SPECIFIC AIMS

The majority of California dairy farms have a high prevalence of endemic zoonotic pathogens in their cattle, namely *Cryptosporidium parvum*, *E. coli* O157:H7, *Salmonella enterica* subsp. *enterica*, *Listeria monocytogenes*, and *Campylobacter jejuni*. Moreover, there are specific cohorts of cattle with higher prevalence and higher intensity of fecal shedding of those pathogens. Contact with these highly infectious cattle substantially elevates the risk of zoonotic transmission to farm workers, particularly if farm workers employ risky personal behaviors, such as improper use of protective equipment, are assigned job tasks that result in sustained fecal exposure, and work on dairy farms with poor safety culture or deficient engineering or administrative controls. To our knowledge identification of these *nodes of high occupational exposure to zoonotic pathogens* has not been conducted previously for western animal agriculture overall and specifically for California's large dairy industry in a comprehensive manner; the lack of this surveillance has likely led to unnecessarily high levels of exposure to zoonotic pathogens among farm workers and the associated human risk of enteric infection. This project will facilitate substantial reduction in farm worker occupational exposure to these enteric pathogens and thereby help reduce the annual incidence of these occupational diseases in California dairy farm workers. The goals for this project are to develop the necessary zoonotic pathogen exposure data, identify worker behavior and protective equipment, along with employer-level controls that will mitigate these risks, and develop and disseminate training materials that will assist dairy farm workers and their employers to reduce occupational exposure to zoonotic infectious pathogens commonly shed by California dairy cattle. These goals, outcomes and impacts will be achieved by addressing the following specific aims:

***Specific Aim 1*. Quantify high-risk bacterial and protozoal zoonotic pathogens shed by infected California dairy cattle that can result in zoonotic pathogen infections in farm workers with direct contact with cattle or their manure.** Previous epidemiological surveys will be combined with new microbiological data from our project to characterize specific cohorts of dairy cattle that exhibit a higher prevalence and higher intensity of fecal shedding of *Cryptosporidium parvum*, *E. coli* O157:H7, *Salmonella enterica* subsp. *enterica*, *Listeria monocytogenes*, and *Campylobacter jejuni*.

***Specific Aim 2*. Identify hierarchical clusters of high occupational exposure to zoonotic pathogens for dairy farm workers.** We will identify combinations of worker occupational task, use of protective equipment, specific personal behaviors, and psychosocial factors, along with engineering and administrative controls associated with dairy farming that collectively affect the risk of sustained exposure to bovine zoonoses for farm workers, using both traditional epidemiological methods and novel network analysis algorithms developed by our research team. To bolster the validity of these findings, these identified clusters of high exposure risk will be cross-referenced against each farm worker's history of clinical symptoms consistent with enteric infection from the studied pathogens (*C. parvum*, *E. coli* O157:H7, etc.). These combinations of worker-level and owner/manager-level factors that cause these clusters of high exposure risk will be targets for our worker and owner/manager training in Specific Aim 3.

***Specific Aim 3.* Develop and disseminate recommendations for reducing zoonotic exposure to dairy workers, farm owners and managers, and allied organizations by conducting outreach and training in California, Nevada and Arizona**. The outreach and training activities, done in collaboration with the WCAHS Outreach Core, will magnify the impact of this project and help promote zoonotic disease prevention among our large population of California dairy farm workers, along with key dairy regions in Nevada (Fallon area) and Arizona (greater Phoenix area).

Outcomes from this project will include a detailed assessment of how animal infection patterns – when combined with high-risk occupational tasks, inadequate personal protective behaviors or equipment, and lack of proper engineering or administrative controls by the employer/owner– result in significant risk of zoonotic pathogen exposure. The knowledge gained and training materials developed for zoonotic disease risk reduction will be readily applicable to farm workers on dairies located not just in California, but also throughout the United States.

