Risk Factors for *Bartonella* Seroreactivity Among Veterinary Professionals in the Pacific Northwest

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Public Health

University of Washington

2021

Committee:

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Program Authorized to Offer Degree:

Environmental and Occupational Health Sciences

University of Washington

Abstract

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Exposure to zoonotic disease is a significant occupational risk in veterinary medicine. In this study, we characterized PPE use, injury frequency, and *Bartonella* seroreactivity in Washington State veterinary workers. Using a job exposure matrix developed to reflect occupational risk factors for exposure to *Bartonella* and multiple logistic regression, we explored determinants of risk for *Bartonella* seroreactivity. Depending on the titer cutoff used, *Bartonella* seroreactivity was between 24.0% and 55.2%. No significant predictors of seroreactivity were found, though the relationship between high-risk status and increased seroreactivity for some *Bartonella* species approached significance. The predictive power of the model was likely limited by the small sample size and high level of exposure to risk factors for~~of~~ most participants. Given the high proportion of veterinarians seroreactive to one or more of the three *Bartonella* spp. known to infect dogs and cats in the United States, and the unclear relationship between occupational risk factors and seroreactivity, more research is needed in this area.

Background

Veterinarians and other veterinary ~~care~~ workers face occupational exposure to zoonotic pathogens on a daily basis. ~~Exposure to zoonotic disease is an occupational risk implicit in veterinary medicine.~~ While zoonotic infections can be subclinical, they can also be severe—even life-threatening—or significantly impact individuals' quality of life. In a survey of members of the Oregon Veterinary Medicine Association, almost one half (47~~.2~~%) of respondents reported having contracted a zoonotic disease during their career (1). In a 2012 study of Canadian veterinarians, 1~~76.7~~% reported that they had been diagnosed with or treated for a zoonotic disease in the past five years (2). ~~Authors of a 2021~~A recent systematic review and meta-analysis of veterinary occupational hazards found a pooled proportion estimate of zoonotic disease infection of 17% among veterinarians~~,~~ with proportions up to about 31%, 26%, and 24% for specific infections such as bartonellosis, Q fever, and viral infection, respectively (3). Despite these risks, surveys have reported ~~Zoonotic infection is of particular concern in veterinarians because of~~ generally low use ~~or~~ of personal protective equipment (PPE) and ~~insufficient~~ lack of comprehensive infection prevention and control (IPC) planning in ~~the profession~~veterinary workplaces (4, 5, 6). According to a survey of over 2,000 veterinarians in the United States, almost all ~~92-98.5% of veterinarians~~(92-99%) engage in needle recapping~~;~~, less than one-quarter r~~(<25%)~~reported using appropriate PPE when handling patients with dermatologic (17.9%), gastrointestinal (21.4%), respiratory (6.3%), or neurologic symptoms (16.5%)~~,~~; and ~~only~~ just over one-half (55~~.2~~%) always wash their hands before eating or drinking at work (7). As a result, veterinary ~~care~~ workers remain ~~While the majority of the zoonotic diseases experienced by veterinarians are relatively mild and treatable, the frequency of infection and exposure in~~

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~~veterinarians indicates that this population is particularly~~ vulnerable to emerging and reemerging zoonotic diseases, although the magnitude of the risk remains poorly understood.

