Zoonotic Infections Among Employees from Great Smoky Mountains and Rocky Mountain National Parks, 2008–2009

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Abstract

U.S. National Park Service employees may have prolonged exposure to wildlife and arthropods, placing them at increased risk of infection with endemic zoonoses. To evaluate possible zoonotic risks present at both Great Smoky Mountains (GRSM) and Rocky Mountain (ROMO) National Parks, we assessed park employees for baseline seroprevalence to specific zoonotic pathogens, followed by evaluation of incident infections over a 1-year study period. Park personnel showed evidence of prior infection with a variety of zoonotic agents, including California serogroup bunyaviruses (31.9%), *Bartonella henselae* (26.7%), spotted fever group rickettsiae (22.2%), *Toxoplasma gondii* (11.1%), *Anaplasma phagocytophilum* (8.1%), *Brucella* spp. (8.9%), flaviviruses (2.2%), and *Bacillus anthracis* (1.5%). Over a 1-year study period, we detected incident infections with leptospirosis (5.7%), *B. henselae* (5.7%), spotted fever group rickettsiae (1.5%), *T. gondii* (1.5%), *B. anthracis* (1.5%), and La Crosse virus (1.5%) in staff members at GRSM, and with spotted fever group rickettsiae (8.5%) and *B. henselae* (4.3%) in staff at ROMO. The risk of any incident infection was greater for employees who worked as resource managers (OR 7.4; 95% CI 1.4,37.5; $p=0.02$), and as law enforcement rangers/rescue crew (OR 6.5; 95% CI 1.1,36.5; $p=0.03$), relative to those who worked primarily in administration or management. The results of this study increase our understanding of the pathogens circulating within both parks, and can be used to inform the development of effective guidelines and interventions to increase visitor and staff awareness and help prevent exposure to zoonotic agents.

Key Words: Incidence—National Park Service—Prevalence—Vector-borne—Zoonoses.

Introduction

THE NATIONAL PARK SERVICE (NPS) AIMS TO PRESERVE the natural resources of its parks while leaving natural ecosystems intact with minimal interference. These ecosystems include a variety of native wildlife and associated arthropods which may carry pathogens that cause zoonotic infections from direct animal or vector-borne contact (Rayor 1985; McLean et al. 1989; New et al. 1993; Taylor et al. 1997; Gese

et al. 1997; Mills et al. 1998; Rhyan et al. 2001; Riley et al. 2004; Reeves 2007; Greger 2007; Winters et al. 2008).

Sporadic cases and outbreaks of zoonotic diseases have been reported previously among NPS employees and visitors. In November 2007, a wildlife biologist from Grand Canyon National Park died of pneumonic plague following transport and necropsy of an infected mountain lion carcass (Wong et al. 2009). Outbreaks of tick-borne relapsing fever have occurred among visitors staying in rustic cabins in Grand

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Canyon National Park (Boyer et al. 1977; Paul et al. 2002), and adjacent to Rocky Mountain National Park (ROMO; Trevejo et al. 1998). NPS employees may have prolonged exposure to wildlife and arthropods in occupational settings, possibly placing them at increased risk for zoonoses circulating within the parks. For visitors, national parks often represent unique, unfamiliar environments, and many might unknowingly participate in high-risk behaviors. However, because infected visitors may not become ill and seek care until they return home, epidemiologic links to where exposure occurred may be missed. Understanding the variety of pathogens impacting NPS staff may therefore help inform the risks for visitors as well as employees.

Materials and Methods

To determine zoonotic disease risks present at both Great Smoky Mountains (GRSM) and ROMO National Parks, we quantified background seroprevalence and measured incident infections to select zoonotic pathogens among a cohort of NPS staff members through two serosurveys conducted a year apart (2008–2009); questionnaires were also administered on potential risk factors and clinical features.

Enrollment

Employees were recruited using informational fliers. Participation was limited to permanent employees expecting to remain at their current location for at least 1 year from enrollment. All participants provided informed consent and the study was conducted under a protocol approved by the Centers for Disease Control and Prevention (CDC) Human Subjects Institutional Review Board.

Serological testing

Approximately 10 mL of whole blood were collected from each participant during each serosurvey. Serum was separated from cells. Samples were labeled with unique identifiers and stored at 4°C until transfer to the CDC's Rickettsial Zoonoses Branch laboratory in Atlanta, Georgia, and the Bacterial Diseases Branch laboratory in Fort Collins, Colorado, from GRSM and ROMO, respectively. At the labs 250–500 µL of serum were aliquotted, stored at –70°C, and shipped to collaborating laboratories for subsequent serological testing.

