Prevalence of *e.coli* O157:H7, *Salmonella*, and *Cryptosporidium* Among Arizona Dairy Workers Using Post-Work Swabbing

Research

Rietta Wagoner[1], Kaitlyn A. Benally[2], Daniela Cabrera[2], Gerardo Lopez[2], Nicolas I. Lopez-Galvez[3], Duarte Diaz[2,*]

[1] Community, Environment, and Policy, University of Arizona, Tucson, Arizona, USA.
[2] School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, Arizona, USA.
[3] Division of Environmental Health, San Diego State University Graduate School of Public Health, San Diego, California, USA.
[*] Correspondence: duartediaz@arizona.edu

HIGHLIGHTS

- Microbial assessment of dairy workers in Arizona, U.S.
- Provides demographic and working information of an underserved group.
- Highlights the need for health and safety assessments and solutions in the dairy industry.

ABSTRACT. *The dairy industry in Arizona, like many other agricultural industries in the United States, is dependent on the labor that migrant farm workers provide. Infections caused by zoonotic pathogens are commonly underreported or misdiagnosed, and possibly more so in migratory workers that face cultural, structural, legal, financial, and geographic barriers to health services. The objectives of this project were to: assess the demographics of Arizona dairy workers, determine the exposure potential of Arizona dairy workers to zoonotic organisms, and inform best management practices. A questionnaire including demographics, work tasks, and household characteristics was administered. Swab samples were collected from the shoulders, knees, and foreheads of employees at two dairy operations at the end of the work shift. The swabs were cultured for E.coli O157:H7 and Salmonella. Molecular DNA isolated from Salmonella and Cryptosporidium was quantified using droplet-digital Polymerase Chain Reaction (ddPCR). Twenty dairy workers were recruited, and 60 samples were collected. The majority of workers were male, preferred to speak Spanish, and identified as Latino/Hispanic (68.8%, 93.8%, and 93.8%, respectively). E. coli O157:H7 was detected in 13% of cultured knee and forehead samples. Salmonella spp. gene copies were detected on 60.0% of samples collected from forehead skin samples; 40.0% of shoulder clothing samples; and 15% of knee clothing samples, as measured via ddPCR. The positive cultural and molecular samples indicate the need for improved post-workday sanitation practices at farms. This study provides surveillance of a largely invisible population, including insights that can be used to create site-specific health and safety protocols for the dairy industry, inform risk assessment models, and foster preventive practices in the dairy industry.*

Keywords. *Agricultural workers, Dairy workers, ddPCR, Exposure, Microbial, Zoonotic.*

Submitted for review on 19 May 2023 as manuscript number JASH 15680; approved for publication as a Research Article by Associate Editor Dr. Farzaneh Khorsandi and Community Editor Dr. Michael Pate of the Ergonomics, Safety, & Health Community of ASABE on 21 December 2023.

  ISSN 1074-7583 https://doi.org/10.13031/jash.15680

Similar to other agricultural industries in the U.S., the dairy industry is dependent on the labor of migrant farm workers (Arcury and Quandt, 2007; Grzywacz et al., 2014; Rosson et al., 2009; Villarejo, 2003). The Southwestern U.S. dairy industry employs an estimated 48,000 workers each year to produce over 30% of the milk in the U.S., commonly utilizing 24-hour production methods in the milking parlor to maximize milk output (Adcock et al., 2015; NASS, 2020). These intensive work tasks create an increased potential for exposure to bovine pathogens through the inhalation of aerosols, direct contact, contact with contaminated fomites, oral ingestion, and vector transmission, which could provoke zoonotic infections (McDaniel et al., 2014; Trevino, 2006; Wilkinson, 2006). Zoonotic pathogen outbreaks can adversely impact public health as well as the food distribution chain (Abdus Sobur et al., 2019). Zoonotic diseases of cattle include anthrax, bovine tuberculosis, bovine spongiform encephalopathy, campylobacteriosis, colibacillosis, cryptosporidiosis, listeriosis, and salmonellosis, among others (CDC, 2019a; Grout et al., 2020; McDaniel et al., 2014; Rahman et al., 2020; Vlasova and Saif, 2021; ISU Center for Food Security and Public Health, 2021). The National Institute of Allergy and Infectious Diseases listed over 10 bovine zoonoses as emerging or re-emerging diseases in 2018 (National Institute of Allergy and Infectious Diseases, 2018). The United States Center for Disease Control and Prevention (CDC) has listed 24 bovine zoonoses as potential bioterrorism agents because of their potential to be used as biological weapons to impact human health, the national food supply, and incite public fear (Khan et al., 2000; McDaniel et al., 2014). These include *Clostridium difficile, Cryptosporidium spp., Escherichia coli,* and *Salmonella spp.,* to name a few (Oliver et al., 2005). Infections caused by zoonotic organisms are commonly underreported or misdiagnosed, and possibly more so in migratory populations that encounter structural and geographic barriers to health services (Jimenez et al., 2014). *Campylobacter*, *Escherichia coli* O157:H7, *Salmonella*, *Giardia, Cryptosporidium parvum*, and *Listeria monocytogenes* are common pathogenic organisms present in dairy herds (Anthony, 2019; Edrington et al., 2004; Genovese et al., 2004; Li et al., 2019; Santín et al., 2008; Santín et al., 2004).

E.coli O157:H7 is found in the intestines of mammals and birds and is excreted in feces. Cattle have been associated with the majority of cases of *E.coli* O157:H7 serotype infections (Munns et al., 2015). There are an estimated 70,000 *E.coli* O157:H7 infections each year in the U.S. (CDC, 2017b). This is most probably an underestimation due to low testing rates, in particular among the asymptomatic population. The true prevalence of disease in the community cannot be exclusively based on positive testing. Infection can occur from exposure to as few as 10 cells, with an incubation period between 1 and 10 days, with an average of 3-4 days of sickness (CDC, 2017b; Kaper, 2005). After exposure, humans may be asymptomatic or can develop one or all of the following symptoms: diarrhea, cramps, vomiting, fever, and progression from diarrhea to bloody diarrhea (CDC, 2017b). After the initial symptoms, 5%-10% of patients develop Hemolytic Uremic Syndrome (HUS), which results in a 3%-5% case mortality rate in the U.S. (CDC, 2017a). HUS is a very serious problem for children. The majority of children with kidney disease on dialysis in the U.S. are related to pre-existing HUS (CDC, 2017b).

Salmonella spp. bacteria are also found in the intestinal tracts of animals and humans and are shed in the feces of infected individuals (Abdus Sobur et al., 2019). Antibiotic resistance in *Salmonella spp.* is an increasing concern for health professionals; drug-resistant nontyphoidal *Salmonella* was labeled as a serious threat in the CDC's 2019 Antibiotic Resistance Threats in the United States Report (CDC, 2019a). A 2000-2001 study of over 1,500 "apparently healthy" dairy cattle in the Southwestern U.S. reported antimicrobial resistance in 17 of 67 isolates, with some isolates indicating multidrug resistance (Edrington et al., 2004). Individuals involved in animal handling, especially young animals, are likely to be exposed to *Salmonella spp.,* as dairy cattle can shed bacteria for a median of 50 days and up to 391 days (Hoelzer et al., 2011). In a 2003 evaluation of households with workers exposed occupationally (e.g., cattle farm, research laboratory, or veterinary clinic experiencing salmonellosis), nearly 30% had culturable *S. enterica* collected from vacuum cleaner contents (Rice et al., 2003). Approximately 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths attributable to *Salmonella spp.* occur in the U.S. each year (CDC, 2019b).