Bartonella is ~~one such reemerginga~~ zoonotic ~~disease~~pathogen that has been associated with occupational veterinary exposures (8, 9). *Bartonella* species are fastidious, slow-growing, gram-negative bacteria that ~~typically~~ cause ~~long-lasting~~ intraerythrocytic and endotheliotropic infections (10). Since its reclassification in 1993, the genus has grown by over forty species and new members are regularly proposed (11). *Bartonella* is of particular relevance to ~~veterinarians~~ veterinary workers because of the presence of these bacteria in blood, tissues, and pathological effusions in companion animals such as dogs, cats, and rodents, and livestock such as cattle (12, 11). *Bartonella* ~~seropositivity~~ is particularly common in domestic cats—studies have documented 30-40% seroreactivity for *B. henselae* and the presence of several other *Bartonella* species (13)~~ in domestic cats~~. In humans, *Bartonella* infection can cause illness with a wide range of severity. *B. henselae* infections are typically characterized by self-limited, regional lymphadenopathy (also known as 'cat scratch disease')~~,~~ but can also cause more severe, disseminated disease, particularly in immunocompromised individuals (14). Other *Bartonella* species have been implicated in chronic rheumatic disease manifestations (15, 16). ~~*Bartonella* has been well-characterized as a cause of acute infection, but little is known about the possible health effects of subclinical infection or regular exposure to the pathogen as an occupational risk.~~ Because of the expanding number of *Bartonella* species, the spectrum of disease they can cause, and their presence in companion animals and livestock, *Bartonella* ~~should be considered~~has been proposed as a serious, ~~previously~~ underrecognized threat, particularly to veterinary workers (17)~~ians~~. ~~(REF).~~

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Sequential and potentially life-time studies are needed to correlate the occupational risk of zoonotic exposure to actual health outcomes. In order to protect veterinary professionals from the threat of emerging zoonotic diseases, we first need to understand the prevalence of and risk factors for zoonotic infection in this population. Many surveys of occupational healthzoonotic risks disease infor veterinary workers have been conducted, however, these surveys do not necessarily include zoonotic disease or investigate specific zoonotic infections. Surveys that do include and specify zoonotic disease often rely on self-reported, (and frequently, self-diagnosed,) symptoms and testing at a single point in time, making the identification of subclinical manifestations difficult and the misclassification of infection likely. . Studies that investigate specific zoonotic diseases and measure infection prevalence more quantitatively are needed. In the current study, we measured seroreactivity to *Bartonella henselae, B. kohlerae,* and *B. vinsonii* subsp. *berkhoffii* in a sample of 96 veterinarians at a from Washington StateVeterinary Medical Association conference. Using a job exposure matrix (JEM) developed from an accompanying survey, we estimated occupational risk of factors for exposure to *Bartonella* to explore determinants of risk for *Bartonella* seroreactivity.

Methods

This study was designed and carried out as a cross-sectional convenience survey of veterinarians in the Pacific Northwest. A self-administered survey of veterinary practice characteristics, work practices, exposure to potentially infectious materials, injuries, and health outcomes was distributed to 96 veterinary professionals at thea 2019 Pacific Northwest Vveterinary medicine Ceonference of by the Washington Veterinary Medical Association. A blood sample was also collected from each participant and analyzed by immunofluorescence

assay (IFA) for antibodies specific to *Bartonella henselae, B. kohlerae,* and *B.vinsonii* subsp. *berkhoffii*.

Laboratory Methods

Bartonella vinsonii subsp. *berkhoffii, B. henselae, and B. koehlerae* antibodies were determined in the Intracellular Pathogens Research Laboratory (IPRL) at North Carolina State University (North Carolina, USA) using cell culture grown bacteria as antigens and following standard immunofluorescent antibody assay (IFA) techniques as previously described (9, 18) ~~(Oteo et al., 2017, Portillo et al 2020)~~. A canine isolate of *B. vinsonii* subsp. *berkhoffii* genotype II (NCSU 95CO-08, Winnie), and feline isolates of *B. henselae* SA2 strain (NCSU 95FO-099, Missy) and *B. koehlerae* (NCSU 09FO-01, Trillium), were passed from agar plate grown cultures into *Bartonella*-permissive cell lines, i.e., the DH82 (a canine monocytoid) cell line for strains *B. henselae* SA2 and *B. koehlerae* and Vero cells (a mammalian fibroblast cell line) for *B. vinsonii* subsp. *berkhoffii* genotype II, for IFA testing. For each antigen, heavily infected cell cultures were spotted onto 30-well Teflon-coated slides (Cell-Line/Thermo Scientific), air-dried, acetone-fixed, and stored frozen. Fluorescein conjugated goat anti-human IgG (Cappel, ICN) was used to detect bacteria within cells using a fluorescent microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY). Serum samples diluted in a phosphate-buffered saline (PBS) solution containing normal goat serum, Tween-20, and powdered nonfat dry milk to block nonspecific antigen binding sites were first screened at dilutions of 1:16 to 1:64. All sera that were reactive at a reciprocal titer of 64 were further tested with two-fold dilutions to an endpoint titer. To avoid confusion with possible nonspecific binding found at low dilutions, ~~a~~ cutoffs of 1:64 and 1:128 ~~were as~~ selected as a seroreactive titers.

