. were isolated from fecal samples of cows (12 positive out of 70 samples tested, 17.1%), sheep (8 out of 46, 17.4%), and goats (2 out of 58, 3.4%) (Table 2). A combined sample of goat and sheep feces was also positive. Four samples were positive for *L. monocytogenes*, one each from cows and goats and two from sheep (Table 2). None of the fecal samples from alpacas or llamas were positive for any *Listeria*. Ivanek et al. *(18)* reported on the dynamics of pathogen fecal shedding, specifically *L. monocytogenes*, and their results indicate that fecal shedding is subtype specific and can vary from 2 to 92%. These authors also found considerable day-to-day variability in fecal shedding of the pathogen and suggested that fecal samples should be collected at least daily in order to calculate the true prevalence within a herd of cattle *(18)*. This finding may explain the low number of animal fecal samples found positive for *L. monocytogenes* in this study, since samples were collected every 2–4 weeks. Nonetheless, overall, 17.1% (12 out of 70) of the fecal samples

TABLE 5. Grouped prevalence of *Listeria* by household type and collection year (number of positive samples/total number of samples collected, [%])

Year	Ruminants	Overall (OP)	Food (FP)	Kitchen Environment (KEP)	Non-kitchen Environment (NKEP)
1	Yes	18/897[a] (2.0)	9/336[a] (2.7)	8/284[a] (2.8)	1/277[a] (0.4)
	No	11/823[a] (1.3)	4/309[a] (1.3)	4/293[a] (1.4)	3/221[a] (1.4)
3	Yes	23/848[a] (2.7)	3/322[a] (0.9)	6/265[a] (2.3)	14/261[a] (5.4)
	No	9/795[a] (1.1)	4/304[a] (1.31)	4/274[a] (1.5)	1/217[a] (0.5)
Total 1+3	Yes	41/1745 (2.3)	12/658 (1.8)	14/529 (2.6)	15/538 (2.8)
	No	20/1618 (1.2)	8/613 (1.3)	8/567 (1.4)	4/438 (0.9)

OP: includes all samples except for human and animal fecal samples
FP: includes all food samples
KEP: includes refrigerator, kitchen sink and kitchen countertop samples
NKEP: includes shoe soles, washing machine, utility sink and farming glove samples
[a]Grouped prevalence with same superscript within a column are not significantly different ($P \geq 0.05$)

from cattle and 9.6% (10 out of 104) of the fecal samples from goats and sheep were positive for *Listeria* spp. However, none of the households with ruminants reported to have had a case of listeriosis in their animals within the 12-month period before sample collection began, indicating asymptomatic carriage of *Listeria* by these animals.

Several studies have reported that ruminant animals may be asymptomatic carriers of *Listeria*, and thus may serve as a reservoir and source of contamination for other animals *(25, 30, 31, 32)*, as well as humans and food manufacturing environments. For example, Wagner et al. *(48)* reported a case in a cheese-producing farm, where *L. monocytogenes* was possibly transmitted from contaminated animal feeds to the milk supply, onto the working surfaces of the cheese-making facility and into humans. In the same study, *L. monocytogenes* was detected two months after the outbreak on the boots and in the feces of a worker *(48)*. As a consequence, a cross-contamination cycle between the worker and the cheese processing environment was established. This may also have been the case in our study, since there were several instances where shoe samples tested positive at the same time as other samples taken from inside the household environment (kitchen sinks, washing machines and refrigerators) (Table 2). The shoes were probably contaminated while being used to work with the animals *(48)*.

In household #2, which had cows on the property, the animal feces sample tested positive for *Listeria* spp. twice during year 1 and all four times during year 3 sample collection (Table 2). Swabs from shoes and the washing machine also tested positive at the same time, indicating a potential scenario of cross-contamination between the animals and the household. In another case, household #5, which had goats on the property, had samples from food (cheddar cheese), and the refrigerator, washing machine and kitchen sink (twice) test positive for *Listeria* spp. in year 1, and samples from the kitchen sink, along with one shoe sample tested positive for *Listeria* spp. during all four visits in year 3 (Table 2). These results may indicate not only potential cross-contamination events but also re-contamination or persistence of *Listeria* within the household environment.

