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Project title:

Developing and validating methods for accurately measuring the shedding of *Escherichia coli* O157:H7 by rangeland cattle and assessing water quality

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Background

Ranching operations in California are amongst the most diverse in the world with 38 million acres of rangeland owned or managed by ranchers. The diverse geography of the state, along with extreme climatic and environmental variability has resulted in a highly complex livestock production industry. Ranching is an important agricultural component of California agriculture that produces high quality and valuable foods to feed the nation and the rest of the world. However, the ranching industry often faces challenges to meet the requirements of local, regional, and national water quality regulations.

The gut of cattle is a natural reservoir for the human pathogen, *Escherichia coli* O157:H7. Prevalence of *E. coli* O157:H7 in cattle have been well documented and therefore, cattle have been considered as a major source of environmental contamination (Cobbaut et al., 2009; Ferens et al., 2011; Sasaki et al., 2011; Smith et al., 2010; Soon et al., 2011). Grazing beef cattle have been frequently reported carrying *E. coli* O157 during grazing on pasture (Branham et al., 2005; Hussein et al., 2003; Kondo et al, 2010; Looper et al., 2009; Thran et al., 2001) and a strong seasonal influence has been demonstrated, with cattle generally shedding more bacteria during the summer months (Kondo et al., 2010; Pearce et al., 2009; Thran et al., 2001; Williams et al., 2010). In addition, *E. coli* O157:H7 have been increasingly considered as a pathogen that impacts water quality and can potentially cause waterborne diseases (Berry et al., 2010; Balbus et al., 2002; Craun et al., 2005; Edge et al., 2012; Vinten et al., 2009;). It is of significance for the ranching industry to develop strategies that will minimize the prevalence and intensity of cattle shedding of *E. coli* O157:H7 and thereby protect water quality. However, such efforts are hampered by the current gap of knowledge related to techniques of accurate enumeration of *E. coli* O157:H7 in cattle feces and in water.

Detection and enumeration of *E. coli* O157:H7 in feces from rangeland cattle and adjacent waterways are the first steps for monitoring environmental contamination and water quality. To generate accurate estimates of bacterial numbers present in a sample, the result of detection assays need to be adjusted by the percent recovery of the particular method. Traditionally, percent recovery was commonly based on bacteria numbers estimated by folds of serial dilution, e.g., 10-fold, from bacterial culture solution. Bacteria concentrations and/or numbers in serial dilution methods are usually determined by counting bacterial colonies on monitoring agar plates and inflated by the dilution factor. As a result, bacteria numbers and concentrations are based on calculated and estimated numbers but not actual counts of bacterial cells. Calculated numbers of bacteria could be far different from actual numbers present and significantly affect the calculated

percent recovery of a method. This could have an important effect on estimates of risk associated with cattle grazing, thereby impacting water quality and range management decisions based on these numbers.

The volumes of water used for detecting bacteria and monitoring water quality varies significantly among researchers and water quality experts (Buchholz et al., 2012; Dahm et al., 2012; Macy et al., 2005; Valtari et al., 2012). It is accepted that using smaller volumes (100 ml to 1 L) decrease and using larger volumes (10 L or more) increase the probability of detecting *E. coli* O157:H7 from water. However, processing volumes of water as large as 10 L is challenging and usually results in losing some bacteria during processing if there is not a suitable method used to concentrate the water prior to analysis.

In summary, estimates of shedding of *E. coli* O157:H7 by rangeland cattle and subsequent bacterial loads in water adjacent to rangeland could be incorrect due to the lack of accurate methods of detecting the pathogen in feces and in water. Accurate detection of *E. coli* O157:H7 in feces from rangeland cattle and in water is among those critical and immediate needs of the industry for correct estimation the risks of water contamination and to protect sustainable growth of the industry. For this project, we propose a method that combines ImmunoMagnetic Separation (IMS), Green Fluorescence Protein (GFP) transformation, flow cytometer enumeration, and ultrafiltration of water for accurate detection of *E. coli* O157:H7 in feces of rangeland cattle and in environmental water adjacent to rangeland.

Objective

Our overall goal is to prevent zoonotic pathogens from contaminating environmental water adjacent to rangeland thus improving water quality. Our specific objectives are to 1) Develop laboratory methods for accurate enumeration of *E. coli* O157:H7 in fecal and water samples; 2) Validate the methods for detection of *E. coli* O157:H7 in fecal sample from rangeland cattle and from environmental water; 3) Apply the methods of detection of *E. coli* O157:H7 in seasonal samples from rangeland cattle and environmental water; 4) Provide knowledge to the cattle industry about *E. coli* O157:H7 shedding in rangeland cattle and adjacent water.

Hypothesis

Our hypothesis is that techniques for accurately enumerating *E. coli* O157:H7 in rangeland cattle feces and in environmental water do not presently exist. Current methods may overestimate or underestimate the real loads of *E. coli* O157:H7 and therefore, shedding of *E. coli* O157:H7 by rangeland cattle and associated water quality have not been correctly assessed.

Scientific significance

Accurate enumeration of *E. coli* O157:H7 shedding by rangeland cattle and in environmental water adjacent to rangeland will provide valuable information to the rangeland cattle industry, water boards, and public health officials. Based on a literature search conducted on September 18, 2012, no publications were found that use GFP transformed and MoFlow cytometer sorted *E. coli* O157 and ultrafiltration and apply these techniques in detecting *E. coli* O157:H7 in rangeland fecal samples and environmental water samples. We propose this collaborative project

to address the important issue of developing an accurate enumerating method for *E. coli* O157:H7 in feces and water.