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Job Exposure Matrix

We created a job exposure matrix (JEM) for occupational exposure risk by combining measures of reported PPE use and reported animal-related injury (including bites and scratches) from the survey (Appendix I). Use score for PPE was determined by assessing reported glove, surgical mask, eye shield, and other PPE use ("other" PPE was rare, but included heavy gloves and squeeze chutes~~define what other could be here~~) for nine tasks where exposure to blood, saliva, needle sticks, or animal bites and scratches could occur, on a 0-4 scale (0 = never use, 4 = always use). Scores on each tasks were summed to create a total PPE use score with a maximum of 144 points. High PPE use was defined as ≥ 37 points (average of > 4 points per task, e.g. multiple pieces of PPE sometimes or one piece of PPE always), moderate PPE use as 19-36 points (average of > 2 and ≤ 4 points per task, e.g. no more than multiple pieces of PPE rarely or one piece of PPE often), and low PPE use as 0-18 points (average of ≤ 2 points per task, e.g. no more than two pieces of PPE rarely or one piece of PPE sometimes). Score for occupational injury was assigned by assessing frequency of animal injury and needle stick injury in the past year on a scale of 0-5 (0 = never, 5 = daily). Scores were summed for a total injury score with a maximum of 10 points. A low injury score was defined as ≤ 3 points (e.g. no more than monthly for one type of injury or every six months for two types), moderate injury score as 4-6 points (e.g. daily for one type of injury or monthly for two types), and high injury score as ≥ 7 points (e.g. at least weekly for one type of injury and monthly for the other). To create a total overall risk score, PPE use scores were reverse coded, with high use assigned a value of 1, moderate use assigned a value of 2, and low use assigned a value of 3. Low injury was assigned a value of 1, moderate injury a value of ~~3~~2, and high injury was assigned a value of 3. The two categories (PPE use and injury) were summed to create the overall risk score. Low overall risk was defined

as 0-32 total points (high PPE use and low injury), moderate total risk as 4 total points, and high total risk as 5-6 total points (low PPE use and high injury).

Logistic Regression Model

We then generated a logistic regression model including total risk level, career length, cat ownership, and dog ownership as possible predictors of overall ~~*bartonella*~~ *Bartonella* seroreactivity at the 1:128 cut-off ($Logit(p) = \beta 0 + \beta 1X_{risklevel} + \beta 3X_{careerlength} + \beta 4X_{petcat} + \beta 4X_{petdog}$). The same model was used also used for the outcomes *B. henselae* seroreactivity, *B. kohlerae* seroreactivity, and *B. vinsonii* subsp. *berkhoffii* seroreactivity. A chi-squared test was used to assess the relationship between *Bartonella* seroreactivity and general health rating.

Results

Participant ~~characteristics~~~~demographiues~~Characteristics

~~at a Washington veterinary con~~Most of the 96 participants were veterinarians, though practice owners, veterinary technicians, practice managers, and students were also included (Table 1). The sample was mostly female and of white race/ethnicity. Categories for jobs and race were not mutually exclusive. Age ranged from 23 to 71 years, with a mean age of 48.2. The mean career length for participants was 22.3~~26~~ years. As Table 1 and 2 show, exposure to cats and dogs was high for the majority of the sample: almost all participants work with cats and/or dogs and most have a pet cat and/or dog. Scratches were the most common occupational injury for ~~most~~ participants. The most common animal source of injury was cats.