Several species of *Cryptosporidium,* a microscopic parasite responsible for diarrheal disease, can also be transferred from animal intestines to humans via direct or indirect fecal exposure. While a large proportion of cryptosporidiosis outbreaks are associated with swimming pools or other recreational water sources, 15% of *Cryptosporidium* outbreaks that occurred between 2009 and 2017 in the U.S. were linked to contact with cattle (Gharpure et al., 2019). Pre-weaned animals (e.g., dairy calves) have the highest prevalence of *C. parvum*, making workers at dairy operations involved in the care of pre-weaned animals most likely to be infected with *Cryptosporidium* (Santín et al., 2008, 2004). Symptoms of cryptosporidiosis begin on average 7 days after infection and can include symptoms of watery diarrhea, stomach cramps or pain, dehydration, nausea, vomiting, and fever (CDC, 2017a). These symptoms could last from several days to several weeks, with most cases lasting one to two weeks. As with other infections, persons with weakened immune systems are at risk for more serious complications and even fatal illness (CDC, 2017a). From 2009 to 2017, 444 cryptosporidiosis outbreaks were reported in the U.S., resulting in 7,465 cases, 287 hospitalizations, and one death (Gharpure et al., 2019).

Infections caused by *E. coli O157:H7*, *Salmonella spp.,* and *Cryptosporidium spp.* can be avoided with the use of low-cost and tailored preventive efforts (Cross et al., 2012; Devamani et al., 2014; Kinyua et al., 2016; Perez-Garza et al., 2017; Rwego et al., 2008). However, agricultural workers in the United States are more likely to accept and remain in hazardous working conditions due to their migratory and documentation status, lack of social support, difficulty accessing healthcare, fear of retribution, and adverse socioeconomic status (Arcury and Quandt, 2007; Furey et al., 2016; NAWS, 2018; Villarejo, 2003). The National Agricultural Workers Survey (NAWS) reports that over half of agricultural workers in the U.S. are foreign-born, a majority have not received education beyond primary school, and approximately half reported working in an undocumented status (Grzywacz et al., 2014; NAWS, 2018). The National Occupational Research Agenda (NORA) Agriculture, Forestry, and Fishing (AgFF) Sector Council prioritized this population in the 2018 National AgFF Agenda Goals, with an emphasis on the characterization of the demographics of agricultural populations and the creation of education, training, and translational interventions (NORA Agricultural Forestry and Fishing Sector Council, 2018). While there have been a handful of global studies of dairy worker and/or dairy farmer exposure to microorganisms (Abdus Sobur et al., 2019; Awadallah et al., 2016; Di Piazza et al., 2013; Gilpin et al., 2008; Leibler et al., 2023; Pierce et al., 2023; Rwego et al., 2008), to date, there has been no surveillance of dairy workers in Arizona or investigations of dairy worker exposure to *E.coli* O157:H7 or *Salmonella* in the U.S. Therefore, the objectives of this study were to: determine the exposure potential of Arizona dairy workers to zoonotic organisms, assess the demographics of Arizona dairy workers, and inform best management practices to reduce the potential risk of transmission.

Materials and Methods

Study Location

Dairy operations with milking cow inventories of between 500 and 10,000 heads in Arizona were contacted for participation in the study in 2017. The project was explained to dairy owners or managers, and site authorizations were completed before visiting each dairy. The study protocol was reviewed and approved by the University of Arizona Human Subjects Protection Program (IRB# 1709797791).

Study Population

A convenience sample of participants was recruited to participate in the study from two farms in 2017. Individuals that met the following criteria were considered for participation in the study: at least 18 years old and working in the milking area of the dairy farm. Participants were recruited by a bilingual (Spanish and English) investigator at the end of the workday with assistance from farm management. A bilingual (Spanish and English) investigator screened potential participants and explained all sampling procedures to interested individuals in the individual's language of choice. To address the risk of increased vulnerability due to non-documentation status, signatures were not required on consent forms. At the conclusion of the study, each participant received a small monetary compensation for their time in the form of a Walmart gift card. A total of 20 participants were recruited from two dairies in the southwestern portion of the U.S. At Farm 1, all 16 workers on shift were approached and agreed to participate. Similarly, all of the 4 workers on shift at Farm 2 agreed to participate. Farm 1 employed approximately 45 workers and maintained a herd of over 6,000 milking cows. Farm 2 employed 10 workers and kept a herd of approximately 500 cows.

Questionnaire

A short survey including questions regarding demographics, work history, tasks completed, personal protective equipment, cleaning practices, and household characteristics was administered orally to all participants in their language of choice (Spanish or English) at the end of a work shift.

Sample Collection and Analysis

Swab samples were collected by one bilingual study staff member from clothing worn over dairy workers' shoulders and knees and directly from the skin of their foreheads at the end of the shift. Samples were collected using Puritan and ESK sampling kits (Fisher Scientific, Chicago, IL) pre-filled with 4 mL of Letheen broth and a polyester-tipped swab for environmental surface sampling. Samples were collected from each 100 square centimeter area by wiping the swab in a Z pattern horizontally, vertically, and then diagonally. Samples were collected aseptically using new gloves for each individual, and the sample collection workstation surface was cleaned after collecting the three samples for each individual. All samples were packaged in ice chests, cooled to approximately 4°C, and then transported to the University of Arizona Laboratory, where they were processed within 24 hours.

Cultural Assays

Enumerations of *E. coli* O157:H7 and *Salmonella* via cultural assay were done as previously described (Ravishankar et al., 2009) with slight modification. Sorbitol MacConkey (SMAC, VWR, Radnor, PA, USA) agar was used for *E. coli* O157:H7 (Feng et al., 2020; March and Ratnam, 1986; Weagant et al., 1995), and Xylose lysine deoxycholate (XLD, VWR) agar for *Salmonella*. All samples were plated using 1 mL over three plates and incubated for 18-24 hours at 37°C.

Positive Control for Salmonella

E. coli O157:H7 (ATCC 960218) and *Salmonella enterica* serotype Newport strain (LAJ160311) were obtained from Dr. Sadhana Ravishankar's Laboratory, University of Arizona, Tucson, AZ. *Cryptosporidium parvum* oocysts were obtained from Dr. Michael Rigg's Laboratory, University of Arizona, Tucson, AZ.

DNA Extraction and Purification

DNA extractions were performed on 200 µL of each swab sample and positive controls, *Salmonella enterica* Newport and *Cryptosporidium parvum,* by first conducting five freeze and thaw cycles, to assist in lysing the *Cryptosporidium* oocysts, followed by the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, resulting in 200 µL of extracted DNA. The same DNA extraction was used for the detection of both pathogens.

Primers and Probes

The target gene sequence used for the detection of *Salmonella* was a 262-bp fragment of the *Salmonella*-specific invasion gene, *invA.* The primer pair sequence and probe sequence were used as previously described (Cheng et al., 2008; Yang et al., 2014). Primers were optimized by using a thermal gradient to determine the best annealing temperatures and interpreted using Quanta-Soft technology. Primers were purchased from Invitrogen (Thermo Fisher Scientific, Carlsbad, CA, USA) and probes from LGC Biosearch (Novato, CA, USA).

Droplet Digital PCR

Salmonella and *Cryptosporidium* gene copies were quantified with the primers and probes described above for the *invA* and 18S rRNA amplicon target, using a QX200 droplet digital PCR system (Bio-Rad) according to the manufacturer's instructions. Reactions for the *Salmonella invA gene* were amplified as previously described, with slight modifications (Cheng et al., 2008). Reactions for the *Cryptosporidium* 18S rRNA gene were also amplified on a C1000 Touch Cycler (Bio-Rad) according to Yang with modifications (Yang et al., 2014). Data were analyzed with QuantaSoft software version 1.7 (Bio-Rad). The concentration of *Salmonella* and *Cryptosporidium* were given in copies/µL, and the concentration was multiplied times 25 µL (total volume of reaction) to obtain the total copy numbers in the reaction/2.5 µL of DNA extraction multiplied by 200 µL of sample extracted multiplied by 4 mL of Letheen volume to give a final copies/3 cm^2 swabbed surface. Positive and negative controls were run on each ddPCR 96-well plate.