Pathogens tested and specific methods of serologic testing for each of the agents evaluated have been described elsewhere (Table 1; Lindsey et al. 1976; Dikken and Kmety 1978; Brown et al. 1981; Dalton et al. 1995; Plikaytis et al. 1996; Nicholson et al. 1997; Johnson et al. 2000, 2005; Martin et al. 2000; Quinn et al. 2002, 2004; Dumler 2004; Semenova et al. 2004; Jones et al. 2007). Baseline seropositivity was interpreted as prior infection. Among persons seronegative at baseline, seroconversions were considered evidence of incident infections during 2008–2009. For positive plaque-reduction neutralization test results for California serogroup viruses of the family Bunyaviridae (La Crosse, Snowshoe hare, Jamestown Canyon, and Trivittatus), or flaviviruses of the Japanese encephalitis antigenic complex of the family Flaviviridae (West Nile and St. Louis encephalitis viruses), the virus with the highest titer in the family was considered to be the most likely infecting virus, providing that this titer was $\geq$ fourfold higher

than titers for all others in the family; otherwise, the results were considered positive at the family-level only.

Data analysis

Data were summarized and park-specific prevalence and incidence were calculated. Incident infection with each agent and groups of agents aggregated by their most typical route of transmission were evaluated as dependent variables, including: (1) tick-borne zoonotic disease agents (*Anaplasma phagocytophilum*, *Borrelia hermsii*, *Borrelia burgdorferi*, Colorado tick fever virus, *Ehrlichia chaffeensis*, *Francisella tularensis*, and spotted fever group rickettsiae); (2) mosquito-borne zoonotic disease agents (Bunyaviridae and Flaviviridae); and (3) other routes of transmission (i.e., flea-borne, soil-borne, direct contact, and food-borne; *Bartonella henselae*, *Brucella* spp., *Coxiella burnetii*, *Leptospira* spp., *Toxoplasma gondii*, typhus group rickettsiae, and *Yersinia pestis*). Logistical regression was used to identify significant associations ($p=0.05$) between questionnaire data and infection incidence. Multivariate models were built using backward stepwise selection; adjusted and unadjusted odds ratios with 95% confidence intervals (CI) were calculated. Analyses were conducted using SAS 9.1 (SAS Institute Inc., Cary, NC).

Results

Study population

Of 460 eligible NPS employees, 141 (31%) enrolled in this study, including 79 (33%) from GRSM and 62 (28%) from ROMO. For the follow-up survey, 117 (83%) participants returned, including 71 (90%) from GRSM and 46 (76%) from ROMO. The median age at enrollment was 47 years (range 24–70 years); 67% were male. The median duration of NPS employment was 9 years (range 0.1–38 years). These attributes did not differ significantly between parks. One-quarter of the participants resided in park housing. Daily work activities were proportionately distributed across NPS divisions (Table 2), with participation in 14 primary job duties (Table 3). Additionally, 102 (87%) participants reported traveling out of state, and 21 (18%) out of country during the study period.

Reported arthropod and animal exposures. Ninety-five (81%) participants reported insect bites during the study period, and 38 (32%) found ticks on their skin or clothes; 52% of resource managers reported finding ticks on themselves. Nearly half (56%) reported contact with animals at work, including rodents (37%), canids (18%), reptiles/amphibians (16%), fish (9%), felids (3%), and other wildlife (24%). Law enforcement rangers/rescue crew workers reported the most animal contact (68%), while administrative staff reported the least (45%). A minority of participants reported higher-risk zoonotic exposures, including contact with dead rodents (20%), canid bites (6%), and felid bites (4%). These data did not significantly differ between parks.

Protective behaviors. Insect repellent was reportedly used when spending time outdoors by 66% of participants, though only 44% reported using repellent at work (ranging from 21% among administrative staff to 55% among resource managers). Nine percent reported specifically treating clothing with insecticide/repellent, though this was higher among