Feces from cows in household #12 tested positive in year 1 (*Listeria* spp.) and again in year 3 (*Listeria* spp. and *L. monocytogenes*; Table 2). In addition, during year 3, multiple food, refrigerator and shoe samples were positive for both *Listeria* spp. and *L. monocytogenes*, in another potential cross-contamination scenario where the most likely source may have been the animal feces. These results point to ruminant animals as an important source of contamination for the household environment, and to a potentially higher exposure of the household members to the microorganism, compared exposure of members of households without ruminants.

Listeria prevalence in the kitchen environment

Listeria spp. and *L. monocytogenes* were isolated from all sampling sites within the kitchen (Tables 2–4). The overall prevalence of *Listeria* in the kitchen environment (KEP; Table 5) was higher in households with ruminants (2.6%) than in those without (1.4%), though not statistically higher ($P \geq 0.05$). As was the

TABLE 6. Number of households with samples positive for *Listeria* by year and household type

	At least one positive sample			Two positive samples			Three or more positive samples		
Ruminants	**Year 1**	**Year 3**	**Both years**	**Year 1**	**Year 3**	**Both years**	**Year 1**	**Year 3**	**Both years**
Yes	11	13	6	4	3	0	5	4	2
No	10	6	3	1	0	0	0	1	0

TABLE 7. Cronbach's alpha coefficient values for the behavioral indices

Index[a]	**Cronbach's alpha coefficient[b]**
Perishable Food Handling and Cooking Index (PFHCI)	0.747
Pathogen Awareness Index (PAI)	0.659
Personal Cleanliness Index (PCI)	0.679
Kitchen and Household Cleanliness Index (KHCI)	0.796
Inside Cross-contamination Index (ICCI)	0.787
Outside Cross-contamination Index (OCCI)	0.823
Risky Foods Procurement Index (RFPI)	0.500

[a]Each index comprises a series of questions from the different instruments used and was calculated as the average for the answers given by participants

[b]Cronbach's alpha coefficient measures the internal consistency or reliability of an instrument, and is a function of the extent to which questions in each index have high commonalities *(3, 4)*

case with samples involving animal feces and shoes, several cases of possible cross-contamination, re-contamination and/or persistent contamination occurred within the kitchen environment of several households. For example, the kitchen sink and two different food samples in household #3 tested positive for *L. monocytogenes* during the same visit (Table 2). In this case, both of the food samples and the kitchen sink were positive for strains of the same serotype (4b). In household #17, two food samples and the refrigerator swab also tested positive for the same *L. monocytogenes* serotype (1/2a) on two consecutive visits. In another case, household #18 had two food samples and the refrigerator test positive for *Listeria* spp., and one of the food samples was positive for *L. monocytogenes* (Table 2*)*. Even when it was not possible to establish the origin of contamination, it was clear that cross-contamination occurred within the kitchen environment, a phenomenon that has been reported before *(38, 47)*. In the present study, cases of potential cross-contamination/re-contamination with multiple samples from different sites testing positive at the same time or throughout the sample collection period occurred more often in households with ruminants (10 out of 30 households) than in households without ruminants (2 out of 28 households) (Tables 2 and 3). Samples positive for *Listeria* in non-ruminant households tended to be isolated (single samples from a given household testing positive for a given visit; Table 6).

Listeria prevalence in non-kitchen environmental samples

In the non-kitchen environment, *L. monocytogenes* was isolated only from one shoe sole and one washing machine sample, both collected from households without ruminant animals (Tables 3 and 4). While the overall *Listeria* prevalence in the non-kitchen environment (NKEP; Table 5) was numerically higher in households with ruminants on their premises than in those without (2.8 and 0.9%, respectively), differences were not significant ($P \geq 0.05$) by ruminant presence or by collection year. However, the interaction between ruminant presence and collection year was significant ($P < 0.05$), primarily due to a large increase from year 1 to year 3 in the number of shoe soles testing positive for *Listeria* spp. in households with ruminant animals (Table 4). Our results show a trend of higher prevalence of *Listeria* in households with ruminant animals on their premises, indicating an increased exposure to the microorganism and a potentially higher risk for listeriosis infection to the household members. Thus, families in households with ruminants on their property should be educated about the potential for increased risk of