Data Analysis

Descriptive statistics were calculated for questionnaire responses, cultured *E. coli* and *Salmonella* detection, *Salmonella spp.* detected by molecular method, and *Cryptosporidium spp.* quantification. Simple linear regression was used to test whether various demographic and workday characteristics significantly predicted the amounts of pathogens present on participants. Statistical analyses were completed using STATA 15 (Stata Statistical Software: Release 15, College Station, TX, USA) and R Studio Software (version 3.5.1, Vienna, Austria).

Results and Discussion

Questionnaire

All 16 individuals from Farm 1 completed the questionnaire after the work shift at approximately 6:00 am, while individuals from Farm 2 opted not to participate in the questionnaire portion of the study due to time constraints. The results from the questionnaire related to participant demographics, work history, tasks completed, personal protective equipment, and household characteristics are summarized in table 1. More than half of the participants (75.0%) reported completing some high school or less, with only four participants reporting having completed high school or having taken some courses at a university or college level. Participants reported various amounts of time spent working at the current farm and working in agriculture. All participants reported working 8 or more hours during each shift. The majority of participants (93.8%) completed milking-related activities during the work shift. Nitrile/plastic/latex gloves, aprons, eye protection, and rubber boots were worn by the majority of participants (93.8%, 81.3%, 62.5%, and 100.0%, respectively) (table 2). The majority (75.0%) of participants reported that they washed their hands at the end of their shift. Half of the participants reported wearing their work boots home, whereas all reported wearing their work clothes home. The majority of participants reported traveling more than 15 minutes from work to home (62.5%) and living with relatives (56.3%). Half of the study participants had children under the age of 18 living in the home.

Table 1. Participant demographics, work history, tasks completed, personal protective equipment, and household characteristics.

		n	(%)
Gender	Male	11	(68.8)
	Female	5	(31.3)
Preferred Language	English	1	(6.3)
	Spanish	14	(87.5)
	Indigenous	1	(6.3)
Race/Ethnicity	Latino/Hispanic	15	(93.8)
	Prefer not to respond	1	(6.3)
Highest level of schooling completed	Completed junior high or less (grades 8 or less)	4	(25.0)
	Some high school (grades 9 - 12)	8	(50.0)
	Completed high school (completed grade 12)	3	(18.8)
	Some university/college	1	(6.3)
Time spent working at current farm	Less than 3 months	4	(25.0)
	3 to 6 months	2	(12.5)
	6 months to 1 year	3	(18.8)
	1 to 5 years	7	(43.8)
Time spent working in agriculture	Less than 3 months	2	(12.5)
	3 to 6 months	1	(6.3)
	6 months to 1 year	2	(12.5)
	1 to 5 years	7	(43.8)
	More than 5 years	4	(25.0)
Hours worked during shift	Less than 8	0	(0.0)
	8 or more	16	(100.0)
Tasks/work activities completed during shift	Milking	15	(93.8)
	Hospital parlor	1	(6.3)
	Moving cattle in corral	1	(6.3)

Table 2. Participant clothing, personal protective equipment, and household characteristics.

		n	(%)
Which clothing are worn during workday?	Nitrile/plastic/latex gloves	15	(93.8)
	Apron	13	(81.3)
	Respirator	1	(6.3)
	Eye protection	10	(62.5)
	Rubber boots	16	(100.0)
	Long sleeves	1	(6.3)
Did you wash your hands at end of your shift?	Yes	12	(75.0)
	No	4	(25.0)
Are the boots worn during shift same as boots worn home?	Yes	8	(50.0)
	No	8	(50.0)
Are clothes worn during shift same as clothes worn home?	Yes	16	(100.0)
	No	0	(0.0)
How long does it take to travel from work to home?	5 to 15 minutes	6	(37.5)
	More than 15 minutes	10	(62.5)
Who lives in your home?	Alone	3	(18.8)
	With family	9	(56.3)
	With other dairy workers	1	(6.3)
	With family and other dairy workers	2	(12.5)
	With partner/spouse	1	(6.3)
Are there children (18 years or younger) in your home?	Yes	8	(50.0)
	No	8	(50.0)
Are there elderly individuals (65 years or older) in your home?	Yes	1	(6.3)
	No	15	(93.8)

Culturable and Viable Samples

E.coli O157:H7

E. coli O157:H7 was detected in 8 out of 60 samples (13%) via culture assay (table 3). Seven of the twenty participants (35%) had detectable levels of *E. coli* O157:H7 on at least one body part, with one individual having detectable levels on two body parts (knees and forehead). Farm 2 had a higher percentage of workers with detectable *E. coli* O157:H7 on the knees (75%), compared to Farm 1 (18.8%) (fig. 1). *E. coli* O157:H7 was not detected on shoulder clothing samples from any of the workers in this study (fig. 1). Finally, 6.3% and 25% of workers from Farm 1 and Farm 2 had detectable levels of *E. coli* O157:H7 on the forehead, respectively (fig. 1). The mean and range of detected cultured *E. coli* O157:H7 on all workers' knees, shoulders, and foreheads are displayed in table 3.

Table 3. Measured Pathogen Loading on Body Parts of Dairy Workers (n = 20 participants, 3 body parts for a total of 60 samples).[a]

	Total n = 60	Knees n = 20		Shoulders n = 20		Forehead n = 20	
Assay and Pathogen	n (%)	n (%)	Mean (range)	n (%)	Mean (range)	n (%)	Mean (range)
Culture Assays							
E. Coli O157:H7 (CFU)	13 (8/60)	6 (30.0)	97.3 (20-240)	0 (0)	ND	2 (10)	114 (8.0-220)
Salmonella spp. (CFU)	0 (0)	0 (0)	ND	0 (0)	ND	0 (0)	ND
ddPCR Assays							
Salmonella spp. (copies/3 cm^2)	38 (23/60)	3 (15)	9.6 (7.2-11.2)	8 (40)	10.8 (4.0-19.2)	12 (60)	13.2 (3.0-50.4)
Cryptosporidium spp. (copies/3 $cm^{2)}$	2 (1/60)	1 (5)	6 (6.0-6.0)	0 (0)	ND	0 (0)	ND

[a] CFU = Colony-forming units; ND = non-detect.

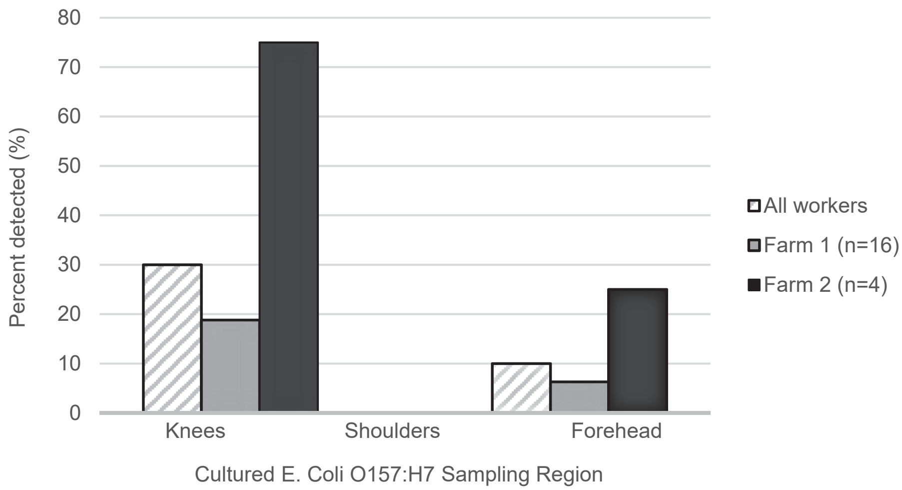

Figure 1. Cultured E.coli O157:H7 samples percent detected by sampling region.

Salmonella spp.