TABLE 1. LIST OF ZOONOTIC PATHOGENS FOR WHICH EACH STUDY PARTICIPANT WAS TESTED FOR EXPOSURE

CDC branch laboratory	Pathogens tested	Laboratory assay/methodology	Criteria for evidence of prior infection	Criteria for evidence of incident infection
Rickettsial Zoonoses Branch (RZB)	<i>Anaplasma phagocytophilum</i> <i>Bartonella henselae</i> <i>Coxiella burnetii</i> <i>Ehrlichia chaffeensis</i> <i>Rickettsia rickettsii</i> (representative of spotted fever group rickettsiae) <i>Rickettsia typhi</i> (representative of typhus group rickettsiae)	IgG antibodies were determined using indirect immunofluorescence assays (IFA) against whole-cell rickettsial antigens using standard procedures (Dumler 2004) with specific buffers and concentrations as detailed by Nicholson and associates (Nicholson et al. 1997); for <i>A. phagocytophilum</i> , samples were first screened by an in-house-developed research enzyme-linked immunosorbent assay (ELISA) using whole cells coated onto polystyrene plates; samples were screened for <i>C. burnetii</i> antibodies using a commercial (PanBio) assay following the manufacturer's instructions; seroreactive samples were titrated to end-point using the appropriate IFA	<i>B. henselae</i> : Positive IgG titer in 2008 ($\geq 1:256$) Other pathogens: Positive IgG titer in 2008 ($\geq 1:64$)	<i>B. henselae</i> : Change of IgG titer from negative in 2008 ($< 1:256$) to positive in 2009 (four-fold titer change with titers $\geq 1:256$) Other pathogens: Change of IgG titer from negative in 2008 ($< 1:64$) to positive in 2009 ($\geq 1:64$)
Bacterial Special Pathogens Branch (BSPB)	<i>Brucella</i> spp. <i>Leptospira</i> spp.	<i>Brucella</i> microagglutination test (BMAT) was used with minor modifications (U-bottom plates, incubation at 28°C, and discontinued use of safranin; Brown et al. 1981); the <i>Brucella</i> antigen used was the <i>B. abortus</i> strain 1119-3, from the National Veterinary Services Laboratories in Ames, Iowa <i>Leptospira</i> microagglutination test (LMAT) was used; live leptospiral cell suspensions representing 20 serovars and 17 serogroups were incubated with serially-diluted serum specimens; the resulting agglutination titers were read using darkfield microscopy; reported titer was highest dilution of serum that agglutinated $\geq 50\%$ of cells for each serovar tested (Dikken 1978)	<i>Brucella</i> : Positive IgG titer in 2008 (≥ 20) ^a <i>Leptospira</i> : Positive IgG titer in 2008 (≥ 100) to one or more serovars	<i>Brucella</i> : $\geq$ Fourfold rise in titer between 2008 and 2009 ^a <i>Leptospira</i> : $\geq$ Fourfold rise in titer between 2008 and 2009 ^b
Parasitic Diseases Branch (PDB)	<i>Toxoplasma gondii</i>	A commercial IgG enzyme immunoassay (EIA; Bio-Rad, Hercules, CA) was used according to the manufacturer's instructions (Jones et al. 2007); diagnostic sensitivity and specificity were each 100%	Positive IgG value in 2008 (≥ 9)	Change of IgG value from negative in 2008 (< 9) to positive in 2009 (≥ 9)
Bacterial Diseases Branch (BDB)	<i>Borrelia burgdorferi</i> <i>Borrelia hermsii</i> <i>Francisella tularensis</i>	Two tier-testing was used: EIA followed by Western Blot (WB)-M and -G of anything that was equivocal or positive (WB)-M and -G of anything that was equivocal or positive CDC in-house EIA was used to detect antibody to <i>B. hermsii</i> antigen in a whole-cell sonicate (WCS); to assess specificity, comparative EIA was run to <i>B. burgdorferi</i> WCS; reactive WCS EIAs were followed by in-house WB CDC in-house microagglutination assay was used; a suspension of stained bacteria was used as an antigen and dilutions of sera were tested; wells were subsequently examined visually for a typically blue color indicating agglutination	Positive WB confirmation of two-tier testing Positive WB confirmation of two-tier testing Positive IgG titer in 2008 ($\geq 1:128$)	Negative testing in 2008 with positive M or G blot in 2009 Negative testing in 2008 with positive M or G blot in 2009 Negative titer in 2008 serum with positive titer in 2009 serum

(continued)

TABLE 1. (CONTINUED)