Approach

We propose a two-year project to develop and validate methods for accurately measuring the shedding of pathogenic *E. coli* O157:H7 by rangeland cattle and assessing adjacent water quality. The objective of the project will be fulfilled by developing and validating the methods in the laboratory and implementing them with fecal samples and environmental water samples.

Methods

Bacteria strain

The *E. coli* O157:H7 Strain that will be used in this project will be the strain isolated from wild pig feces from ground during the 2006 *E. coli* O157:H7 spinach outbreak near Salinas, CA. The strain ID is FDLBF06M-01066-1 coded by California Department of Public Health. As determined by pulsed-field gel electrophoresis (PFGE), the strain is identical to other strains isolated from environmental samples (including some from nearby range cattle) during the investigation. The strain was also confirmed positive for Shiga toxin 1 and 2 (Paton and Paton 2003).

Construction of competent bacteria and transformation of Green Fluorescence Protein (GFP)

The use of GFP in *E. coli* O157:H7 has been shown to have no effect on intrinsic characteristics of the cells. We will transform the GFP to *E. coli* O157:H7 so that the bacteria can be counted by flow cytometer and their growth visualized in a UV light box.

1. Preparation of competent bacteria:
    1) A single colony of freshly retrieved *E. coli* O157:H7 will be inoculated into 5 ml of LB broth and incubated overnight in a shaking incubator (200rpm).
    2) One ml of the bacteria broth will be inoculated into 100ml LB broth and incubated again until the optical density (OD) value of the solution is 0.3 - 0.5, then immediately cooled down by placing the culture solution on ice for 10 min.
    3) The solution will be centrifuged at 1500g for 10min at 4°C.
    4) The supernatant will be discarded, and pellets resuspended with 20 ml of cold and sterilized 0.1mol/L $CaCl_2$ followed by incubation on ice for 10min
    5) Repeat steps 3) and 4)
    6) Add cold and sterilized glycerol to a final concentration of 20% glycerol.
    7) Mix well, distribute to 1.5ml tubes with 100ul in each tube, and store at -80°C.
2. Transformation:
    1) Take a tube of competent bacteria from -80°C and immediately place on ice.
    2) Inoculate 10µl of Plasmid DNA (pGREEN) to the 100µl competent bacteria.
    3) Gently mix, incubate on ice for 30min.
    4) Incubate at 42°C in a water bath for 90 s and then incubate on ice for 10min.

5) Add the solution to 800 µl LB broth; incubate at 37°C for 2 - 3 h in a shaking incubator (200rpm).
6) Centrifuge at 1500g for 5min at 4°C.
7) Discard 800µl of supernatant, mix the rest of the supernatant with the sediments.
8) Incubate at 37C overnight.
9) Streak the GFP transformed *E. coli* O157 on Rainbow agar or Sorbitol MacConkey Agar plates and incubate at 37°C overnight. *E. coli* O157 colonies with green fluorescence can be visualized using a UV light box (Figure 1). Transformed *E. coli* O157:H7 bacteria will be grown on the plates again and keep stock at -80°C for use in methods development and validation.

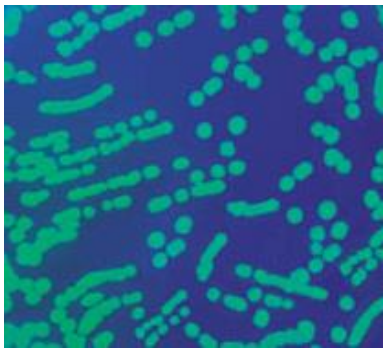

Figure 1. *E. coli* transformed with GFP

Flow Cytometry

The GFP-transformed *E. coli* O157:H7 will be grown in Tryptic Soy Broth (TSB) (BD Becton Sparks, MD USA) at 37°C in a shaking incubator (100rpm) for 3 h or until sample has reached an absorbance of at least 0.1 ABS (absorbance) at the wavelength of 600nm. An aliquot of 1mL of TSB will be transferred into a microcentrifuge tube and mixed with 50µL CountBright Beads (Invitrogen). A trained flow cytometer technician will run the TSB using Cytomation MoFlo Cell Sorter (MoFlo) (Beckman Coulter Cytomation Collins, CO, USA) which sorts the *E. coli* O157:H7 using UV excitation through a multi-line UV laser at 351 nm. The threshold is set on log side scatter, which has a forward scatter in log, and can detect the GFP fluorescence with a 488 nm laser and a 530/30 band pass filter. The MoFlo can sort and count a single GPF-transformed *E. coli* O157:H7 bacteria.