No samples collected had detectable levels of *Salmonella spp*. measured via culture assay. However, a total of 23 (38%) samples were positive for *Salmonella spp.* DNA. One participant had *Salmonella spp.* DNA on all three sampling body areas, two workers had detectable levels of *Salmonella spp.* DNA on knees and shoulders, four had *Salmonella spp.* DNA on shoulders and forehead, one participant had *Salmonella spp.* DNA only on the shoulders, and 7 participants had *Salmonella spp*. DNA on the forehead only. A total of 15 participants (75%) had detectable levels of *Salmonella spp.* DNA on any body part (table 3). Farm 2 had a higher percentage of workers with detectable *Salmonella spp*. DNA on the knees (25%) compared to Farm 1 (12.5%) (fig. 2). Conversely, Farm 1 had a higher percentage of workers with detectable levels of *Salmonella spp.* DNA on the forehead (44%) compared to Farm 2 (25%) (fig. 2). Finally, 75% of workers from Farm 1 had detectable levels of *Salmonella spp*. DNA on their shoulders compared to none of the workers from Farm 2 (fig. 2). The mean and range of *Salmonella spp.* DNA on all workers' knees, shoulders, and foreheads can be found in table 3. When compared to those who reported having completed junior high school or less, individuals that completed high school or college had an increase of 20.0 *Salmonella spp.* bacteria/ 3cm^2 on swabs collected from the forehead (Model $R^2 = 0.39$, $p = 0.01$, table A1).

Cryptosporidium spp.

One sample collected from a participant's knee at Farm 1 tested positive for *Cryptosporidium spp.* detected by molecular method. (mean = 3 bacteria/ 3cm^2).

Discussion

The study suggests dairy milking parlor workers may be exposed to *E. coli* O157:H7, *Salmonella spp*., and *Cryptosporidium spp.* during the workday. While the study was not designed to diagnose disease, it aims to provide evidence of the potential health problems that the presence of a pathogen among "apparently healthy workers" could eventually cause among children, older individuals, or family members with immunocompromised systems.

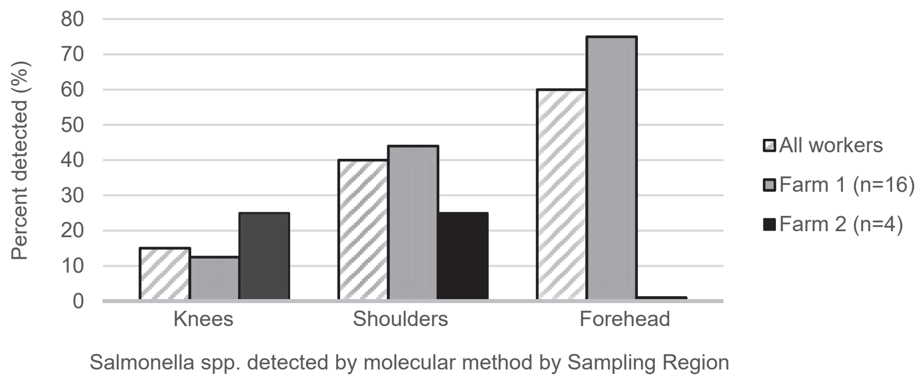

Figure 2. *Salmonella spp.* detected by molecular method samples percent detected by sampling region.

As has been observed, most Hispanic workers share dwellings composed of extended members, such as the elders and children. The exposure is consistent with findings from a multi-dairy study in Bangladesh of antibiotic-resistant *E.coli*, and *Salmonella* spp., where milker's hand wash tested positive for total viable bacterial count (TVC), *E.coli*, and *Salmonella* spp. in all dairies assessed (Abdus Sobur et al., 2019). In the present study, *Salmonella* was the most prevalent pathogen, occurring in 38% (23/60) of environmental samples (table 3). The forehead was found to have the greatest risk, with 60% (12/20) of the dairy workers having *Salmonella spp.* on the forehead, followed by the shoulders with 40% (8/20) and the knees with 15% (3/20) (table 3). The CDC estimates that *Salmonella* causes approximately 1.35 million illnesses, over 26,0000 hospitalizations, and 420 deaths in the United States every year (CDC, 2019b). Although no positive samples were cultured for Salmonella spp. due to either low concentrations or being in a viable but nonculturable (VBNC) state, a total of 23 (38%) samples were positive droplet digital PCR molecular detection. Previously thought to be in a dormant state, bacteria have measurable metabolic activity in a VBNC state (e.g., uptake of amino acids) (Ramamurthy et al., 2014). Additionally, bacteria in a VBNC state can be "revived" when the required conditions are present (e.g., an appropriate environment or energy source) (Ramamurthy et al., 2014). In a 2021 study of Colorado dairy workers, absorbent materials were placed on workers' clothing, gloves, and boots and assessed for *Salmonella ser. Dublin*, *Campylobacter spp.,* and *E. coli* using PCR (Palomares Velosa et al., 2021). Samples were 44%, 21%, 22%, and 6% positive for *Salmonella ser. Dublin*, *Campylobacter jejuni*, and *E.coli*, respectively (Palomares Velosa et al., 2021). No samples in the study were positive for *Cryptosporidium*. Interestingly, positive *Salmonella* and *Campylobacter* samples occurred concomitantly (Palomares Velosa et al., 2021).

Otherwise, in phase one of this study, *E. coli O157:H7* was cultured and found in 13% (8/60) of samples but was not processed by ddPCR. Knees were found to harbor *E. coli O157:H7* 30% (6/20), forehead 10% (2/20) (table 3). It is important to highlight the difference between the two farms. Farm 2 was a smaller dairy operation, employing a relatively small number of workers. Smaller operations may have less access to preventive and protective controls, which may partially explain the higher proportion of cultured bacteria on workers' body parts, in comparison to Farm 1, underscoring the need to include large and small operations in future studies. The sample size of this study was relatively small, limiting our ability to compare exposure between the two farms of varying sizes. Because of this, it is unclear whether the higher proportion of cultured bacteria on Farm 2 workers' body parts in comparison to Farm 1 is a true difference or sample variance. Future research should investigate whether farm size is associated with access to preventive and protective controls. Organisms found on the knees and foreheads of dairy parlor workers may be explained by direct contact or splash with feces, etc., or as it is transferred to the forehead (e.g., touching the forehead with their hands). Thus, it is important to understand the bacterial loading on workers' hands/gloves and their transfer (contacts) with other body parts/clothing. Additionally, it is important to recognize the public health concern brought that even a low dose of *E. coli* O157:H7, approximately 100-200 cells, can cause disease in the general public and that 10 cells can cause severe disease in susceptible populations (ISU Center for Food Security and Public Health, 2016); Msolo et al., 2016). These findings are similar to a study in South Africa that assessed the prevalence of *E. coli* O157:H7 in three dairies found very low prevalence of isolates on worker's hands (7%) compared to 55% of cattle udders (Msolo et al., 2016). Similarly, in a study at five small-scale dairy farms in Egypt, *E. coli* was isolated in only 11% of workers' hand swab samples (Awadallah et al., 2016).

The organism *Cryptosporidium* was found once on the knees of 2% (1/60) of the participants and appears to pose the least risk of exposure for dairy workers in this study (table 3). In general, employees at dairy operations that are involved in the care of pre-weaned animals are most likely to be infected with *Cryptosporidium* (Santín et al., 2008). In larger dairy operations, there is much less cross-functional work (e.g., parlor employees working outside of the parlor), so it is surprising that no samples were detected at the smaller farm and that one *Cryptosporidium* positive sample was found at the larger farm (Farm 1). Finally, 20% (4/20) of dairy workers had two or more pathogens detected on the sampling sites, and one had all three pathogens detected on them, increasing the risk of infection.