CDC branch laboratory	Pathogens tested	Laboratory assay/methodology	Criteria for evidence of prior infection	Criteria for evidence of incident infection
Arboviral Diseases Branch (ADB)	<i>Yersinia pestis</i>	Passive hemagglutination/inhibition assay (PHA/HI) was used for anti- <i>Y. pestis</i> F1 antibody titers	Positive IgG titer in 2008 ($\geq 1:16$)	Negative titer in 2008 serum with positive titer in 2009 serum
	Colorado tick fever virus	Plaque reduction neutralization tests (PRNT) were performed on the latest available sample for each participant, with 90% plaque reduction end-points used; where a second sample was positive, the first was tested to assess for seroconversion ^b	PRNT ≥ 10 in 2008	Negative titer in 2008 serum with positive titer in 2009 serum
	Bunyaviruses (including La Crosse virus, Jamestown Canyon virus, Trivittatus, and Snowshoe hare virus)	PRNTs were performed on the latest available sample for each participant, with 90% plaque reduction end-points used (Martin et al. 2000; Johnson et al. 2000; Lindsey et al. 1976); for cases with positive PRNTs, immunoglobulin M (IgM)-capture enzyme-linked immunosorbent assays (MAC-ELISA) were performed	PRNT ≥ 20 or IgG-positive in 2008	Negative titer in 2008 serum with positive titer in 2009 serum; in a given serum sample, the homologous serotype was assumed to be any virus associated with a titer $\geq$ fourfold higher than titers against all other viruses in the same serogroup used in the tests
Meningitis and Vaccine Preventable Diseases Branch (MVPDB)	Flaviviruses	West Nile virus (WNV)/St. Louis Encephalitis virus (SLEV) IgG ELISA, and WNV/SLE IgM duplex microsphere-based immunoassay (MIA) assays were performed on all samples; if IgM was non-specific or positive, PRNTs were performed on all samples for that participant; 90% plaque reduction end-points were used (Martin et al. 2000; Johnson et al. 2000, 2005)	PRNT ≥ 20 or IgG-positive in 2008	Negative titer in 2008 serum with positive titer in 2009 serum; in a given serum sample, the homologous serotype was assumed to be any virus associated with a titer $\geq$ fourfold higher than titers against all other viruses in the same serogroup
	<i>Bacillus anthracis</i>	Anti-protective antigen (PA) ELISA was used; data were analyzed using a four-parameter logistic-log curve-fitting model with "ELISA for Windows" software (Plikaytis et al. 1996); a calibration factor (109.4 μ g/mL anti-PA IgG) for standard reference serum AVR801 was used to determine the concentration of anti-PA IgG in μ g/mL of test serum; the lower limit of quantification of the anti-PA ELISA was 3.7 mg/mL anti-PA IgG in undiluted serum samples; diagnostic sensitivity was 98.6% and diagnostic specificity was 98.4% (Quinn et al. 2002, 2004; Semenova et al. 2004)	Anti-PA IgG concentration $\geq$ to the assay lower limit of quantification (3.7 μ g/mL) or $\geq$ fourfold change in anti-PA IgG concentration compared to the value reported for the 2008 sample	Anti-PA IgG concentration $\geq$ to the assay lower limit of quantification (3.7 μ g/mL), or $\geq$ fourfold change in anti-PA IgG concentration compared to the value reported for the 2008 sample

^aSuggestive of prior exposure though not confirmatory; may also reflect cross-reactivity with other bacteria (Al Dahouk et al. 2003a, 2003b).

^bCriteria for incident exposure might not be met if infection occurred early during the study period, as titers may decline over time.

TABLE 2. DISTRIBUTION OF STUDY PARTICIPANTS AND ALL ELIGIBLE EMPLOYEES BY NATIONAL PARK SERVICE DIVISION AT THE GREAT SMOKY MOUNTAINS (GRSM) AND ROCKY MOUNTAIN (ROMO) NATIONAL PARKS, 2008–2009

Division	Total participants (% of eligible employees)	ROMO participants (% of eligible employees)	GRSM participants (% of eligible employees)
Superintendent	5 (46)	2 (50)	3 (43)
Interpretation/education	12 (33)	6 (35)	6 (32)
Administration	10 (30)	5 (26)	5 (36)
Visitor and resource protection	26 (28)	13 (28)	13 (28)
Resource management	39 (42)	11 (27)	28 (53)
Facility management	46 (24)	22 (23)	24 (25)
Other	3 (–) ^a	3 (–) ^a	0 (0)
Total	141 (31)	62 (28)	79 (33)

^aUnknown or cannot be calculated.

law enforcement rangers/rescue crew workers (21%) and resource managers (14%).

Medical history. Illness resulting in fever was reported among 33 (28%) participants during the study period; 6 (5%) also reported a rash. Fifty (43%) participants used antibiotics during the study, and 4 (4%) specifically used doxycycline, an antibiotic commonly prescribed for suspected tick-borne infections.

Laboratory analysis

Baseline seropositivity. Among 135 participants with sufficient serum at enrollment, 104 (77%) were seropositive

for ≥ 1 of the agents tested for, including 62 (83%) at GRSM and 42 (70%) at ROMO. Agent profiles differed between parks (Table 4). Baseline seropositivity was noted at both parks for *B. anthracis* (2%), flaviviruses (2%), *A. phagocytophilum* (8%), *Brucella* spp. (9%), *T. gondii* (11%), spotted fever group rickettsiae (22%), *B. henselae* (27%), and California serogroup *Bunyaviridae* (30%). In ROMO, 22% of participants were reactive to Jamestown Canyon virus and none to La Crosse virus; in GRSM, 7% were reactive to Jamestown Canyon virus and 23% to La Crosse virus. Seropositivity to *C. burnetii* (1%), *F. tularensis* (1%), *Leptospira* spp. (1%), and *E. chaffeensis* (3%), was limited to GRSM employees, while seropositivity to Trivittatus virus (2%), Snowshoe Hare virus (2%), West Nile virus