Qualitative and quantitative detection of E. coli O157:H7

Qualitative: A previously described enrichment and ImmunoMagnetic Separation (IMS) method (Paton and Paton, 2003) will be used for the detection of *E. coli* O157:H7. Ten grams of feces are placed into 100 ml Tryptic Soy Broth (TSB) and incubated in a Multitron programmable shaking incubator for 2 h at 25°C followed by 8 h at 42°C and held at 6°C overnight. After the incubation, 1.0 ml of the enrichment solution will be used for IMS isolation of *E. coli* O157 and 100 µl final solutions will be obtained after IMS. The IMS isolation is performed using anti-*E. coli* O157 beads (Invitrogen, Carlsbad, CA) with a Dynal Bead Retriever (Thermo, Finland) according to manufacturer's instructions. After IMS, 50 µl of the final solutions are streaked onto Rainbow agar (Biolog, Hayward, CA) and the rest 50 µl on Sorbitol MacConkey Agar (BD Becton, Sparks, MD) for isolation of *E. coli* O157:H7. The plates will be incubated for 24 h at 37°C. The number of bacterial colonies on all positive plates will be counted and recorded. GFP-transformed *E. coli* O157:H7 will be visualized using a UV light box on both plates. Positive samples will be confirmed with PCR using *E. coli* O157 specific primers

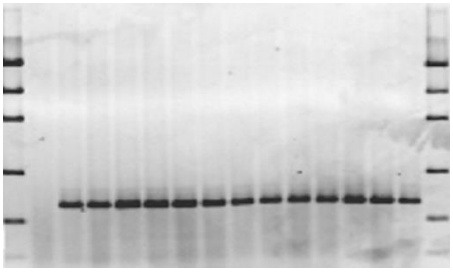

Figure 2. Confirmation of PCR products by electrophoresis

5’ CGG ACA TCC ATG TGA TAT GG 3’(forward) and 5’ TTG CCT ATG TAC AGC TAA TCC 3’(reverse). The 50µl PCR reaction mix will be composed of 1X PCR Buffer, 200µM of each DNTP, 1.5mM $MgCl_2$, 0.4µM Forward Primer, 0.4µM Reverse Primer and 1.25Units/reaction AmpliTaq Polymerase. PCR reaction started 95°C for 1 min to denature the DNA followed by 30 cycles of denaturation at 94°C for 15 sec, annealing at 55°C for 15 sec and extension at 72°C for 1 min. The PCR products will be stained with ethidium bromide and visualized on a 2% agarose gel (Figure 2).

Quantitative: A MPN (most probable number) procedure will be implemented on positive samples immediately after PCR confirmation. A regimen of 10 g (×4 replicates), 1 g (×4 replicates), and 0.1g (×4 replicates) samples (stored at 4°C) will be processed with the same IMS methods as above. The numbers of positive reactions of each weight and each replicate will be used for calculating *E. coli* O157:H7 concentrations using computer software based MPN calculator (Mike Curiale).

Spike trial for accurate detection of E. coli O157:H7 from fecal samples

We will first collect fecal samples from rangeland cattle and screen for the presence of *E. coli* O157:H7 using the methods described above. Then 10 g of negative fecal sample will be spiked using MoFlo sorted GFP-transformed *E. coli* O157:H7 at concentrations of 1, 10, 50, 100, 1000, and 10000 bacterial cells per gram of feces. Each spike trial will be repeated in triplicate. This method will be directly compared to the traditional method of spiking based on serial dilution estimated bacterial numbers. Detection of *E. coli* O157:H7 in all spiked samples will be performed as qualitative detection and numbers of detectable spiked GFP-transformed *E. coli* O157 will be determined by using the MPN methods as described above. Percent recovery for both spiking methods will be calculated by comparing numbers of recovered *E. coli* O157:H7 to the numbers of spiked *E. coli* O157:H7.

Spike trial for accurate detection of E. coli O157:H7 from water samples

We will first collect environmental water samples in a seasonal stream adjacent to the collaborating producer. Water will be autoclaved and completely cooled to 4°C before the spike trial. Then we will spike MoFlo sorted GFP-transformed *E. coli* O157 to 10 L water at concentrations of 1, 10, 50, 100, 500, 1000 bacteria cells per 100ml respectively. Similar to the fecal trial described above, this method will be directly compared to the traditional method of spiking based on serial dilution estimated bacterial numbers. Spiked water will be filtered using hollow-

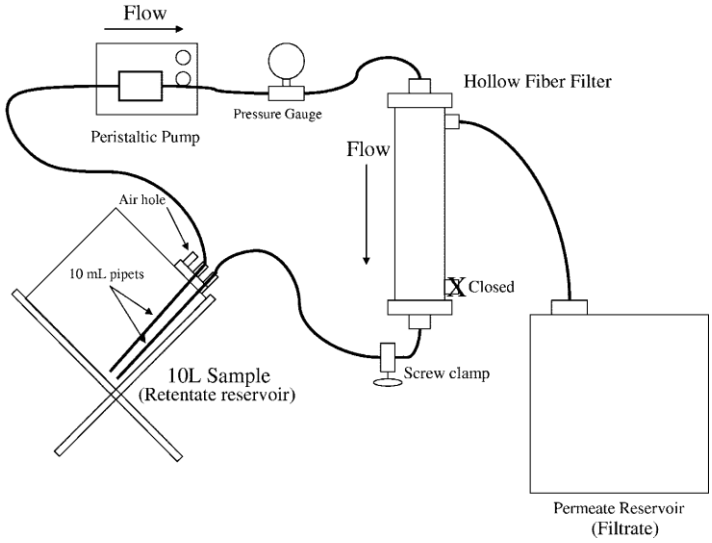

Figure 3. Schematic of 10-liter ultrafiltration setup

fiber ultrafiltration (UF) technique (also called tangential flow) that has been reported to be effective for recovering a diverse array of microbes from water (Hill et al, 2005). F200NR filters will be used for the ultrafiltration and 1000ml of concentrated water (retentate) will be obtained. The MPN methods will be used to determine concentrations of spiked *E. coli* O157:H7 in retentate water with a regimen of 200 ml (×4 replicates), 20 ml (×4 replicates), and 2 ml (×4 replicates). Numbers of spiked *E. coli* O157:H7 in spiked water and percent recoveries will be calculated by comparing numbers of recovered *E. coli* O157:H7 to that of spiked *E. coli* O157:H7.