The demographic characteristics of the participants in this study are similar to results obtained from dairy farms in Wisconsin that participated in a 2012 study (n = 836 workers, n = 67 farms) (Juárez-Carrillo, 2017). The majority of workers in the Wisconsin dairy study were male (86%), had attended school for 7-12 years (59%), and preferred that training be in Spanish (96%) (Juárez-Carrillo et al., 2017). However, the majority of Wisconsin dairy workers had been working at the dairy for 6 to 10 years, whereas the workers in this study reported working at the dairy for 1 to 5 years or less. A 2015 study of dairy worker respiratory health in California also provided similar questionnaire results (n = 205) (Mitchell et al., 2015). In the California study, dairy workers were predominantly Latino (90.6%), completed interviews in Spanish (98.0%), and worked a mean of 9.3 hours per day (Mitchell et al., 2015). As for education, 55.2% of the Californian dairy workers had attended sixth grade or less, and 44.8% had completed more than sixth grade (Mitchell et al., 2015). In a group of 55 dairy workers interviewed in Colorado, eighty percent of the participants were male, 35 years old on average, and had completed 9 years of schooling (Menger-Ogle et al., 2020). A majority of the participants were from Mexico (69.1%) and identified Spanish as their native language (94.5%). When asked to describe their English proficiency, the most commonly selected response was "I understand it well, but have trouble speaking it" (46.3%), followed by "None" (35.2%) (Menger-Ogle et al., 2020). In another study of dairy workers in Colorado, the majority had only primary education, and 34% had been enrolled up to middle school (Palomares Velosa et al., 2021). Of the group interviewed, 63% were men, and 50% were younger than 30 years old (Palomares Velosa et al., 2021). Nearly ninety percent of participants (88.1%) had migrated from Latin-American countries, including Mexico, Guatemala, Honduras, and Colombia (Palomares Velosa et al., 2021). Similarly, in a 2016 study of 44 dairy workers at farms in both South Dakota and Colorado, approximately half of the participants were from Mexico, and the rest were from Central America (primarily Guatemala), Peru, and Puerto Rico (Menger et al., 2016a). In a multi-dairy intervention study in Texas, nearly 60% of participants reported Mexico as their country of birth, followed by Guatemala (19.4%), the U.S. (15.1%), and Honduras (5.8%) (Rodriguez et al., 2020). Over 30% of workers interviewed reported having arrived in the U.S. within the last 5 years (Rodriguez et al., 2020). Thus, management recommendations based on this study are generalizable to most dairy farms in this region (Southwestern U.S.) that are handled mostly by undereducated Hispanic workers.

Although the U.S. Department of Labor 2015-2016 NAWS does not include livestock workers, their survey findings from vegetable, horticulture, and fruit and nut workers (n = 5342) are also similar to those presented in this study. Similarly to the present study, 83% of crop workers self-identified as belonging to a Hispanic group, such as Mexican or Mexican American (NAWS, 2018). Sixty-seven percent of farmworkers had one or two children under the age of 18 living with them (NAWS, 2018). As for distance to work, 11% reported living on the farm/worksite, 70% lived 25 miles or less from the work site, and 16% lived over 25 miles away from the worksite (NAWS, 2018). Finally, the NAWS includes hours worked weekly, with an average of 5 days worked per week for an average of 45 hours (approximately 9 hours per day) (NAWS, 2018).

Strengths and Limitations

This study provides the first surveillance of an important but largely invisible and vulnerable population: dairy workers in Arizona. More specifically, this study has provided insight into the demographics, work history, tasks completed during the workday, personal protective equipment and post-shift hygiene practices, and household characteristics of dairy workers. The information derived from this study can be used to determine sample size for future exposure and epidemiologic assessments. It provides evidence of the need to strengthen the partnership between the dairy industry and academic investigation on animal health and economic production to also design research projects aimed at improving worker health and safety. Another strength of this study is that it included both male and female respondents, which can be difficult in agricultural settings. While there is no comparison of male versus female exposures and practices in the study, female livestock workers are an underrepresented group in agricultural health studies and face unique health and safety concerns (e.g., sexual harassment, modified work practices such as working in pairs, equipment design that does not accommodate height or arm length). Anecdotally, we have observed an increase in the number of females working in milking parlors in the Southwest over the past ten years. The inclusion of female workers in the study improves our general understanding of the makeup of dairy worker populations, which can help guide health and safety practices. The results from this project can inform risk management models, where the exposure assessment piece is oftentimes missing. This study is also a catalyst for building culture and discussion around health and safety in the Arizona dairy industry and can inform risk management practices as well as health and safety programs. Finally, this study demonstrates the importance of careful selection of where to collect samples from and how collecting samples from multiple locations on workers can lead to innovative and creative control solutions. The forehead, an infrequently utilized sampling area in risk assessment, was identified as a useful location, thus revealing the importance of considering cultural habits in study designs. As dairy parlor workers complete their tasks throughout the workday, bovine feces may be transferred to the face through hand-to-face contact, indirect contact from hats or other items worn on the head/face, through inhalation or ingestion of bioaerosols, or direct contact from semi-liquid feces that splashes onto the face. Swab samples collected from the forehead may represent the concentration of zoonotic exposures to the conjunctiva and orifices of the face (i.e., eyes, nose, and mouth).

This study also has several limitations. The relatively small sample size limits the generalizability and interpretation of the results. Notably, the lack of questionnaire participation from Farm 2 limits our ability to fully characterize the participants and interpret the results. Additionally, home hygiene practices were not included in the questionnaire, limiting our understanding of the risk of take-home exposure as well as the possibility that pathogens originate in the home environment. Furthermore, we did not collect pre-shift samples from workers' clothing and skin to compare to our post-shift samples, nor did we collect environmental samples, limiting our ability to isolate the source of the contaminants. Additional exposure pathways include contact with contaminated food or media (e.g., air, soil, water, sediments, or biota), or with infected persons/animals outside of the workplace. Additional questions on the questionnaire regarding PPE provision and use, specifically the type of respirators used and face/eye coverings, would have been useful to include. Samples were only collected from three areas of the workers' bodies/clothing: foreheads, shoulders, and knees. The inclusion of additional body parts or clothing (e.g., hats, eyewear, boots, hands/gloves) would have provided useful information. Finally, this study was conducted in a relatively limited geographic area, further limiting the generalizability of the results.

Recommendations

Recommendations for controlling dairy workers' exposure to zoonotic organisms should follow the industrial hygiene hierarchy of controls (NIOSH, 2020): elimination (i.e., physically removing the hazard from the workplace), substitution (i.e., replacing the hazard with a less harmful substitution), engineering (i.e., isolate workers from the hazard), administrative (i.e., change the way people work), and personal protective equipment (PPE). Where elimination and substitution of the hazard are not possible, engineering controls in a dairy milking parlor include barriers or shields between cow and worker, increased general ventilation, and localized ventilation. Ventilation can control airborne exposure to organisms, as this route of exposure is an important vehicle for exposure (Mitchell et al., 2015; Sanz et al., 2015). Potential administrative controls include providing: sick time to limit cross-transmission to other workers; training workers on recognition of zoonotic disease transmission; establishing protocols to keep worker clothes (overalls, aprons, uniforms) and boots at work with employer that will wash them at 40°C with biological detergent; enforcing that handwashing stations and mandatory handwashing pre-shift as well as at end of shift and before breaks are taken; mandatory training upon beginning employment on workers' rights, hazards in the dairy farm, how to protect self and others, etc.; and increased surveillance of herd health. Trainings should focus on understanding and involving the workers (e.g., use material that is in the language and has an adequate literacy level, embrace core cultural beliefs, and provide multiple avenues for participation). Training should also include basic workers' rights and available resources (e.g., the General Duty Clause, the state department of occupational safety and health, migrant worker law clinics); maximize worker engagement (e.g., ask workers for input as to the best training methods and/or treat safety and health as an ongoing movement rather than a one-off event); utilize novel and effective training methods (e.g., community health workers (*promotoras*), reinforce content with short surveys and games); and evaluate the impact of the program on a regular basis (Juárez-Carrillo et al., 2017; Liebman et al., 2016; Menger et al., 2016b; Robert Hagevoort et al., 2013; Rovai et al., 2016; Schenker and Gunderson, 2013). Finally, the following PPE should be provided and worn by workers to provide a barrier between the worker and zoonotic organisms: goggles, face shields, aprons, gloves, etc. It is important to mention that these physical barriers can be useful for controlling the spread of other infections, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as they can be spread through respiratory droplets and contact with contaminated fomites (WHO, 2020).