TABLE 3. DESCRIPTION AND DISTRIBUTION OF THE PRIMARY JOB DUTIES OF STUDY PARTICIPANTS BY NATIONAL PARK SERVICE DIVISION AT THE GREAT SMOKY MOUNTAINS (GRSM) AND ROCKY MOUNTAIN (ROMO) NATIONAL PARKS, 2008–2009

Primary job duties	Job duty description	Percentage of participants engaged in job duty	Percentage at GRSM	Percentage at ROMO
Office/administration	Human resources and staff support	52	51	54
Supervisory/ management	Division or branch managers	31	27	37
Emergency medical service/search and rescue	First responders and search and rescue	9	10	7
Custodial	Provide janitorial services	7	10	2
Building maintenance/ utilities	Maintain buildings and operate water, wastewater, and electrical systems	16	13	22
Trail maintenance	Repair and maintain off-road trails	10	11	9
Roads/grounds maintenance	Repair and maintain roads and landscapes	16	15	17
Wildlife management	Conduct research on terrestrial wildlife species and manage exotic diseases	12	8	17
Vegetation management	Conduct research on plants and vegetation and remove invasive or exotic species	16	15	17
Fisheries	Conduct research on aquatic wildlife species	5	4	7
Wildland fire response	Suppress fires	4	6	2
Campground/fees	Run campgrounds and collect gate or recreational use fees	5	4	7
Cultural resources/ archeology	Research and preserve cultural artifacts, structures, and materials	5	6	4
Resource education	Lead interpretive programs and educate visitors on park resources	10	11	9

Employees may engage in more than one listed job duty.

TABLE 4. PRIOR AND INCIDENT ZOO NOTIC INFECTIONS AMONG GREAT SMOKY MOUNTAINS (GRSM) AND ROCKY MOUNTAIN (ROMO) NATIONAL PARKS EMPLOYEES, 2008–2009

Zoonotic disease agent	Evidence of prior infection (2008)			Evidence of incident infection (2008–2009)		
	Overall (n = 135) [n (%)]	GRSM (n = 75) [n (%)]	ROMO (n = 60) [n (%)]	Overall (n = 110) [n (%)]	GRSM (n = 66) [n (%)]	ROMO (n = 44) [n (%)]
Tick-borne pathogens						
<i>Anaplasma phagocytophilum</i>	11 (8.1)	7 (9.3)	4 (6.7)	0 (0)	0 (0)	0 (0)
<i>Borrelia burgdorferi</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Borrelia hermsii</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Colorado tick fever virus	5 (3.7)	0 (0)	5 (8.1)	0 (0)	0 (0)	0 (0)
<i>Ehrlichia chaffeensis</i>	4 (3.0)	4 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Francisella tularensis</i> ^b	1 (0.7) ^a	1 (1.3) ^a	0 (0)	0 (0)	0 (0)	0 (0)
Spotted fever group rickettsiae	30 (22.2)	16 (21.3)	14 (23.3)	5 (4.5)	1 (1.5)	4 (9.1)
Mosquito-borne pathogens						
La Crosse virus	17 (12.6)	17 (22.7)	0 (0)	1 (0.9)	1 (1.5)	0 (0)
Jamestown Canyon virus	18 (13.3)	5 (6.7)	13 (21.7)	0 (0)	0 (0)	0 (0)
Trivittatus virus	1 (0.7)	0 (0)	1 (1.7)	0 (0)	0 (0)	0 (0)
Snowshoe hare virus	1 (0.7)	0 (0)	1 (1.7)	0 (0)	0 (0)	0 (0)
Bunyavirus ^c	6 (4.4)	4 (5.3)	2 (3.3)	0 (0)	0 (0)	0 (0)
West Nile virus	2 (1.5)	0 (0)	2 (3.3)	0 (0)	0	0 (0)
Flavivirus ^c	3 (2.2)	2 (2.7)	1 (1.7)	0 (0)	0 (0)	0 (0)
Pathogens transmitted by other routes (direct, flea-borne)						
<i>Bacillus anthracis</i>	2 (1.5)	1 (1.3)	1 (1.7)	1 (0.9)	1 (1.5)	0 (0)
<i>Bartonella henselae</i>	36 (26.7)	24 (32.0)	12 (20.0)	6 (5.5)	4 (6.1)	2 (4.5)
<i>Brucella</i> spp. ^d	12 (8.9)	7 (9.3)	5 (8.3)	0 (0)	0 (0)	0 (0)
<i>Coxiella burnetii</i> ^b	1 (0.7)	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Leptospira</i> spp.	1 (0.7)	1 (1.3)	0 (0)	4 (3.6)	4 (6.1)	0 (0)
Typhus group <i>Rickettsiae</i>	3 (2.2)	0 (0)	3 (5.0)	0 (0)	0 (0)	0 (0)
<i>Toxoplasma gondii</i>	15 (11.1)	8 (10.7)	7 (11.7)	1 (0.9)	1 (1.5)	0 (0)
<i>Yersinia pestis</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