TABLE 8. Behaviors associated with increased *Listeria* prevalence in rural households

	Covariate effect	β[a]	*P*-value	exp (β)[b]
Overall Prevalence (OP)	Perishable Food Handling and Cooking Index (PFHCI)	-0.9064	0.0288	0.4040
	Associated behaviors:			
	Refrigeration of leftover foods within 2 h of preparation			
	How full is the refrigerator?			
	Refrigerator temperature			
	Use of thermometer for cooking of whole chicken, ground beef, steaks and roasts			
	Coverage of leftovers inside fridge			
	Presence of visible spoiled food, odors, spills and/or dripping inside the fridge			
Non-kitchen Environment Prevalence (NKEP)	Personal Cleanliness Index (PCI)	-1.0450	0.0337	0.3517
	Associated behaviors:			
	Hand wash after farming/pet activities			
	Boots change after farming activities			
	Clothes change after farming activities			
	Location, frequency and technique of hand wash after farming activities			
	Use of an automatic dryer for clothes			

[a]β: measures the degree of association between the probability of any given household having a positive sample and the value of a particular index *(33)*

[b]exp (β): the odds ratio of any given household having a positive sample when an specific index changes one unit

exposure to *Listeria* and about preventive measures that can be applied during and after farming activities, with special attention to personal cleanliness habits, such as hand washing and change of clothing and shoes, after animal care. This type of educational campaign may help in preventing cross-contamination, re-contamination and persistent contamination of the household environment and food supply.

Risk factors associated with increased *Listeria* prevalence

Cronbach's alpha coefficients for the seven behavioral indices developed ranged from 0.500 to 0.823 (Table 7), indicating an acceptable level of relatedness between the questions in each index *(3, 4)*. High relatedness between questions is desirable since it indicates that variance in the responses is due to individual differences between the subjects providing the answers *(3)*.

Logistic regression analysis *(40)* found that only two of the seven behavioral indices correlated with any of the four *Listeria* prevalence factors (OP, FP, KP, and NKEP). For this study, all recovered *Listeria* spp. were considered for the analysis of risk factors, since other species of *Listeria* may share the same ecological niches in the environment with *L. monocytogenes* (including food, vegetation and soil) *(24)* and may grow faster than *L. monocytogenes (9, 35)*. Further, the detection of any *Listeria* spp. within the household environment may be cause for concern, since *Listeria* in general is used as a hygiene indicator in all stages of the food processing chain *(20)*.

Table 8 shows the two behavioral indices that correlated with prevalence of *Listeria* in the households. The Beta coefficients are negative, meaning that as the mean value of the index increases, the predicted prevalence will be reduced. This indicates that households that apply more desirable behaviors will have a decrease in the prevalence of *Listeria* in the environment.

The Overall Prevalence (OP) of *Listeria* was significantly ($P < 0.05$) affected only by a negative score on the Perishable Food Handling and Cooking Index (PFHCI) (Table 8). This suggests that the way people handle and cook perishable foods at home is very important in the prevention of *Listeria* contamination. A high score for this index included using a thermometer to ensure adequate cooking of chicken and meat products, refrigerating leftovers within 2 h of preparation, covering refrigerated leftovers and keeping the refrigerator cold, clean and not too full. This is good advice for all consumers, but is especially important for persons at increased risk for listeriosis,

including the elderly, pregnant women, neonates, and the immunocompromised *(25)*. From data in Table 5, it can be calculated that 29 and 40% (12 out of 41, and 8 out of 20, respectively) of the *Listeria*-positive samples came from food samples in households with and without ruminants, respectively. More specifically, 50% (7 out of 14) and 46.1% (6 out of 13) of the samples positive for *L. monocytogenes* (Table 4) were food samples in households with and without ruminants, respectively, pointing to foods as the main source for the pathogen in the household, regardless of the presence of ruminants on the property, and stressing the importance of carefully handling foods commonly contaminated with *Listeria.*