Potential for Future Research

Future research endeavors could include the implementation of interventions and the subsequent evaluation of said interventions. Exposure assessments can inform risk assessment models for dairy workers, family members, or other household members. One could further explore "what-if scenarios" such as: what would the probability of annual infection be if the dairy worker had washed their hands at the conclusion of the workday or if the worksite administration washed the worker's clothing at 40°C with biological detergent? It would also be useful to identify target intervention areas by assessing the surface and airborne transfer efficiency of microorganisms throughout the dairy environment via phage tracers and/or collecting samples from additional parts of the body (e.g., hands, boots) or areas within the workplace. An innovative direction of future research would be to investigate dairy or other livestock workers' changes in microbiome over time, assessing health outcomes such as resistance to zoonotic infections. Finally, this project underscores the need for increased surveillance of dairy workers' access to healthcare, utilization of healthcare services, barriers to receiving healthcare, zoonotic disease transmission, and healthcare needs. Assessments of return-on-investments for policy changes, such as the inclusion of sick time in employee contracts, would be useful for engaging with and educating dairy producers.

Conclusions

This study reinforces that dairy workers, a largely invisible population in Arizona, are exposed to zoonotic organisms during the workday on areas of their body/clothing that are not covered by aprons or coveralls at the end of the workday. Some of these clothing items are worn at home, creating the potential for the transfer of organisms to others, including those sharing vehicles or household members. Immunocompromised, young, or otherwise vulnerable individuals in contact with workers may be at risk for infections. These infections can also have a direct potential financial impact on laborers, as they will stay home sick or care for relatives and potentially lose out on work income, as many dairies in the Southwestern U.S. do not offer sick leave. This issue is further compounded by the possibility of outbreaks occurring within the dairy worker population. We emphasize the importance of research and policies directed at improving the health and safety of dairy workers in the U.S., with recommendations on how to effectively control exposure using the hierarchy of controls.

Acknowledgments

- This work was supported by the U.S. NIOSH Western Center for Agricultural Health and Safety under Grant #2U54OH007550.
- We would like to thank the Arizona dairy industry for their advice throughout the study and our participants, without whom this study could not have happened.

- We thank the 2017 Lopez lab interns for their work on sample collection, preparation, and processing, as well as data entry.

References

Abdus Sobur, M., Al Momen Sabuj, A., Sarker, R., Taufiqur Rahman, A. M., Lutful Kabir, S. M., & Tanvir Rahman, M. (2019). Antibiotic-resistant *Escherichia coli* and *Salmonella spp*. associated with dairy cattle and farm environment having public health significance. *Vet. World, 12*(7), 984-993. https://doi.org/10.14202/vetworld.2019.984-993

Adcock, F., Anderson, D., & Rosson, P. (2015). The economic impacts of immigrant labor on US dairy farms. Center for North American Studies.

Anthony, K. (2019). Protecting dairy workers from infectious disease. UC Davis, Western Center for Agricultural Health and Safety. Retrieved from https://aghealth.ucdavis.edu/news/protecting-dairy-workers-infectious-disease#:~:text=coliO157%3AH7%2CSalmonella%2C,%2CListeriamonocytogenes%2CandCampylobacter

Arcury, T. A., & Quandt, S. A. (2007). Delivery of health services to migrant and seasonal farmworkers. *Annu. Rev. Public Health, 28*(1), 345-363. https://doi.org/10.1146/annurev.publhealth.27.021405.102106

Awadallah, M. A., Ahmed, H. A., Merwad, A. M., & Selim, M. A. (2016). Occurrence, genotyping, shiga toxin genes and associated risk factors of *E. Coli* isolated from dairy farms, handlers and milk consumers. *Vet. J., 217*, 83-88. https://doi.org/10.1016/j.tvjl.2016.09.014

CDC. (2017a). *Cryptosporidium*, General information for the public. Retrieved from https://www.cdc.gov/parasites/crypto/general-info.html

CDC. (2017b). *E. Coli*, Symptoms. Retrieved from https://www.cdc.gov/ecoli/ecoli-symptoms.html

CDC. (2019a). Antibiotic resistance threats in the United States, 2019. Retrieved from https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

CDC. (2019b). *Samonella* homepage. Retrieved from https://www.cdc.gov/salmonella/index.html

Cheng, C.-M., Lin, W., Van, K. T., Phan, L., Tran, N. N., & Farmer, D. (2008). Rapid detection of *Samonella* in foods using real-time PCR. *J. Food Prot., 71*(12), 2436-2441. https://doi.org/10.4315/0362-028X-71.12.2436

Cross, P., Edwards-Jones, G., & Rigby, D. (2012). Eliciting expert opinion on the effectiveness and practicality of interventions in the farm and rural environment to reduce human exposure to *Escherichia coli* O157. *Epidemiol. Infect., 140*(4), 643-654. https://doi.org/10.1017/S0950268811001257

Devamani, C., Norman, G., & Schmidt, W.-P. (2014). A simple microbiological tool to evaluate the effect of environmental health interventions on hand contamination. *Int. J. Environ. Res. Public. Health, 11*(11), 11846-11859. https://doi.org/10.3390/ijerph111111846

Di Piazza, F., Di Benedetto, M. A., Maida, C. M., Glorioso, S., Adamo, G., Mazzola, T., & Firenze, A. (2013). A study on occupational exposure of Sicilian farmers to *Giardia* and *Cryptosporidium*. *J. Prev. Med. Hyg., 54*(4), 212-217.

Edrington, T. S., Hume, M. E., Looper, M. L., Schultz, C. L., Fitzgerald, A. C., Callaway, T. R.,... Nisbet, D. J. (2004). Variation in the faecal shedding of *Samonella* and *E. Coli* O157:H7 in lactating dairy cattle and examination of *Samonella* genotypes using pulsed-field gel electrophoresis. *Lett. Appl. Microbiol., 38*(5), 366-372. https://doi.org/10.1111/j.1472-765X.2004.01495.x

Feng, P., Weagant, S. D., & Jinneman, K. (2020). Chapter 4: Diarrheagenic *Escherichia coli*. In *FDA's Bacteriological Analytical Manual (BAM).* Retrieved from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4a-diarrheagenic-escherichia-coli

Furey, E. M., O'Hora, D., McNamara, J., Kinsella, S., & Noone, C. (2016). The roles of financial threat, social support, work stress, and mental distress in dairy farmers' expectations of injury. *Front. Public Health, 4*. https://doi.org/10.3389/fpubh.2016.00126

Genovese, K. J., Jung, Y. S., McReynolds, J. L., Anderson, R. C., & Nisbet, D. J. (2004). Antimicrobial resistance and serotype prevalence of *Samonella* isolated from dairy cattle in the Southwestern United States. *Microb. Drug Resist., 10*(1), 51-56. https://doi.org/10.1089/107662904323047808

Gharpure, R., Perez, A., Miller, A. D., Wikswo, M. E., Silver, R., & Hlavsa, M. C. (2019). Cryptosporidiosis outbreaks - United States, 2009-2017. *MMWR - Morb. Mortal. Wkly. Rep., 68*(25), 568-572. https://doi.org/10.15585/mmwr.mm6825a3

Gilpin, B. J., Scholes, P., Robson, B., & Savill, M. G. (2008). The transmission of thermotolerant *Campylobacter* spp. to people living or working on dairy farms in New Zealand. *Zoonoses Public Health, 55*(7), 352-360. https://doi.org/10.1111/j.1863-2378.2008.01142.x

Grout, L., Baker, M. G., French, N., & Hales, S. (2020). A review of potential public health impacts associated with the global dairy sector. *GeoHealth, 4*(2), e2019GH000213. https://doi.org/10.1029/2019GH000213

Grzywacz, J. G., Alterman, T., Gabbard, S., Shen, R., Nakamoto, J., Carroll, D. J., & Muntaner, C. (2014). Job control, psychological demand, and farmworker health: Evidence from the national agricultural workers survey. *J. Occup. Environ. Med., 56*(1), 66-71. https://doi.org/10.1097/jom.0000000000000025