RESEARCH STRATEGY

Significance

Over the past twenty years the dairy industry in the western United States (US) has experienced a variety of structural changes ranging from a large increase in herd size, intensification of the overall farming operation, technological innovation in milking parlor design and equipment, strengthened regulations for manure management systems, and a growing dependence on a large immigrant workforce that is increasingly specialized for specific on-farm tasks (e.g., milking parlor, calf feeding, manure management, nutrition). In addition to these structural and operational changes that have occurred in the West and across the US (Douphrate et al., 2013), numerous zoonotic pathogens are endemic in the dairy cattle population and are distributed throughout the dairy environment (Mohammed et al., 2009), posing a constant threat to human and animal health (Suresh et al., 2012). For example, the following five zoonotic pathogens (herein referred to as the 5P) have a long history of causing mild to severe gastrointestinal illness and occasional human mortality through direct contact with infectious manure, contaminated food or water: *Salmonella enterica* subsp. *enterica*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni*, and the highly infectious protozoal parasite, *Cryptosporidium parvum*. Each of these five microorganisms exhibit unique virulence factors and host-pathogen adaptations that make them well suited as bovine-derived zoonotic pathogens that annually infect thousands to millions of humans in the US and internationally (Scallan et al., 2011; Suresh et al., 2012). Specifically, the CDC estimates that *Campylobacter* spp. and nontyphoidal *Salmonella* spp. each cause over one million domestically acquired illnesses per year, with *E. coli* O157:H7, *L. monocytogenes* and *Cryptosporidium* spp. together causing an additional several hundred thousand illnesses (Scallan et al., 2011). It is currently unknown what proportion of this annual disease burden in the US is attributable to farm workers, but without question workers at animal agricultural facilities such as dairies are at very high risk of exposure and subsequent infection with these enteric diseases due to their daily and sustained occupational contact with infectious cattle and manure matrices. For example, dairy calves in California that become infected with *Cryptosporidium parvum* can shed up to 10^7 oocysts (infectious stage) per gram feces (Moore et al., 2003; Atwill et al., 2012), with a cumulative incidence within the first 30 days of age exceeding 90% for a typical California dairy (Harp et al. 1996). Given that the infectious dose for infecting 50% of healthy humans (ID_{50}) is estimated to be as few as 160 bovine-derived *C. parvum* oocysts (Dupont et al., 1995; see Figure 1), this indicates that less than 1 mg of highly infective calf feces ($160/10^7 = 1.6\times10^{-6}$ grams) is sufficient for fecal-oral transmission of this parasite into dairy workers from exposure pathways such as aerosolization during pressure washing or handling an infected calf and then inadvertently licking one's lips after such tasks. Although reliable data on the annual incidence of the 5P in dairy workers is currently lacking for reasons explained below, we expect occupational exposure and subsequent disease to be a serious but neglected human health issue that can be mitigated by the interventions identified during this project.

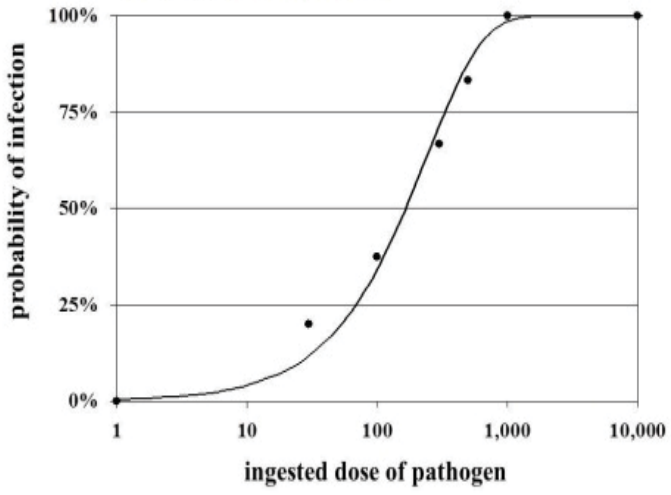

FIGURE 1. Human dose-response for *C. parvum*. Raw data and expected infectious dose curve for healthy human volunteers ingesting *C. parvum* oocysts originally obtained from an infected calf in Iowa (Dupont et al., 1995).

There is a well-documented and long history of the 5P as causative agents of community-wide human outbreaks, endemic human illness, and occasional mortality from food and waterborne transmission and direct human contact with infected cattle and their manure (e.g., Hunter and Thompson, 2005; Scallan et al., 2011; Suresh et al., 2012). Despite the proven ability of these enteric zoonoses to cause extensive human morbidity and sporadic mortality throughout the US, there is a paucity of on-farm research and proven intervention strategies for reducing human occupational exposures on Western US dairy farm operations to these

microbiological hazards. For example, the Migrant Clinicians Network at the University of Wisconsin provides a limited amount of information on pathogen exposures to dairy workers. Our project will substantially add to these current recommendations and provide a detailed assessment of where zoonotic pathogen exposure is highest within the dairy farm environment and how worker personal behavior, use of personal protective equipment, psychosocial factors, and higher-level employer controls, such as the safety culture and administrative controls interact to modulate worker occupational exposure to these microbiological hazards.