Implementation of the methods for accurate detection of E. coli O157:H7 in feces from rangeland cattle and associated water

Through our extension collaborators, we will enroll three cattle producers from the foothill regions of the Central Valley that have adjacent environmental water streams. We will visit each rangeland four times respectively in spring, summer, fall and winter. Ten 50-g fecal samples and three 10-L water samples will be collected from each rangeland during each visit. Methods developed as described above will be implemented for detection of *E. coli* O157:H7 from fecal samples and water samples.

Data analysis

Percent recovery will be calculated by dividing the number of bacteria recovered from a sample by the known number spiked into the sample. Prevalence and intensity of bacteria in fecal and water samples will be determined and values between herds and between seasons will be compared with using a chi-square analysis (prevalence) and a two-sided t-test (intensity). Also, a random effects logistic regression model (using STATA® for Windows Release 11), with herd entered as a random effect will be developed to determine associations between herd and environmental risk factors and prevalence of infection.

Project timeline

Timeline	Activities
1/1/2013 – 2/28/2013	Project preparation
3/1/2013 – 4/31/2013	GFP validation
5/1/2013 – 6/31/2013	MoFlo validating
7/1/2013- 8/31/3013	Ultrafiltration validating
9/1/2013 – 2/28/2014	Fecal spike trials
3/31/2014 – 8/31/2014	Water spike trials
September 2014	Seminar on knowledge of detection of *E. coli* O157:H7 in rangeland cattle feces and environmental water
9/1/2014 – 8/31/2015	Implementation of methods to rangeland cattle and environmental water samples
9/1/2015 – 9/30/2015	Data analysis
10/1/2015 – 10/30/2015	Drafting and submitting project report
11/1/2015 – 12/31/2015	Drafting and submitting manuscripts to journals
December 2015	Seminar on knowledge of *E. coli* O157:H7 in rangeland cattle and

	environmental water

Relevance to the mission of the Russell L. Rustici Rangeland and Cattle Research Endowment

Rangeland cattle production is one of California's most significant and visible agricultural components. At the same time, the rangeland cattle industry faces strict regulations related to water quality. Environmental water contamination by rangeland cattle is becoming one of the critical issues and challenges facing the industry. Water quality (under "Rangeland Ecosystem Services") is one of the research priorities listed in the 2012 Call of the Russell L. Rustici Rangeland and Cattle Research Endowment. This project will develop and validate methods for accurate enumeration of *E. coli* O157:H7 in fecal samples from rangeland cattle and from adjacent environmental water. Having the ability to accurately determine the presence (or absence) of this important bacterial pathogen will help the industry as we move forward to providing practical answers to the critical issue of water quality. The objectives of our proposed project meet the mission and the goal of the Russell L. Rustici Rangeland and Cattle Research Endowment. Results from the project will benefit the California range cattle producers and the entire California public. We intend to extend the results of this research to a broad audience, including producers at local and State-level Cattlemens Association meetings (such as the Yolo County Cattle and Woolgrowers Association and the CCA annual meeting), extension specialists at Cooperative Extension seminars, and veterinarians at events such as the UC Davis Veterinary Practitioners Seminar. Additionally, we will host two seminars at UC Davis and present findings from the project. We will also attend professional meetings or conferences. In addition, we expect scientific publications to result that will undoubtedly stimulate further research efforts by colleagues that will further benefit the industry.

Qualifications of the principal investigators

Xunde Li, MS, PhD is a research microbiologist at the Department of Population Health and Reproduction (PHR) and a scientist at the Western Institute for Food Safety and Security (WIFSS) , School of Veterinary Medicine. Dr. Li is a trained microbiologist who has extensive experience in detection and characterization of waterborne zoonotic pathogens. In this project, Dr. Li will have primary responsibility for ensuring that samples are processed according to established protocols. He will also analyze the resulting microbiological data.

Bruce Hoar, DVM, PhD, Dip ACVPM has greater than 20 years of clinical experience as a food animal veterinarian. He is also trained as an epidemiologist and has held a research position in the School of Veterinary Medicine for the past 8 years. He has studied the presence of *E. coli* O157:H7 in beef cattle (Kondo et al., 2010) and in cull cattle destined for slaughter (Maier et al., IN PRESS). His experience and training will enable him to perform all the animal-related tasks associated with the project including collection of fecal samples.

Rob Atwill, DVM, PhD is a professor of PHR, the director of WIFSS and the director of Extension of School of Veterinary Medicine. Dr. Atwill will coordinate data analysis and manuscript editing. Once data is fully available, Dr. Atwill will present our findings at extension meetings throughout the state.

Daniel Myers, MBA has approximately 20 years' experience in Ranch Management and has a multitude of experience with stress related weight loss, lameness, and illness due to cattle shipments. This experience has been gained from shipping calves, yearlings, and cull cows and bulls numerous times throughout the year. Mr. Myers will contribute his knowledge and experience in animal management, production and welfare.

Morgan Doran is the County Director of the University of California Cooperative Extension for Solano County, and serves as Livestock and Natural Resources Advisor for Napa, Yolo, Solano, and Sacramento Counties. The primary focus of his program is to disseminate research-based information that helps livestock producers make informed decisions on livestock management. He also conducts research projects that provide useful information for local producers and resource managers. Mr. Doran will provide extension and outreach activities to cattle producers through local and regional meetings, such as Yolo County Cattlemen and Woolgrowers Association annual meeting.