^aThe serum met criteria for positivity in 2008, though it was subsequently negative in 2009 for two possible reasons: (1) the result reflected a measurement aberration, as the two samples only had one dilution-level difference, or (2) it was a case of waning seropositivity after recent infection.

^bPathogens with multiple known routes of transmission grouped according to the most common route.

^cThe specific virus could not be identified.

^dNot confirmatory for prior exposure; may also reflect cross-reactivity with other bacteria (Al Dahouk et al. 2003a, 2003b).

(3%), typhus group rickettsiae (3%), and Colorado tick fever virus (4%), was limited to ROMO employees.

Incident infections. Among 110 employees who participated in the follow-up, 18 incident infections were detected (Table 4). Twelve GRSM participants showed evidence of incident infection with one of six different agents: *B. anthracis*, *B. henselae*, California serogroup viruses, *Leptospira* spp., spotted fever group rickettsiae, and *T. gondii*. Five ROMO participants showed evidence of incident infection with *B. henselae* or spotted fever group rickettsiae; one was infected with both. Eighty-eight percent of participants with incident infections recalled an illness during the preceding year, though the symptoms reported did not significantly differ in frequency from participants without evidence of incident infection.

Risk factors for incident infections

Any zoonotic agent. Risk of incident infection (Table 5) with any agent was significantly greater among participants who worked as resource managers (adjusted odds ratio [AOR] 7.4; 95% CI 1.4,37.5; $p=0.02$), or law enforcement rangers/rescue crew members (AOR 6.5; 95% CI 1.1,36.5;

$p=0.03$), relative to those who worked in administration and/or management. Contact with tissues of fish also increased the odds of incident infection (AOR 4.4; 95% CI 1.2,16.4; $p=0.03$). The median age of participants with incident infection was 46 years (range 30–70 years); most were male (70%), and worked in the park for a mean of 11 years (range 2–25 years), with 71% from GRSM.

Tick-borne diseases. All incident tick-borne infections were with spotted fever group rickettsiae. Risk increased with increasing age: each additional 5 years doubled the odds of incident infection (AOR 2.2; 95% CI 1.1,4.5; $p=0.02$). These participants were a median of 55 years old (range 49–70 years), worked at the park for an average of 14 years (range 2–25 years), and spent a mean of 26% (range 5–75%) of their work hours outdoors. Eighty percent were male ROMO employees.

Mosquito-borne diseases. One individual had incident infection with a mosquito-borne pathogen serologically identified as La Crosse virus. This participant was a male GRSM administrative employee who also reported working outdoors 38% of the time.

TABLE 5. SIGNIFICANT SELF-REPORTED RECREATIONAL, WORK-RELATED, AND MEDICAL RISK FACTORS FOR INCIDENT ZOONOTIC INFECTIONS AMONG EMPLOYEES FOR GREAT SMOKY MOUNTAINS (GRSM) AND ROCKY MOUNTAIN (ROMO) NATIONAL PARKS EMPLOYEES, 2008–2009

Zoonotic pathogen with which the employee was infected	Risk factor	Odds ratio	95% Confidence interval	p Value	Adjusted odds ratio	95% Confidence interval	p Value
≥1 agent	Job type:						
	Administration/management	Referent	–	–	–	–	–
	Resource manager	7.4	1.4,37.5	0.02	6.6	1.3,34.7	0.03
	Law enforcement ranger/rescue crew	6.5	1.1,36.5	0.03	6.5	1.1,37.5	0.04
Spotted fever group rickettsiae	Contact with fish tissue/fluids	4.9	1.4,17.0	0.01	4.4	1.2,16.4	0.03
	Age (5-year increments)	2.3	1.0,5.0	0.04	–	–	–
	Contact with deceased canid	17.8	2.3,138.4	0.006	–	–	–
	Contact with reptile	34.0	3.5,329.6	0.002	–	–	–
<i>Bartonella henselae</i>							
<i>Leptospira</i> spp.	bodily fluids/feces						