Valid and precise estimates of the size of the farm worker population on these animal agricultural operations in the West are difficult to obtain, but using a recent estimate of one worker per 80 to 100 cattle for large dairy operations in New Mexico (Cabrera et al., 2008), we can extrapolate that 18,000 to 22,500 farm workers are needed for the estimated 1.8 million dairy cattle on 1470 dairies comprising today's California dairy industry alone (CDFA, 2014a; USDA, 2013). Similarly, the California Department of Food and Agriculture estimate from their annual survey of cost of milk production that there were about 122 dairy cattle for each dairy laborer (excluding crop farming labor) in 2013-14, which translates to about 14,700 dairy workers for California's dairy industry (CDFA, 2014b). Similar estimates could be projected throughout the Western US for the size of the farm worker population for dairy industries located in such states as Washington, Oregon, Nevada, Arizona, and New Mexico.

The lack of knowledge regarding zoonotic disease in this extensive worker population is partly the result of cultural, socioeconomic and political challenges to implementing a targeted zoonotic disease surveillance program for the primarily immigrant dairy farm worker population in the West. Additional factors contributing to our poor understanding of the incidence and contributing factors for zoonotic infections among immigrant dairy farm workers might include lack of access to health care in rural communities, reluctance to self-refer to a health clinic, low and sporadic income, and language barriers (Baker et al., 2012; Schenker and Gunderson, 2013). As a consequence, the annual incidence of enteric zoonoses attributed to occupational exposure is relatively unknown for the dairy farm worker population and their families, who can be secondarily exposed through the index infection (Pereira et al., 2002). This proposed project will begin addressing this long-standing deficiency by identifying key risk factors for sustained pathogen exposure from infected cattle and their manure and disseminating training materials that will assist dairy farm workers and their employers to reduce occupational exposure to zoonotic infectious diseases commonly shed by dairy cattle. Given the very large dairy industry in California, we will focus this project on the large milkshed in Central California's San Joaquin Valley, which has a large immigrant farm worker population.

We propose to conduct a pair of longitudinal observational studies that together will characterize the primary zoonotic exposures occurring on large commercial dairies in California's San Joaquin Valley from infectious cattle (Specific Aim 1) and identify workplace controls, personal behaviors and personal protective equipment (PPE) that with proper training and appropriate use can substantially reduce the risk of worker infection from the 5P dairy cattle zoonoses (Specific Aims 2 and 3). This project will also identify psychosocial and other personal factors (worker level) along with higher level engineering and administrative controls (employer level) that modulate dairy worker exposure to these 5P and reduce the risk of subsequent enteric illness (Specific Aim 2). In this manner our multidisciplinary team will build trust among the worker community and the dairy industry in general, generate detailed data on critical zoonotic exposure pathways, and collaborate with the WCAHS Outreach Core to develop and disseminate training materials that promote occupational health and safety targeted to the unique needs of California's dairy farm worker population.

It can be argued that the field of *Occupational Health & Safety for Agricultural Zoonotic Disease* is not well developed for our animal agricultural sectors in the US. As mentioned above, the Migrant Clinicians Network at the University of Wisconsin provides limited information on pathogen exposures to dairy workers, but with little detail in terms of where within the dairy farming environment are zoonotic risks maximal and how workplace controls can best reduce these risks. As a consequence, our proposal is well positioned to significantly contribute to this area of occupational health and safety and to address the unique cultural and socioeconomic challenges of delivering occupational health programs to a mainly Latino workforce for our large and intensified dairy industry. As described below in the *Innovation* section, we will utilize a novel combination of quantitative microbiology, data on worker and employer controls, and epidemiologic and computational network analyses to identify *hierarchical clusters of high occupational exposure to zoonotic pathogens* and to use this information to design and disseminate targeted educational and training materials that will allow California's dairy worker population and associated employers to significantly reduce the risk of on-farm enteric zoonotic disease.