Hagevoort, G. R., Douphrate, D. I., & Reynolds, S. J. (2013). A review of health and safety leadership and managerial practices on modern dairy farms. *J. Agromed., 18*(3), 265-273. https://doi.org/10.1080/1059924X.2013.796905

Hoelzer, K., Moreno Switt, A. I., & Wiedmann, M. (2011). Animal contact as a source of human non-typhoidal salmonellosis. *Vet. Res., 42*(1), 34. https://doi.org/10.1186/1297-9716-42-34

ISU Center for Food Security and Public Health. (2016). Enterohemorrhagic *Escherichia coli* and other *E. Coli* causing hemolytic uremic syndrome. Retrieved from https://dr.lib.iastate.edu/handle/20.500.12876/14157

ISU Center for Food Security and Public Health. (2021). Zoonotic diseases of cattle. Center for Food Security and Public Health, ISU. Retrieved from https://www.cfsph.iastate.edu/Assets/zoonotic-diseases-of-cattle-table.pdf

JBS International. (2018). Findings from the National Agricultural Workers Survey (NAWS), 2015-2016: A demographic and employment profile of United States farmworkers. *Vol. 13*.

Jimenez, C. A., Ishimoto, T., Lanaspa, M. A., Rivard, C. J., Nakagawa, T., Ejaz, A. A.,... Johnson, R. J. (2014). Fructokinase activity mediates dehydration-induced renal injury. *Kidney Int., 86*(2), 294-302. https://doi.org/10.1038/ki.2013.492

Juárez-Carrillo, P. M., Liebman, A. K., Reyes, I. A., Ninco Sánchez, Y. V., & Keifer, M. C. (2017). Applying learning theory to safety and health training for hispanic immigrant dairy workers. *Health Promot. Pract., 18*(4), 505-515. https://doi.org/10.1177/1524839916683668

Kaper, J. B. (2005). Pathogenic *Escherichia coli*. *Int. J. Med. Microbiol., 295*(6), 355-356. https://doi.org/10.1016/j.ijmm.2005.06.008

Khan, A. S., Levitt, A. M., & Sage, M. J. (2000). Biological and chemical terrorism: Strategic plan for preparedness and response. *MMWR - Morb. Mortal. Wkly. Rep., 49*(RR04). Retrieved from https://www.cdc.gov/mmwr/preview/mmwrhtml/rr4904a1.htm

Kinyua, M. N., Wald, I., Camacho-Céspedes, F., Izurieta, R., Haas, C. N., & Ergas, S. J. (2016). Does the use of tubular digesters to treat livestock waste lower the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia*? *J. Water Health, 14*(5), 738-753. https://doi.org/10.2166/wh.2016.032

Leibler, J. H., Abdelgadir, A., Seidel, J., White, R. F., Johnson, W. E., Reynolds, S. J.,... Schaeffer, J. W. (2023). Influenza D virus exposure among US cattle workers: A call for surveillance. *Zoonoses Public Health, 70*(2), 166-170. https://doi.org/10.1111/zph.13008

Li, X., Flores, K. A., Barry, S., Becchetti, T. A., Doran, M., Finzel, J. A.,... Atwill, E. R. (2019). Statewide cross-sectional survey of *Cryptosporidium* and *Giardia* in California cow-calf herds. *Rangeland Ecol. Manag., 72*(3), 461-466. https://doi.org/10.1016/j.rama.2019.01.004

Liebman, A. K., Juarez-Carrillo, P. M., Reyes, I. A., & Keifer, M. C. (2016). Immigrant dairy workers' perceptions of health and safety on the farm in America's Heartland. *Am. J. Ind. Med., 59*(3), 227-235. https://doi.org/10.1002/ajim.22538

March, S. B., & Ratnam, S. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J. Clin. Microbiol., 23*(5), 869-872. https://doi.org/10.1128/jcm.23.5.869-872.1986

McDaniel, C. J., Cardwell, D. M., Moeller, R. B., & Gray, G. C. (2014). Humans and cattle: A review of bovine zoonoses. *Vector Borne Zoonotic Dis., 14*(1), 1-19. https://doi.org/10.1089/vbz.2012.1164

Menger, L. M., Pezzutti, F., Tellechea, T., Stallones, L., Rosecrance, J., & Roman-Muniz, I. N. (2016a). Perceptions of health and safety among immigrant Latino/a dairy workers in the U.S. *Front. Public Health, 4*. https://doi.org/10.3389/fpubh.2016.00106

Menger, L. M., Rosecrance, J., Stallones, L., & Roman-Muniz, I. N. (2016b). A guide to the design of occupational safety and health training for immigrant, Latino/a dairy workers. *Front. Public Health, 4*. https://doi.org/10.3389/fpubh.2016.00282

Menger-Ogle, L. M., Pezzutti, F., Menger-Ogle, A. D., Stallones, L., Rosecrance, J., & Roman-Muniz, I. N. (2020). Occupational safety and health of foreign-born, Latinx dairy workers in Colorado. *J. Occup. Environ. Med., 61*(1), 61-68. https://doi.org/10.1097/jom.0000000000001472

Mitchell, D. C., Armitage, T. L., Schenker, M. B., Bennett, D. H., Tancredi, D. J., Langer, C. E.,... Mitloehner, F. M. (2015). Particulate matter, endotoxin, and worker respiratory health on large Californian dairies. *J. Occup. Environ. Med., 57*(1), 79-87. https://doi.org/10.1097/jom.0000000000000304

Msolo, L., Igbinosa, E. O., & Okoh, A. I. (2016). Prevalence and antibiogram profiles of *Escherichia coli* O157:H7 isolates recovered from three selected dairy farms in the Eastern Cape Province, South Africa. *Asian Pac. J. Trop. Dis., 6*(12), 990-995. https://doi.org/10.1016/S2222-1808(16)61170-2

Munns, K. D., Selinger, L. B., Stanford, K., Guan, L., Callaway, T. R., & McAllister, T. A. (2015). Perspectives on super-shedding of *Escherichia coli* O157:H7 by cattle. *Foodborne Pathog. Dis., 12*(2), 89-103. https://doi.org/10.1089/fpd.2014.1829

NASS. (2020). Milk production. USDA, NASS. Retrieved from https://www.nass.usda.gov/Publications/Todays_Reports/reports/mkpr0420.pdf

National Institute of Allergy and Infectious Diseases. (2018). NIAID emerging infectious diseases/ pathogens. Retrieved from https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens

NIOSH. (2020). Hierarchy of controls. Retrieved from https://www.cdc.gov/niosh/topics/hierarchy/default.html

NORA Agricultural Forestry and Fishing Sector Council. (2018). National Occupational Research Agenda for Agriculture, Forestry and Fishing - National Institute for Occupational Sfaety and Health. Retrieved from https://www.cdc.gov/nora/councils/agff/pdf/National_Occupational_Research_Agenda_for_AgFF_May_2018.pdf.