Josh Davy is a Livestock, Range, and Natural Resources Advisor for Glenn, Colusa, and Tehama Counties. His program provides research and educational support in the areas of livestock, range, irrigated pasture and natural resource management. Mr. Davy will also participate in outreach and extension activities related to this project.

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Budget and Budget Justification

Budget

We request a total of $76,740 for the two year project as detailed in the following table

Category	Description	Costs
GFP transformation	5 GFP plasmids @$200 each plus supplies for growing, transforming, and stocking	$2,000
Flow Cytometry (MoFlo)	36 Moflo @ $100 each	$3,600
Ultrafiltration	72 filtration @ $40 each	$2,880
Immunomagnetic Separation (IMS)	228 IMS @ $25 each	$5,700
PCR	228 PCR @ $20 each	$4,560
Most probable number (MPN)	100 MPN @ $30 each	$3,000

Media and agar plates	1400 agar plates @ $2.0 each	$2,800
Laboratory supplies	Miscellaneous	$2,000
Labor support	25% time for 24 months	$24,600
In-state fee for graduate student	50% Ph.D. for 24 months	$12,000
Seminars for extending knowledge	2 seminars @ $3,500 each	$7,000
Travel	Travel to rangelands, 12 trips @ $250.00 each	$3,000
	Travel to professional meetings, 2 trips @ $1,800 each	$3,600
Total		$76,740

Justification

Our budget total of $76,740 reflects costs that are detailed below:

GFP transformation:

We will select and purchase 5 different GFP plasmid (Invitrogen, Qiagen) and compare their capability to transform into *E. coli* O157:H7 cells and select one for the project. Average cost of a plasmid is $200, therefore the plasmids will cost $1,000. Other laboratory supplies for growing, transforming and stocking transformed *E. coli* O157:H7 is estimated as $1000. Therefore, the cost for GFP transformation is $2000.

Flow cytometry (MoFlo):

Cost of materials and maintenance for conducting a MoFlo is $100. In total we will perform 36 MoFlo for both spike trials (6 concentrations for feces and 6 for water equal 12, triplication will be 36). The cost will be $100 x 36=$3,600.

Ultrafiltration:

In total we will conduct 72 ultrafiltration (triplication of 6 concentration of water spike trial by both MoFlo and serial dilution will be 36, plus 36 real environmental samples will be 72) including spike trials and detection of *E. coli* O157:H7 from environmental water samples. Cost of each ultrafiltration is approximately $40 including the single use filters. Total cost will be $40 x 72=$2,880.

Immunomagnetic Separation (IMS):

Each IMS will cost $25 including anti-*E. coli* O157 bead antibodies and single use retriever tubes etc. We will conduct 228 IMS (72 spike trials plus 120 real fecal samples and 36 real water samples equal to 228) and the cost will be $25 x 228=$5,700

PCR:

Each PCR will cost $20 including primers, PCR buffers, PCR tubes, and electrophoreses etc. We will conduct 228 PCR (72 spike trials plus 120 real fecal samples and 36 real water samples equal to 228) and the cost will be $20 x 228=$4,560.

Most probable number (MPN):

Each MPN cost $30 including deep well reservoir plates and solution etc. We will conduct approximately 100 MPN (36 for fecal spike trial and 36 for water spike trial plus esteimated 28 positive out of 156 environmental samples, approximately 100) and the cost will be $30 x 100=$3,000

Media and agar plates:

We will need approximately 700 Rainbow agar plates and 700 MacConkey Agar plates (approximately 500 plates for spike trials and 200 plates for detection E. coli o157:H7 from rangeland samples). Each plate cost approximately $2.0 and the cots will be $2,800.

Laboratory supplies:

We estimate that routine laboratory supplies including but not limited to pippets, tubes, reagents, gloves etc will cost $2,000

Labor support:

To aid with laboratory procedures, a Lab Assistant at 50% time will work in this project for a total of 24 months, the cost will be $24,600 for salary and benefits.

In-state fee for graduate student:

To aid the laboratory procedures and assist in data analysis, a Ph.D. student will work in this project for 50% time for 24 months and we will pay $6,000 tuition each year and $12,000 in total during the two year project period.

Seminars for extending knowledge:

We will host two seminars at UC Davis. Each seminar will cost $3,500 including renting conference rooms, printing seminal materials and presentations. The cost will be $3,500 x 2=$7,000.

Travel to rangelands for sampling:

We will use a UC Davis Fleet Services vehicle to travel from Davis to rangelands for sampling. Each trip will cost $250 and the cost will be $250 x 12=$3,000.

Travel to professional meetings:

We request budget for travelling to two professional meetings/conference to present findings of this project. Each travel will cost $1,800 including registration, flights, hotels, transportation and foods. The two travels will cost $3,600.