Diseases transmitted by other routes (e.g., flea-borne or direct transmission). Twelve incident infections occurred by pathogens transmitted through neither tick nor mosquito bites. Exposure to *B. henselae* was significantly associated with contact with deceased canids (OR 17.8; 95% CI 2.3,138.4; $p=0.006$), while *Leptospira* spp. infection was significantly associated with contact with reptile bodily fluids and feces (OR 34.0; 95% CI 3.5,329.6; $p=0.002$). Seventy-five percent of participants with incident *Leptospira* spp. infection also reported contact with rodents and rodent feces, which approached significance (OR 7.3; 95% CI 0.7,73.0; $p=0.09$). Single cases of incident infection were also detected to *B. anthracis* and *T. gondii* in male GRSM employees who reported extensive animal and soil contact at home and work, and had noticeable illnesses in the preceding year.

Discussion

This study described prior and incident zoonotic infections among employees at the GRSM and ROMO National Parks during 2008–2009. Though other studies have investigated zoonotic disease risks among national parks (Boyer et al. 1977; McLean et al. 1989; New et al. 1993; Gese et al. 1997; Mills et al. 1998; Paul et al. 2002; Reeves 2007; Levine et al. 2008; Wong et al. 2009), none have addressed the breadth of pathogens evaluated here. These parks were selected due to their popularity among visitors and the likelihood of the presence of local zoonoses. GRSM is the most visited U.S. national park, averaging 10 million visitors per year and employing >300 permanent staff. It spans parts of Tennessee and North Carolina, states which report the greatest numbers of Rocky Mountain spotted fever (RMSF) cases (Adjemian et al. 2009; Openshaw et al. 2010). ROMO also receives millions of visitors each year, and maintains >230 permanent staff. Zoonoses previously detected in ROMO include Colorado tick fever virus, plague, tularemia, and tick-borne relapsing fever (Trevejo et al. 1998).

NPS employees have unique occupational risks for acquiring zoonotic diseases. Here, NPS employees reported spending 40% of their workday outdoors, and with primary job duties that included vegetation management, road and grounds maintenance, wildlife management, trail maintenance,

and search and rescue. Similarly to other outdoor occupations, NPS employees had substantial and often prolonged exposure to ticks and mosquitos, as evidenced by noticeable and frequent insect bites (80%), and the presence of ticks on their skin or clothes (32%; Piacentino and Schwartz 2002; Cinco et al. 2004; Buczek et al. 2009). Despite this, insect repellent use was only rarely reported (Bartosik et al. 2008). As was previously observed in forestry workers, the risk of infection increased with age (Buczek et al. 2009). Additionally, NPS workers demonstrated the potential for direct zoonotic exposure through contact with dead rodents (20%), and felid or canid bites (4–6%), as is often seen in wildlife officers and veterinarians. Notably, NPS resource managers and law enforcement ranger/rescue crew members were at highest risk for incident zoonotic infections, suggesting a possible need for division-specific prevention measures.

We found evidence of high rates of prior zoonotic infections among NPS employees, in many cases exceeding expected seroprevalence rates for the general population. The seroprevalence of California serogroup viruses (31%), *B. henselae* (25%), spotted fever group rickettsiae (21%), and flaviviruses (5%), were greater than what is reported in the literature (Yevich et al. 1995; Reisen and Chiles 1997; Spach and Koehler 1998; Hilton et al. 1999; McCall et al. 2001; Marshall et al. 2003; Stramer et al. 2005). California serogroup bunyavirus infections detected in ROMO predominantly showed specific serological reactivity to Jamestown Canyon virus, consistent with the regional distribution of snow pool mosquito vectors, whereas those detected in GRSM were predominantly seroreactive to La Crosse virus, consistent with the distribution of *Aedes triseriatus* vectors (Campbell et al. 1992). Prior infection with several rare and unexpected pathogens was also identified, including the causative agents of anthrax, tularemia, and Q fever. Agents detected at levels similar to those previously reported included *T. gondii* (Jones et al. 2007), Colorado tick fever virus (McLean et al. 1989), *E. chaffeensis*, and *A. phagocytophilum* (Yevich et al. 1995; Demma et al. 2005), though *A. phagocytophilum*, which was observed in 7% of ROMO participants, is not known to occur in Colorado (Demma et al. 2005). While >7% of participants demonstrated possible prior infection with *Brucella* spp., these results could not be confirmed due to the potential for cross-reactivity with

other bacteria, given the titer levels observed (Al Dahouk et al. 2003a, 2003b). As expected, there was no serologic evidence of prior infection with *B. burgdorferi*, which is not endemic in either park (Bacon et al. 2008), *B. hermsii*, which is most commonly transmitted to persons staying in poorly maintained rustic cabins (Boyer et al. 1977; Trevejo et al. 1998; Paul et al. 2002), or *Y. pestis*, which is rare, with <10 U.S. human cases reported annually (Gage and Kosoy 2005). While we cannot infer whether baseline infections occurred at the current park of employment, the background seroprevalence provides a unique cross-sectional view of this population.