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Table 1, Demographic characteristics.

Demographic characteristics	n (%)~~Percent~~	Mean	SD
Age		46.6	13.1
Gender			
Female	82 (85.4%)		
Male	12 (12.5%)		
Other	2 (2.1%)		
Race/ethnicity*			
White	82 (84.4%)		
Black	0		
Hispanic	2 (2.1%)		
Asian	3 (3.1%)		
American Indian/Alaska Native	0		
Native Hawaiian/Pacific Islander	1 (1.0%)		
Other	1 (1.0%)		
Pet ownership*			
Cat	71 (74.0%)		
Dog	72 (75%)		

*Participants could check more than one category; some participants did not answer~~Participants were veterinary professiona~~

Table 2, Occupational characteristics.

Occupational characteristics	n (%)~~Percent~~	Mean	SD
Length of career (years)		22.3	12.6
Job title*			
Owner	27 (28.1%)~~0~~		
Veterinarian	76 (79.2%)		
Veterinary technician	9 (9.4%)		
Veterinary assistant	0		
Veterinary student	1 (1.0%)		
Practice manager	3 (3.1%)		
Other**	6 (6.3%)		
Practice type*			
Small animal	81 (84.4%)		
Large animal	3 (3.1%)		
Mixed practice	13 (13.5%)		
Other**	11 (11.5%)		

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Occupational cat/dog exposure*	
Cat	~~85.4~~94 (97.9%)
Dog	92 (95.8%)~~12.5~~
Most common injury type*	
Bite	8 (8.3%)
Scratch	70 (72.9%)
Kick	5 (5.2%)
Needle stick	16 (16.7%)
Other	6 (6.3%)~~22~~
Most common animal injury source*	
Cat	50 (52.1%)
Dog	29 (30.2%)~~27.1~~
Other	16 (16.7%)

*Participants could check more than one category

**Other jobs included technical services vet, state/army vet, department chair/instructor, public health vet; other practice types included exotic animals, zoos

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Overall, most participants (93.8%) reported being in excellent or good health (Table 3~~)~~ ~~.~~). However, many~~ost~~ participants also reported being diagnosed with a health condition such as allergies, arthritis, or a chronic musculoskeletal disorder (Table 4). ~~(Table 1).~~ Only one participant reported being ~~formally~~ diagnosed with bartonellosis.

Table ~~1~~3, Reported health status.

Self-reported health	n (%)*~~Percent~~
Health rating	
Excellent	35 (36.5%)
Good	55 (57.3%)
Fair	6 (6.3%)
Poor	0
Diagnoses*	
Allergies	42 (43.8%)
Chronic Musculoskeletal Disorder	34 (35.4%)
Arthritis	27 (28.1%)

Zoonotic infection**	18 (18.8%)
Asthma	16 (16.7%)
Dermatitis	12 (12.5%)
Immunocompromising Disorder	6 (6.3%)
Other	10 (10.4%)

*Participants could check more than one category

***Zoonotic infections included ringworm, cryptosporidiosis, salmonellosis, psittacosis, roundworms, and bartonellosis.

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Bartonella Seroreactivity

At the 1:64 titer cutoff, 5~~4~~5.2% of participants were seroreactive to at least one *Bartonella* species (32.3% for *B. henselae,* 36.5% for *B. kohlerae,* and 24.0% for *B. vinsonii* subsp. *berkhoffii*). At the 1:128 cutoff, 24.0% of participants were seroreactive to ~~positive for~~ at least one *Bartonella* species (11.5% for *B. henselae,* 15.6% for *B. kohlerae,* and 8.3% for *B. vinsonii* subsp. *berkhoffii*). 26 (27.1%), 17 (17.7%) and 10 (10.4%) participants were reactive to one, two or three *Bartonella* species, respectively. Forty-four 44 (46.0%) participants were not seroreactive ~~(IFA titers <1:64)~~ to any of the three ~~IFA antigens~~Bartonella species.