XUNDE LI'S BIOGRAPHICAL SKETCH

NAME: Li, Xunde		POSITION TITLE Project Scientist/Research Scientist	
eRA COMMONS USER NAME: none			
EDUCATION/TRAINING:			
INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Jilin Agricultural University, Changchun, China	B.S.	1987	Animal Science
Changchun University of Agriculture and Animal Sciences, Changchun, China	M.S.	1990	Biology
University of Rouen, Rouen, France	Ph.D.	2000	Parasitology

Contact information
Western Institute of Food Safety and Security
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University of California, Davis
Phone: (530) 752 0877
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Haring Hall, UC DavisDavis, CA 95616
Email: xdli@ucdavis.edu

Positions and Employment

1990-1997: Instructor, Department of Biology, Changchun University of Agriculture and Animal Sciences, Changchun, China

2000-2001: Visiting Postdoctoral Scientist, Environmental Microbial and Food Safety Laboratory, Beltsville Agricultural Research Center, ARS, USDA, Beltsville, Maryland.

2001-2003: Postdoctoral Researcher, Veterinary Medicine Teaching and Research Center, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California

2003-2007: Staff Research Associate, Veterinary Medicine Teaching and Research Center Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California

2008+: Project Scientist/Research Scientist, Western Institute for Food Safety and Security; Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California

2009+: Adjunct Professor, Department of Food Science and Technology, Jilin University Heping Campus, China

2012: Research Microbiologist, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California

Honors

2009 Scientific Cooperation Exchange Program between USDA and China's Ministry of Agriculture, invited Expert of U.S. Team #4: "Rapid detection technologies for food safety, environmental and agricultural applications", October 10-25.

1998-2000 "Normandy Association of Parasitology Research" Scholarship, France

Ten Relevant or significant Peer-reviewed Publications

2004 E.R. Atwill, R. Phillips, M.D. Pereira, **X. Li**, B. McCowan. Seasonal Shedding of Multiple *Cryptosporidium* Genotypes in California Ground Squirrels (*Spermophilus beecheyi*). Applied and Environmental Microbiology. 70: 6748–6752.

2005 **X. Li**, E.R. Atwill, L.A. Dunbar, T. Jones, J. Hook, K.W. Tate. Seasonal temperature fluctuations induce rapid inactivation of *Cryptosporidium parvum*. Environmental Science & Technology. 39: 4484-4489.

2006 C.P. Raccurt, P. Brasseur, R.I. Verdier, **X. Li**, E. Eyma, C. Pannier Stockman, P. Agnamey, K. Guyot, A. Totet, B. Liautaud, G. Nevez, E. Dei-Cas, J.W. Pape. Human cryptosporidiosis and *Cryptosporidium* spp. in Haiti (Cryptosporidiose humaine et espèces en cause en Haïti). Tropical Medicine & International Health. 11: 929-934.

2006 **X. Li**, R. Fayer. Infectivity of Microsporidian Spores Exposed to Temperature Extremes and Chemical Disinfectants. The Journal of Eukaryotic Microbiology. 53: S77-79.

2006 **X. Li**, K. Guyot, E. Dei-Cas, J.P. Mallard, J.J. Ballet, P. Brasseur. *Cryptosporidium* oocysts in mussels (*Mytilus edulis*) from Normandy (France). International Journal of Food Microbiology. 108:321-325.

2009 C. Xiao, **X. Li**, W. Wang, R. Meng, Q. Meng, Z. Song, H. Liu, Y. Cai, Y. Luo, N. Wang, S. Wang. Development and application of an immuno-enrichment brush method for rapid detection of four species of pathogenic bacteria. *Chin. J. Health Lab. Tech.* 19(10A): 1-3, 5.

2009 **X. Li**, R. Fayer, R. Palmer, J. Trout, C. Xiao. Strains of *Encephalitozoon cuniculi*, *Encephalitozoon intestinalis*, and *Encephalitozoon hellem* fail to experimentally infect food animals. *Chin J Vet Med*. 11, 1419-1423.

2010 **X. Li**, E.R. Atwill, L.A. Dunbar, K. W. Tate. Effect of daily temperature fluctuation during the cool season on the infectivity of *Cryptosporidium parvum*. *Applied and Environmental Microbiology*, 76(4): 989-993

2010 Pereira M, **X. Li**, B. McCowan, R.L. Phillips, E.R. Atwill. Multiple unique *Cryptosporidium* isolates from three species of ground squirrels (*Spermophilus beecheyi, S. beldingi, and S. lateralis*) in California. Applied and Environmental Microbiology. 6(24): 8269-9276

2011 A. Unc, M. J. Goss, S. Cook, **X. Li**, E.R. Atwill, T. Harter. Matrix effects critical to microbial transport through the vadose zone to groundwater (submitted to Journal of Water Resources)

Synergistic Activities

Journal reviewer: International Journal of Food Science and Technology (2009-present)

Minor advisor for Ph.D. candidates: Elizabeth Antaki; Tamara Vodovoz (current)

Committee: Member of Academic Federation Committee on Research, UC Davis, 2010-2011. Member of Regents' Scholarship Administrative Advisory Committee, UC Davis, 2009-2011.