Oliver, S. P., Jayarao, B. M., & Almeida, R. A. (2005). Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications. *Foodborne Pathog. Dis., 2*(2), 115-129. https://doi.org/10.1089/fpd.2005.2.115

Palomares Velosa, J. E., Salman, M. D., Roman-Muniz, I. N., Reynolds, S., Linke, L., Magnuson, R.,... Rao, S. (2021). Socio-ecological factors of zoonotic diseases exposure in Colorado dairy workers. *J. Agromed., 26*(2), 151-161. https://doi.org/10.1080/1059924X.2020.1725700

Pérez-Garza, J., García, S., & Heredia, N. (2017). Removal of *Escherichia coli* and *Enterococcus faecalis* after hand washing with antimicrobial and nonantimicrobial soap and persistence of these bacteria in rinsates. *J. Food Prot., 80*(10), 1670-1675. https://doi.org/10.4315/0362-028X.JFP-17-088

Pierce, E. S., Jindal, C., Choi, Y. M., & Efird, J. T. (2023). The evidence for *Mycobacterium avium* subspecies paratuberculosis (MAP) as a cause of nonsolar uveal melanoma: A narrative review. *Transl. Cancer Res., 12*(2), 398-412. https://doi.org/10.21037/tcr-22-2540

Rahman, M. T., Sobur, M. A., Islam, M. S., Ievy, S., Hossain, M. J., El Zowalaty, M. E.,... Ashour, H. M. (2020). Zoonotic diseases: Etiology, impact, and control. *Microorganisms, 8*(9), 1405. https://doi.org/10.3390/microorganisms8091405

Ramamurthy, T., Ghosh, A., Pazhani, G. P., & Shinoda, S. (2014). Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. *Front. Public Health, 2*. https://doi.org/10.3389/fpubh.2014.00103

Ravishankar, S., Zhu, L., Olsen, C. W., McHugh, T. H., & Friedman, M. (2009). Edible apple film wraps containing plant antimicrobials inactivate foodborne pathogens on meat and poultry products. *J. Food Sci., 74*(8), M440-M445. https://doi.org/10.1111/j.1750-3841.2009.01320.x

Rice, D. H., Hancock, D. D., Roozen, P. M., Szymanski, M. H., Scheenstra, B. C., Cady, K. M.,... Chudek, P. A. (2003). Household contamination with *Samonella enterica*. *Emerg. Infect. Dis., 9*(1), 120. https://doi.org/10.3201/eid0901.020214

Rodriguez, A., Douphrate, D., Gimeno Ruiz de Porras, D., Prot, E., Perez, A., Hagevoort, R., & Nonnenmann, M. (2020). Bovine tuberculosis case intervention using the T.SPOT.TB assay to screen dairy workers in Bailey County, Texas. *Front. Public Health, 8*. https://doi.org/10.3389/fpubh.2020.00479

Rosson, P., Adcock, F., Susanto, D., & Anderson, D. (2009). The economic impacts of immigration on U.S. dairy farms. AgriLife Research, CNAS. Retrieved from https://agecoext.tamu.edu/wp-content/uploads/2021/02/CNAS-pub-The-Economic-Impacts-of-Immigration-onf-U.S-Dairy-Farms.pdf

Rovai, M., Carroll, H., Foos, R., Erickson, T., & Garcia, A. (2016). Dairy tool box talks: A comprehensive worker training in dairy farming. *Front. Public Health, 4*. https://doi.org/10.3389/fpubh.2016.00136

Rwego, I. B., Gillespie, T. R., Isabirye-Basuta, G., & Goldberg, T. L. (2008). High rates of *Escherichia coli* transmission between livestock and humans in rural Uganda. *J. Clin. Microbiol., 46*(10), 3187-3191. https://doi.org/10.1128/jcm.00285-08

Santín, M., Trout, J. M., & Fayer, R. (2008). A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age. *Vet. Parasitol., 155*(1), 15-23. https://doi.org/10.1016/j.vetpar.2008.04.018

Santín, M., Trout, J. M., Xiao, L., Zhou, L., Greiner, E., & Fayer, R. (2004). Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. *Vet. Parasitol., 122*(2), 103-117. https://doi.org/10.1016/j.vetpar.2004.03.020

Sanz, S., Olarte, C., Martínez-Olarte, R., Navajas-Benito, E. V., Alonso, C. A., Hidalgo-Sanz, S.,... Torres, C. (2015). Airborne dissemination of *Escherichia coli* in a dairy cattle farm and its environment. *Int. J. Food Microbiol., 197*, 40-44. https://doi.org/10.1016/j.ijfoodmicro.2014.12.010

Schenker, M., & Gunderson, P. (2013). Occupational health in the dairy industry needs to focus on immigrant workers, the new normal. *J. Agromed., 18*(3), 184-186. https://doi.org/10.1080/1059924X.2013.797375

Trevino, I. (2006). Practical applications for managing biological risks - Zoonotic Transmission Control for Dairy Producers. Ames, IA: Center for Food Security and Public Health, ISU. Retrieved from http://www.cfsph.iastate.edu/Zoonoses/assets/English/Zoonotic_dairy_speakernotes.pdf

Villarejo, D. (2003). The health of U.S. hired farm workers. *Annu. Rev. Public Health, 24*(1), 175-193. https://doi.org/10.1146/annurev.publhealth.24.100901.140901

Vlasova, A. N., & Saif, L. J. (2021). Bovine immunology: Implications for dairy cattle. *Front. Immunol., 12*. https://doi.org/10.3389/fimmu.2021.643206

Weagant, S. D., Bryant, J. L., & Jinneman, K. G. (1995). An improved rapid technique for isolation of *Escherichia coli* O157:H7 from foods. *J. Food Prot., 58*(1), 7-12. https://doi.org/10.4315/0362-028X-58.1.7

WHO. (2020). Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations. Retrieved from https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations
Wilkinson, C. W. (2006). Zoonotic disease risk for livestock production workers. University of Alberta.
Yang, R., Paparini, A., Monis, P., & Ryan, U. (2014). Comparison of next-generation droplet digital PCR (ddPCR) with quantitative PCR (qPCR) for enumeration of *Cryptosporidium* oocysts in faecal samples. *Int. J. Parasitol., 44*(14), 1105-1113. https://doi.org/10.1016/j.ijpara.2014.08.004

Appendix

Table A1. Univariate Analysis of *Salmonella spp.* levels on Samples Collected from Participant's Foreheads with Demographic and Work Characteristics

	Coef.	Std. Error	t	p-value	95% Conf Interval		R^2 Value
Gender							0.03
Male	Ref.	-	-	-	-	-	-
Female	-4.72	6.95	-0.68	0.51	-19.60	10.20	
Language							0.03
English	Ref.						
Spanish	7.02	13.88	0.51	0.62	-22.95	37.00	
Indigenous	2.00	18.96	0.11	0.92	-38.95	42.95	
Highest level of schooling completed							0.84
Completed junior high or less (grades 8 or less)	Ref	-	-	-	-	-	
Some High School	4.34	6.52	0.67	0.52	-9.74	18.42	
Completed High School or College/University	20.00	7.53	2.66	**0.02**	3.74	36.26	
Time spent working at current farm							0.33
Less than 3 months	Ref.						
3 to 6 months	-12.10	10.01	-1.21	0.25	-33.91	9.71	
6 months to 1 year	9.34	8.83	1.06	0.31	-9.89	28.57	
1 to 5 years	-7.47	7.24	-1.03	0.32	-23.26	8.31	
Time spent working in agriculture							0.30
Less than 3 months	Ref.						
3 to 6 months	-5.20	15.15	-0.34	0.74	-38.55	28.15	
6 months to 1 year	20.76	12.37	1.68	0.12	-6.47	47.99	
1 to 5 years	1.66	9.92	0.17	0.87	-20.18	23.49	
More than 5 years	3.50	10.71	0.33	0.75	-20.08	27.08	
Did you wash your hands at end of your shift?							0.15
Yes	Ref.						
No	10.97	6.97	1.57	0.14	-3.98	25.92	
Are the boots worn during shift same as boots worn home?							0.12
Yes	Ref						
No	-8.54	6.14	-1.39	0.19	-21.70	4.62	
How long does it take to travel from work to home?							0.15
5 to 15 minutes	Ref.						
More than 15 minutes	-9.91	6.22	-1.59	0.13	-23.26	3.43	
Who lives in your home?							0.07
Alone	Ref.						
With family	5.60	9.52	0.59	0.57	-15.36	26.56	
With other dairy workers	-1.07	16.49	-0.06	0.95	-37.36	35.23	
With family and other dairy workers	3.13	13.04	0.24	0.81	-25.56	31.83	
With partner/spouse	-4.35	16.49	-0.26	0.80	-40.64	31.95	
Are there children (18 years or younger) in your home?							0.01
Yes	Ref.						
No	-1.96	6.53	-0.30	0.77	-15.96	12.04	