The Personal Cleanliness Index (PCI) showed a significant ($P < 0.05$) effect on the Non-kitchen Environment Prevalence (NKEP) of *Listeria* spp. (Table 8). The behaviors included in this index are associated with personal hygiene, especially after farming activities and before entering the house (Table 8). Practices that should be followed after farming chores include changing footwear and clothing to avoid tracking of soil and dirt into the house. This was found to be especially important for households with ruminants, where prevalence of *Listeria* on shoes was nearly four times that on shoes from non-ruminant households (5.6 and 1.5%, respectively). Personal cleanliness is generally important in reducing the spread of bacteria, and based on this study, has special importance in reducing the spread of *Listeria* from the farm to the household environment.

E. coli O157:H7 and *Salmonella* prevalence

While this study focused on *Listeria* prevalence, environmental samples were also tested for *E. coli* O157:H7 and *Salmonella*. None of the samples tested positive for *E. coli* O157:H7. *Salmonella* was isolated from samples taken from the refrigerator (1 out of 421 samples; *Salmonella* Senftenberg), farming gloves (1 of 34 samples; *Salmonella* Infantis), washing machine (1 of 419 samples; *Salmonella* Cerro), and shoes (2 of 422 samples; *Salmonella* Typhimurium var. Copenhagen and *Salmonella* Cerrro). All samples positive for *Salmonella* were recovered from households with ruminants. With the exception of one household, all of the *Salmonella*-positive samples were collected in households that also had multiple samples positive for *Listeria* in both years 1 and 3 (Table 2). These results support the theory of potential cross-contamination, re-contamination and/or persistence discussed earlier. There was a trend for households with ruminants to have more positive shoe samples, as well as multiple samples being positive at the same time as the shoes, indicating that it is highly likely that contamination of the household may have occurred from shoes that tracked dirt inside the house from animal pens.

An association between dirt and dust contamination with *Salmonella* and salmonellosis infection, especially in young children, has been reported *(13, 14, 42)*. Haddock and Nocon *(14)* found that vacuum cleaners used in homes of infants with confirmed salmonellosis infection were more likely to contain *Salmonella* than those used in control households. In another study, Haysom and Sharp *(16)* found *Salmonella* counts recovered from vacuum cleaner dust to be significantly higher in samples from rural than urban environments and in households where pets were present. These authors concluded that major sources of bacterial contamination in rural areas were livestock, manure and soil that could be introduced into the domestic environment on footwear, the feet of pets and air currents *(16)*. These findings and the fact that a high proportion of cases of *Salmonella* infection are reported in children 5 years of age or younger *(13, 16)*, make the study of the household environment a priority in the search for control measures for prevention of this disease. The results presented here stress the importance of educating household members, especially in rural households with ruminant animals, about appropriate hygiene habits for cleaning of clothes and farming shoes after animal care and before entering the house.

LIMITATIONS

One limitation of this study is the small sample size of households, which may be the reason that statistically significant differences or effects were not detected, even when the differences in trends were clear between households with and without ruminant animals on their premises. Another limitation is the bias that is inevitable when working with human subjects and self-reported behaviors. It is possible to have recall bias, in which the individual reporting a specific behavior may not correctly remember the details. Also, human subjects given options (which was the case in most data collection instruments used in this study) tend to report the behavior they think is the best, rather than their actual behavior. Differences between self-reported and current behavior have been observed *(29, 37)*. Also, the results of this study are limited to a specific area with its specific conditions, such as climate, that may have affected the prevalence of *Listeria*.

CONCLUSIONS

Households with ruminant animals tended to have higher prevalence of *Listeria* and *Salmonella* in the environment, potentially leading to higher exposure of household members to these pathogens and increasing their risk of infection. Results point to foods as a potentially important source of *L. monocytogenes* for the household environment. Furthermore, findings suggested that cross-contamination, re-contamination and/or persistent contamination may have occurred in some cases with both microorganisms. Handling of perishable foods and personal cleanliness practices immediately after farm animal care play important roles as potential routes for contamination. Education on better cleanliness habits regarding shoes, clothing and hand washing after animal handling may reduce the risk of contamination to those households.

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