Correlation of Seroreactivity and Risk Factors

No significant predictors of seroreactivity were found using the initial logistic regression model, likely because of the small sample size, the lack of variability in risk factors, and the limited sensitivity of *Bartonella* IFA. In a model comparing high risk to both low and moderate risk instead of all risk categories to each other, values approaching or achieving significance were found for the relationship between high risk and general *Bartonella* seroreactivity, as well as *B. kohlerae* seroreactivity (Table 2). A significant inverse relationship between career length and general *Bartonella* seroreactivity was observed in this high-risk model (OR = 0.51 [0.27-

0.98], $p = 0.043$). No significant relationship between *Bartonella* seroreactivity and general health rating was found.

Table 4, Association between high total risk status and *Bartonella* status.

Species	OR	95% CI	P-value
All *Bartonella*	2.95	0.99-8.79	**0.052**
B. henselae	2.03	0.49-8.32	0.33
B. koehlerae	3.35	1.01-11.18	**0.049**
B. vinsonii subsp. *berkhoffii*	2.00	0.41-9.84	0.39

Discussion:

Because few serosurveys of *Bartonella* in veterinary workers have been conducted, it is difficult to compare our findings to existing literature. However, our seroreactivity findings are comparable to a 2017 serosurvey of *Bartonella* in veterinary workers by Oteo et al. (9). At the 1:64 cutoff point, we found that 32.7% of participants were seroreactive to *B. henselae* and 36.5% were seroreactive to *B. kohlerae*, similar to Oteo's findings of 37.1% and 41.6%. Our results differed for *B. vinsonii* subsp. *berkhoffii*. We found that 24.0% of participants were seroreactive to *B. vinsonii* subsp. *berkhoffii,* while Oteo found a much higher percentage of 56.2. At the 1:128 cutoff, we found similar seroreactivity to *B. henselae* (11.5% vs. 10.1%), higher seroreactivity to *B. kohlerae* (15.6% vs. 10.1%), and lower seroreactivity to *B. vinsonii* subsp. *berkhoffii* (8.3% vs. 12.4%).

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Both these sets of findings support the claim that seroreactivity to *Bartonella* may be higher in veterinarians than in the general population, though seroreactivity can only be directly compared for *B. henselae* due to the lack of human serosurveys of *B. koehlerae* and *B. vinsonii* subsp. *berkhoffii*. A serosurvey of healthy adults in Korea found an overall prevalence of 15.0% (1:64 cutoff), and a serosurvey of children in Jordan found a prevalence of 11% (1:64 cutoff) (19, 20). However, a serosurvey of healthy students in Germany found that 30% (1:64 cutoff) were seroreactive to *B. henselae*, similar to the 32.3% prevalence in our sample of veterinary workers (21). Further research will be necessary to determine more definitive estimates of seroprevalence, particularly for different geographical area before a confident comparison can be made.

Overall, relationships between hypothesized risk factors and seroreactivity were not significant in our original logistic regression model. However, there was a trend toward increasing seroreactivity with increasing risk level. This trend was more visible when comparing high risk participants to all other participants (combining low and moderate risk participants), and significant or near-significant relationships were found between high risk level and general *Bartonella* seroreactivity (OR = 2.95, $p = 0.052$) as well as *B. kohlerae* seroreactivity (OR = 3.35, $p < 0.05$). Cat and dog ownership were not significant predictors of seroreactivity. In the high risk model, a significant inverse relationship was found between career length and seroreactivity (OR = 0.51, $p = 0.043$). While this is contrary to the expected trend of higher seroprevalence for more experienced veterinarians because of greater cumulative exposure, it reflects serosurveys in cats which have shown that older cats tend to have lower rates of seroreactivity than younger cats (22). This could be due to less robust immune responses to *Bartonella* in older cats and humans. However, since little is known about *Bartonella* antibody

kinetics, it is also possible that *Bartonella* antibodies are not very long-lasting and are more common in younger veterinarians or that older veterinarians are mounting a weaker immune response. Further research into the durability of *Bartonella* antibodies is needed.