Bruce R. Hoar, DVM, PhD, Dip ACVPM

Livestock and Food Safety Epidemiologist

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Education and Training

University of Saskatchewan, Saskatoon, Canada	DVM	1985
University of Saskatchewan, Saskatoon, Canada	MVetSci (Epidemiology)	1996
University of California, Davis	PhD (Epidemiology)	2001

Positions and Employment

2010 - Livestock and Food Safety Epidemiologist, Western Institute of Food Safety and Security, University of California, Davis

2003-2010 Assistant Professor, School of Veterinary Medicine, University of California, Davis

2001-2003 Postdoctoral Researcher, School of Veterinary Medicine,University of California, Davis

2000-2001 California Epidemiologic Investigation Service Fellow, California Department of Public Health

1996-2000 Veterinary Graduate Academic Fellow, School of Veterinary Medicine, University of California, Davis

1988-1994 Veterinary Practitioner

Other Experience and Professional Memberships

2003- Diplomate, American College of Veterinary Preventive Medicine

2007- Associate Editor, California Agriculture

Selected Peer-reviewed Publications most relevant to the current application

1. Maier GU, Hoar BR, Stull CL, Kass PH, Villanueva V, Maas J. Effect of a Nutritional Reconditioning Program for Thin Dairy Cattle on Body Condition, Meat Quality, Fecal Pathogen Shedding and Health Parameters. Journal of the American Veterinary Medical Association. ** IN PRESS **

2. Kilonzo C, Atwill ER, Mandrell R, Garrick M, Villanueva V, Hoar BR. 2011. Prevalence and molecular characterization of Escherichia coli O157:H7by multiple locus variable number tandem repeat analysis and pulsed field gel electrophoresis n three sheep farming operations in California. Journal of Food Protection, 74: 1413-1421.

3. Kondo S, Hoar BR, Mandrell R, Atwill ER. 2010. Longitudinal prevalence and molecular typing of Escherichia coli O157:H7 using Multiple-Locus Variable-Number Tandem-Repeats Analysis and Pulsed Field Gel Electrophoresis in a range cattle herd in California. American Journal of Veterinary Research, 71(11): 1349-1347.

4. Hoar BR, Paul RR, Siembieda J, Periera M, Atwill ER. 2009. Giardia duodenalis in feedlot cattle from the central and western United States. BMC Veterinary Research, 5(37).

5.Atwill ER, das G.C. Pereira M, Herrara Alonso L, Elmi C, Epperson WB, Smith R, Riggs W, Carpenter LV, Dargatz DA, Hoar BR. 2006. Environmental load of Cryptosporidium parvum oocysts from cattle manure in feedlots from the Central and Western United States. Journal of Environmental Quality, 35: 200-206.

Additional recent publications

Pires AFA, Hoar BR, Sischo WM, Olson SC. 2011. Serological response to administration of *Brucella abortus* strain RB51 vaccine in beef and dairy heifers using needle-free and standard needle-based injection systems. Bovine Practitioner, 45: 102-107.

Favetto PH, Hoar BR, Myers DM, Tindall JE. 2010. Breeding efficiency in pre-pubertal beef heifers treated with an intra-vaginal progesterone releasing device. California Agriculture Journal, 64(2): 106-111.

Ludwick TP, Poppenga RH, Green PG, Puschner B, Melton LA, Hoar BR, Nyberg NL, Maas J. 2008. The correlation of potassium content and moisture in bovine liver samples analyzed for trace mineral concentrations. J Vet Diagn Invest, 20(3): 314-20.

Maas J, Hoar BR, Myers DM, Tindall J, Puschner B. 2008. Vitamin E and Selenium concentrations in month-old beef calves. Journal of Veterinary Diagnostic Investigation, 20(1): 86-89.

Hoar BR, Bell TC, Villanueva V, Davy J, Forero L, Maas J. 2008. Herd-level management and biosecurity factors associated with measures of reproductive success in California beef cow-calf herds. Bovine Practitioner, 42(2): 132-138.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Atwill, Edward Robert	Director, Professor, and Specialist of Environmental Animal Health and Medical Ecology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, San Diego	B.A.	1985	Animal Physiology
University of California, Davis	D.V.M.	1990	Veterinary Medicine
University of California, Davis	M.P.V.M.	1991	Epidemiology
Cornell University	Ph.D.	1994	Infectious Disease Epidemiology

Contact Information

Edward R. Atwill, D.V.M., M.P.V.M., Ph.D.
Director, Western Institute for Food Safety and Security
Director, Veterinary Medicine Extension
Professor of Environmental Animal Health and Medical Ecology
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University of California-Davis

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Davis, CA 95616-8734
TEL: 530-754-2154
FAX: 530-752-5845
EMAIL: ratwill@ucdavis.edu

Positions & Employment

1994-99 Assistant Veterinarian (50%), Department of Population Health and Reproduction, School of Veterinary Medicine and Assistant Cooperative Extension Specialist (50%), Veterinary Medicine Extension, University of California, Davis

1999-03 Associate Professor (50%), Department of Population Health and Reproduction, School of Veterinary Medicine and Associate Cooperative Extension Specialist (50%), Veterinary Medicine Extension, University of California, Davis

2003+ Professor (30%) and Veterinarian in AES (20%), Department of Population Health and Reproduction, School of Veterinary Medicine and Specialist (50%), Veterinary Medicine Extension, University of California, Davis. Research and extension activities focused on the microbial interaction between livestock, wildlife, the environment, and public health

2009+ Director, Western Institute of Food Safety and Security, University of California, Davis

Honors

2001 Award for Outstanding Achievement in UC Cooperative Extension, Friends of Agricultural Extension, California. Finalist

2005 Pfizer Animal Health Award for Research Excellence, School of Veterinary Medicine, University of California, Davis

2011 Outstanding Achievement Award - Research/Academia, Society for Range Management

Examples of Editorial & Grant Review Service

2001-05 Associate Editor, California Agriculture, Journal of the Division of Agriculture and Natural Resources, University of California

2006-07 Ad hoc Reviewer, Applied and Environmental Microbiology

2008-09 Ad hoc Reviewer, Applied and Environmental Microbiology

2008-09 Ad hoc Reviewer, Journal of Environmental Quality

2009-10 Ad hoc Reviewer, Comparative Microbiology, Immunology, & Infectious Diseases