Incident infections with spotted fever group rickettsiae were noted in both parks. GRSM runs through Tennessee and North Carolina, which are among the top states for the reported incidence of RMSF (Openshaw et al. 2010). While a focus of unusually severe and fatal RMSF has been noted in western Tennessee, a milder focus has been identified in North Carolina, fueling speculation that some RMSF reports in this state may actually reflect less pathogenic spotted fever group rickettsiae circulating in the area, such as *R. parkeri* or *R. amblyomii* (Apperson et al. 2008; Adjemian et al. 2009). Spotted fever group rickettsiae also demonstrate cross-reactivity with *R. typhi* and *R. prowazekii*, both typhus group rickettsiae agents (Hechemy et al. 1989).

Incident infection was also detected for *Leptospira* spp., which causes leptospirosis and ranges from subclinical or mild (~90% of infections) to severe illness (Stern et al. 2010). Leptospiral infection occurred in 6% of GRSM participants, and was significantly associated with reptile exposures and mildly associated with rodent contact. While small mammals are considered to be the only primary maintenance hosts that can transfer *Leptospira* to humans, *Leptospira* have been identified in >180 species, including reptiles (Guerra 2009). Incident infection with *B. henselae*, a flea-borne pathogen, was 18 times greater among those reporting contact with deceased canids. When an animal dies, ectoparasites that can carry *B. henselae* might jump to the nearest host available, including humans, facilitating disease transmission. Infection can also occur from contact with infected bodily fluids or tissues (Breitschwerdt et al. 2010). While canids can be infected with *B. henselae* (Diniz et al. 2009), their role as a reservoir for *Bartonella* spp. remains unclear (Chomel et al. 2006). It is possible that this finding may also reflect serologic cross-reactivity with other *Bartonella* spp., such as *B. rochalimae* and *B. vinsonii berkhoffii*, which have close associations with wild and domestic canids (Henn et al. 2009).

Isolated incident infections with zoonotic pathogens were also detected. Incident infection with *B. anthracis*, the cause of anthrax, occurred in an individual from GRSM, though *B. anthracis* is not known to occur in this geographic region, suggesting that exposure likely occurred elsewhere (Blackburn et al. 2007). Lastly, incident exposure to *T. gondii*, which causes toxoplasmosis, occurred in a GRSM participant who reported owning a cat at home. Cat feces or undercooked meat are the main sources of *T. gondii* exposure (Elmore et al. 2010), though transmission is relatively common (Dubey and Jones 2008), making it difficult to distinguish how exposure occurred.

When evaluated collectively, contracting any incident infection during the study period was significantly more likely to occur among NPS resource managers and law enforcement ranger/rescue crew members, and in individuals with contact

with fish tissues. Given that these factors were not associated with increased risk for any one particular agent, it is likely that they serve as a proxy for other factors that are more important in the causal pathway of exposure to zoonotic diseases. Resource managers and law enforcement ranger/rescue crew members are probably more likely to spend time participating in higher-risk outdoor areas and activities given the nature of their job duties, while individuals who reported contact with fish tissues likely spend more recreational time participating in outdoor activities such as fishing, thus increasing their risk for exposure.

This study is subject to several important limitations. The small sample size limited our ability to detect incident infections and meaningfully analyze some risk factors for exposure. The exposures detected were not necessarily confined to the parks where the participants worked, as NPS employees travel often due to transfers between parks, for personal travel, and because they have a predilection for outdoor activities. Additionally, while exposures to some zoonoses induce lifelong immunity, an absence of antibody responses does not exclude prior exposure, particularly if it occurred many years ago. Though inferences about geographic distribution and other risk factors for staff are limited, park-specific similarities and differences are interesting to note and may help guide future research.

Conclusions

While risks to park visitors do not necessarily equal those of NPS employees, the prevalence and incidence described here increase our understanding of pathogens potentially circulating within both parks, and can be used to inform public health interventions for both staff and visitors. Although most zoonotic disease prevention programs rely on environmental control interventions (e.g., area-wide acaricides and animal control), and personal protection (e.g., the use of repellent and protective clothing) to reduce risk, NPS employees must almost always rely on personal protective behaviors to maintain the NPS mission of preserving the park unimpaired. Therefore, educational programs emphasizing routine insect repellent use and protective clothing should target both visitors and staff. Such training programs should be tailored to specific occupations or job categories, and all should highlight the common zoonoses detected here and their associated high-risk activities in order to increase awareness.

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