Because of the small sample size and homogeneity of exposure to risk factors in the sample, it is not surprising that we were unable to identify few significant risk factors for *Bartonella* seroreactivity. A post-hoc power calculation revealed that even using the more sensitive 1:64 cutoff, our sample size was insufficient to detect significant relationships for a true odds ratio of less than or equal to two (23). Despite this limitation, we still observed positive odds ratios, ranging from 2.X to 3.X, ~~of about two to three~~ for multiple types of *Bartonella* seroreactivity according to increasing JEM risk level, indicating that low PPE use and high frequency of animal injury and needle sticks could be risk factors for *Bartonella* seroreactivity. Using a more heterogenous sample with respect to exposure to risk factors and including practice type or frequency of occupational exposure to cats and dogs in future JEMs could also help clarify these relationships.

One of the strengths of this study is the use of serology instead of self-reported illness or PCR to estimate *Bartonella* infection, since *Bartonella* infection can be very mild and may not prompt a visit to the doctor. Serology likely captures more previous infections. This study is also strong in its inclusion of *B. kohlerae* and *B. vinsonii* subsp. *berkhoffii* as well as *B. henselae*. Including these species of *Bartonella* reveals that *B. kohlerae* may be even more common in veterinary workers than *B. henselae* and should be considered when assessing veterinary *Bartonella* exposures. Future research should use serology and include these less-studied species to give a clearer picture of the risk posed to veterinary workers by *Bartonella*. Another key strength is the use of a risk assessment framework based on PPE use and occupational injury

frequency. This is a novel approach—examining the health risks of veterinary workers based on occupational characteristics—and is important for understanding how we may prevent *Bartonella* infections rather than just detecting them.

Overall, this study supports the claim that *Bartonella* is an emerging infectious disease that should be further explored and monitored in this population. While reducing illness from *Bartonella* is worthwhile in itself, *Bartonella* can also be used as an indicator of risk posed by other emerging infectious diseases and to evaluate the effectiveness of PPE use and injury prevention against blood and saliva-borne zoonotic infections in general, making this work important for public health. Future research that includes multiple *Bartonella* species, uses serology in addition to self-report or PCR ~~measures~~testing, and compares veterinary workers with a wide range of work practices and animal exposures will hopefully clarify the role of *Bartonella* in human and animal health, ~~and guide~~inform interventions that improve the safety of veterinary workplaces, and ultimately decrease the burden of *B~~b~~artonella* in this population, positively impacting occupational and public health.~~.~~

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Acknowledgments

Research reported in this thesis was supported by the National Institute for Occupational Safety and Health (NIOSH) under Federal Training Grant T42OH008433. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIOSH.

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Appendix Table I. PPE scoring system for the job exposure matrix.

Task	**Glove use**	**Surgical mask use**	**Eye shield use**	**Other PPE use**	**PPE score**
Cystocentesis	0 = never 1 = rarely 2 = sometimes 3 = often 4 = always	0-4	0-4	0-4	0-16
Drawing blood					
Prepping blood work					
Restraining patient					
Placing/removing IV					
Setting up/examining ear or skin cytology					
Cleaning surgical suites/instruments					
Performing dentistry					
Monitoring anesthetized patients					
Total PPE score					0-144

Appendix Table 2. Injury scoring system for the job exposure matrix.

Injury type	**Daily**	**Weekly**	**Monthly**	**Every 6 months**	**Once a year**	**Never**	**Injury score**
Animal injury	5	4	3	2	1	0	0-5
Sharps injury							
Total injury score							0-10