2009-10 Ad hoc Reviewer, California Agriculture

2009-10 Ad hoc Reviewer, Water Environment Research

2010-11 Grant Review Panel, Food-borne Pathogen-Plant Interactions & Practical Approaches to Food Safety, Agriculture and Food Research Initiative, NIFA-USDA

2010-11 Ad hoc Reviewer, Applied and Environmental Microbiology

Examples of Professional Service

2003-04 Ad hoc Reviewer, Food Safety Program, National Research Initiative Competitive Grants Program, CSREES, USDA

2009-10 Scientific technical review panel for the Pathogen Catchment Budget Model, Portland Water Bureau, Portland, Oregon

2010 Grant review panel, Food Safety Foundational Program, Agricultural Food Research Initiative, National Institute for Food and Agriculture, USDA

2010 Advisory Group, Potomac River Basin Drinking Water Source Protection Partnership, US Environmental Protection Agency Region III

2010-11 Scientific reviewer. Office of Scientific Quality Review, Agricultural Research Service, United States Department of Agriculture. Provide scientific reviews of proposed projects within ARS National Program 108 Food Safety

Ten Relevant or Significant Peer-reviewed Publications (from a list of 129 peer-reviewed journal articles or books, 204 proceedings and abstracts)

1999 **Atwill, E.R**., E. Johnson, M. Das Graças C. Pereira. Association of herd composition, stocking rate, and calving duration with fecal shedding of *Cryptosporidium parvum* oocysts in beef herds. Journal of the American Veterinary Medical Association 215(12): 1833-1838.

2001 Hoar, B., **E.R. Atwill**, C. Elmi, T.B. Farver. An examination of risk factors associated with beef cattle shedding pathogens of potential zoonotic concern. Epidemiology and Infection 127(1):147-155.

2002 **Atwill, E.**R. L. Hou, B.M. Karle, T. Harter, K.W. Tate, R.A. Dahlgren. Transport of *Cryptosporidium parvum* through vegetated buffer strips and estimated filtration efficiency. Applied and Environmental Microbiology 68(11):5517-5527.

2004 Tate, K.W., M. Das Gracas C. Pereira, **E.R. Atwill**. Efficacy of vegetated buffer strips for retaining *Cryptosporidium parvum*. Journal of Environmental Quality 33(6):2243-2251.

2006 **Atwill, E.R.,** K.W. Tate, Maria das Gracas Cabral Pereira, J. Bartolome, G. Nader. Efficacy of natural grassland buffers for removal of Cryptosporidium parvum in rangeland runoff. Journal of Food Protection 69(1):177-184.

2006 Tate, K.W., **E.R. Atwill**, J. Bartolome, G. Nader. Significant *Escherichia coli* attenuation by vegetative buffers on annual grasslands. Journal of Environmental Quality 35:795-805.

2007 Knox, A.K., K.W. Tate, R.A. Dahlgren, **E.R. Atwill**. Wetland filters, irrigation and grazing management can reduce *E. coli* concentrations in pasture runoff. California Agriculture 61(4):159-165.

2008 Miller, W.A., D. Lewis, M. G.C. Pereira, M.S. Lennox, P.A. Conrad, K.W. Tate, **E.R. Atwill**. Farm factors and beneficial management practices associated with reducing *Cryptosporidium* loading in storm runoff from dairy high use areas. Journal of Environmental Quality 37(5):1875-1882.

2009 Lewis, D.J., **E.R. Atwill**, M.S. Lennox, M.D.G. Pereira, W.A. Miller, P.A. Conrad, K.W. Tate. Reducing microbial contamination in storm runoff from high use areas on California coastal dairies. Water Science Technology 60:1731-43 doi:10.2166/wst.2009.561.

2010 Kondo, S., B.R. Hoar, R. Mandrell, **E.R. Atwill**. Longitudinal prevalence and molecular typing of Escherichia coli O157:H7 using multiple-locus variable-number tandem-repeats analysis and pulsed field gel electrophoresis in a range cattle herd in California. American Journal of Veterinary Research 71(11):1339-1347.

Budget and Budget Justification

Budget

We request a total of $77,490 for the two year project as detailed in the following table:

Category	**Description**	**Year 1 Cost ($)**	**Year 2 Cost ($)**
GFP transformation	5 GFP plasmids @$200 each plus supplies for growing, transforming, and stocking	2,000	0
Flow Cytometry (MoFlo)	Moflo @ $100 each	3,600	0
Ultrafiltration	Each filtration @ $40 each	1,440	1,440
Immunomagnetic Separation (IMS)	Each IMS @ $25 each	1,800	3,900
PCR	Each PCR @ $20 each	1,440	3,120
Most probable number (MPN)	Each MPN @ $30 each	2,160	840
Media and agar plates	Each agar plate @ $2.0 each	2,000	800
Laboratory supplies	Miscellaneous	1,000	1,000
Labor support	25% time for 24 months	12,300	12,300
In-state fee for graduate student	50% Ph.D. for 24 months	6,000	6,000
Seminars for extending knowledge	Each seminar @ $3,500 each	3,500	3,500
Travel to rangeland to collect water and feces	Travel to rangelands, each trip @ $250.00 each	750	3,000
Travel to profession meeting to present results	Each trip @1,800	1,800	1,800
Budget for each year		**39,790**	**37,700**